3IMEBE

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THE THIRD INTERNATIONAL MEETING ON ENVIRONMENTAL BIOTECHNOLOGY AND ENGINEERING

Edited by:

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Carme Garau, Joana Maria Luna, Joan March, Antoni Martorell
Margalida Colombas, Jaume Vadell, Jeroni Vera
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IMEBE, INTERNATIONAL MEETING ON ENVIRONMENTAL BIOTECHNOLOGY AND ENGINEERING

WHAT IS IMEBE?

WHY IMEBE?

The best person to explain and answer to the questions about IMEBE is the person who found this series of meeting, Dr. Héctor Poggi Varaldo from Cinvestav (Mexico). In the next text, he explains us the importance of Biotechnology and Engineering for our societies

The synergistic interaction of Environmental Biotechnology and Environmental Engineering has a tremendous potential for making outstanding contributions to the sustainable development and sustainable management of resources in modern societies. To a great extent, we expect that these contributions will also positively impact on societies' organization and improve people's conscience, education and habits. Sustainable development should become the basis for the life of future generations as opposed to over-exploitation of non-renewable energy and material resources and the shortening of life cycles.

Dr. Héctor Poggi Varaldo

Thanks to Dr. Poggi for inviting us to organize the 3rd edition of the IMEBE meeting series, because the main objective of IMEBE is the care of the earth, through the environment with two very important tools Biotechnology and Engineering.

The environment does not understand about countries, politics, only about changes, impacts, risk, pollution, degradation. The earth is the best example of a homeostatic process. We must help through Biotechnology and Engineering, because they are excellent tools to restore the equilibrium and good health of the planet.

Dra. Isabel Sastre Conde

Terrestrial and aquatic ecosystems are affected by the interaction of factors from natural or anthropogenic origin. Both of them have direct or indirect impact on the environment causing deterioration of soil and water which are fundamental matrices for the vital cycles of the ecosystems and therefore, the earth's general equilibrium. To counteract this damage, a series of strategies must be defined to protect and remediate the terrestrial and aquatic systems. These strategies should focus on microbial ecology by promoting vital reactions of the ecosystems' matrices (soil, sediments, and water).

The congress intends to translate fundamental knowledge on the microbial ecology of impaired ecosystems into technological solutions for the restoration of terrestrial and aquatic environments. The unification of fundamental and applied research is of utmost importance because they are interdependent and connected to the action-reaction of the environment. Then the main objective of the congress will be to integrate the different technology oriented research (engineering, biotechnology) in such a way that their future action will be based on fundamental research (ecology).

The action on the environment must be interactive with the participation of different disciplines having common objectives:

- One of the common objectives must be to preserve energy resources and create renewable ones.
- Other common objective must be the re-use of waste which should be valorized and not seen any more as waste but better as "new resources".
- Finally, the last but not the least common objective must be to minimize pollution and discharge to the environment in order to protect its ecology.

All this will be achieved only with the development of cleaner and cheaper technologies both on an environmental and an energy point of view as well as with an adequate environmental education and a sustainable development.

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El congreso desea agradecer sinceramente el trabajo de aquellos investigadores que luchan por mejorar las condiciones ambientales, y especialmente aquellos que no olvidan nunca que son parte de la naturaleza, y como tal la protegen. La co-chairwoman de este congreso quiere puntualizar y dirigir su vista y la de muchos otros, con el afecto y admiración que se merecen, hacía dos personas que brindan su vida al trabajo, y al buen trato personal. Esas personas sobre quien quiero llamar la atención son el Dr. Fernando Esparza García y la Maestra Elvira Ríos. Ellos dedican su vida a trabajar en las temáticas de Biotecnología e Ingeniería ambiental. Disciplinas estas que no se debe olvidar que estas como tal no serían nada sin la ecología, sin la labor conjunta de todos los responsables de esa gran pirámide ecológica que existen dentro de cada ecosistema. Pirámides igualmente representadas en los laboratorios de investigación, donde los investigadores, estudiantes, maestros, auxiliares, etc. son todos juntos parte de esa labor en investigación ambiental. El Dr. Fernando Esparza García es un buen ejemplo de trato ecológico, de cohexión entre las distintas disciplinas de investigación en el departamento de Biotecnología ambiental del Cinvestav, y por supuesto entre los distintos miembros de la pirámide de investigación en su laboratorio. Finalmente mí reconocimiento a la labor ambiciosa del Dr. Hector Poggi de proponer ideas como el IMEBE, integradoras de todos los medios que componen el medio ambiente (suelo, agua, atmósfera etc). Labor que nunca podría haberse realizado sin la ayuda de personas con tanto tesón y esfuerzo como la Maestra Elvira. Finalmente dedico el 3IMEBE, desde mí aportación personal a todos aquellos que creen en la verdadera investigación desde el esfuerzo de trabajar para el conocimiento.

The congress would like to sincerely thank the work of all researchers who fight to improve the environment, particularly those who never forget that they are part of this environment and as such try to protect it. The co-Chairwoman of this congress would like to focus and turn her eyes and those of several others, with the tender and admiration that they deserve, to two persons who dedicated their life to work for the environment and personal relationship, Dr. Fernando Esparza and MSc. Elvira Ríos. Within Environmental Biotechnology and Engineering, we should not forget that these disciplines would be nothing without Ecology since they only reproduce the natural processes and that these processes would not be possible without the cooperation of all the members of the pyramid involved in them. In the same way, research would not be possible without the common work of researchers, students, technicians, etc. Dr. Fernando Esparza is a model of an ecological behaviour within the different research fields in his Environmental Biotechnology Department from Cinestav and of course within the different members of the research pyramid of his laboratory. Finally my recognition goes to the ambitious work of Dr. Hector Poggi and among this work to propose ideas such as the IMEBE congress series which tries to integrate all elements composing our environment (soil, water, atmosphere, etc). Such an idea would never have been turned into reality without the efforts and tenacity of persons such as MSc Elvira Rios. Finally, with my personal contribution, I dedicate 3IMEBE to all persons who believe in true research and who work first of all because they are thirsty of knowledge.

Dra. Isabel Sastre Conde
Co-chairwoman of 3IMEBE

ORGANIZATION, concretely the chairpersons thank:

CONSELLERIA D'AGRICULTURA I PESCA DE LES ILLES BALEARS

IRFAP
IBABSA
SERVEI DE MILLORA AGRARIA

THOSE WHO ALLOWED US TO SET UP 3IMEBE IN MAJORCA

Maria José Suasi Amengual (Conselleria d'Agricultura i Pesca)
Antoni Martorell (IRFAP, Conselleria d'Agricultura i Pesca)

DIFFERENT SPECIAL PEOPLE

- The Scientific Members of the International and National Committees
- The students with fellowship, because some of them made a great effort to get their visa for Spain
- The authors of the different works and all persons attending 3IMEBE, because they are, through their projects, the objectives of the meeting
- The persons of Dr Poggi's group who in different moments made possible the 1st and 2nd IMEBE in Mexico, specially Rafael Hernández.

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MONDAY, 22 SEPTEMBER

TUESDAY, 23 SEPTEMBER

WEDNESDAY, 24 SEPTEMBER

THURSDAY, 25 SEPTEMBER

THE THIRD INTERNATIONAL MEETING ON ENVIRONMENTAL BIOTECHNOLOGY AND ENGINEERING

MONDAY 22 SEPTEMBER

ORAL COMMUNICATIONS

I. Risk assessment and environmental impact

II. Wastewater and solid waste management, treatment and reuse

MONDAY 22 SEPTEMBER 2008 ORAL COMMUNICATION

I RISK ASSESSMENT AND ENVIRONMENTAL IMPACT

Pollution of the Aquatic Ecosystems

Land degradation processes

GUEST LECTURE

2896 MEASURING BIOLOGICAL PARAMETERS IN RIVERS: RELEVANCE OF THE SPATIAL SCALE.

Anna María Romani
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The analyses of biological parameters in river ecosystems have been traditionally used as indicative of water quality with the advantage over chemical or physical analyses that they integrate the effects of punctual as well as long term effects. However, analyses of biological parameters (such as biomass and metabolism) performed at different spatial scales (from the microbial communities to the whole river) inform about different key processes. At the finer scale, microbial interactions and the structure of the microbial community (biofilm microbial biomass, three dimensional structure, and relevance of polysaccharide matrix) can be detected. At the reach scale, the different streambed substrata (sediment, rocks, and particulate organic matter accumulation) are shown to play differential and specific roles on the processing of organic and inorganic materials in the flowing water. Most studies show the importance of preserving streambed patches diversity for the river ecosystem function. In large rivers, the relevance of biological parameters at the planktonic versus the benthic fraction needs to be considered. In whole river ecosystem studies, the changing physical and chemical parameters along the river (such as discharge, temperature and nutrient content) affect on biological parameters. In whole river studies, comparison of different stream reaches includes not simply those predicted by the river continuum concept (increasing nutrient content, changing organic matter quality, changing autotrophic/heterotrophic metabolism) but many times includes also anthropogenic effects (such as flow regulation, toxicity and increasing nutrients due to urban, agriculture and/or industrial activities). Examples of studies of biological parameters in rivers performed at different spatial scales show the different but interacting conclusions that can be obtained when using different scale approaches.

Acknowledgments: Most data included in the talk were thanks to the projects RIORGFUNC, GLOBRIO, MODELKEY and KEYBIOEFFECTS

2402 AN INTEGRATED APPROACH TO AQUATIC HEALTH ASSESSMENT: WATER QUALITY INDEX AND MULTIBIOMARKER RESPONSE

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The pollution of water bodies reduces their quality and is stressful to their biota. In a river, water usually is of the highest quality in its headwaters reaches, becoming dirtier along its length as it passes through different land uses. Therefore, the aquatic environment should be assessed using physicochemical and biological features in order to provide a full spectrum of aquatic ecosystem health. Water Quality Indexes can be used to aggregate data on water quality parameters and to translate this information into a single value. The use of biomarkers as indicators of toxicity delineates the effects of xenobiotics before the appearance of diseases in aquatic organisms. The use of a battery of biomarkers may be useful to evaluate the various responses to mixtures of pollutants. We evaluate the environmental quality of the Champotón river in the upper portion, considering two approaches: the water quality index (WQI) and a battery of biomakers: lipid peroxidation (LPOX), glutation S tranferase (GST) and ethoxyresorufin-Odeethylase (EROD). The Champotón river in Campeche, Mexico, is one of the few water courses of the southeast of Mexico. Four surveys to three study sites were conducted in 2007. Water quality parameters were recorded in situ using a Quanta probe, and water samples were taken for chemical analyses and calculate the WQI. In all study sites, fish specimens of Astyanax aeneus were captured using a electrofishing unit. The fish were immediately dissected to get liver and transported to laboratory in liquid nitrogen. A discriminant analysis allowed the integration of the two approaches and clustered the three study sites along dominant gradients of WQI, LPOX, EROD and GST. The study site in the headwater showed the lowest WQI scores, highest values of EROD and intermediate LPOX values. The highest value of GST it was in the most far away study site.

2563 VALVE MOVEMENT RESPONSE OF THE MUSSEL, *Mytilus galloprovincialis* TO CYPERMETHRIN (PYRETHROID – INSECTICIDE)

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The Moroccan lagoons are economically important and ecologically very sensitive. They are affected by pollution from a range of sources, especially by agricultural pollution from the adjacent agricultural areas that use huge quantities of organic pesticides. These products can be harmful to non target organisms by drainage. A biological monitor for the water quality surveillance using mussels is described. It was designed to characterize the toxicity of the Cypermethrin (pesticide – pyrethroid) to the coastal environment by assessing its sublethal effect on valve movement of the mussel *Mytilus galloprovincialis*. In this method, the valve behaviour of exposed mussels to Cypermethrin is compared with normal valve opening (control) and the reduction in the time of normal opening is used as a rapid response to detect contaminants. The specimens of *M. galloprovincialis* were collected from a reference area (Cap Beddouza) and exposed to a concentrations ranging from 25 to 800 μg/l. The activity was then monitored for 4 hours. The results show that Cypermethrin induced a reduction in the time of normal opening and that this effect is concentration-dependent. The lowest effect concentration was determined at 100 μg/l of Cypermethrin and all reductions in the time of normal opening were statistically significant (100 μg/l). Prolonged closures of mussels were also observed for the exposures to 400 and 800 μg/l. Reductions in the normal opening were of 26.97%; 39.86%; 56.12%; 56.12% and 79.35% respectively for 100; 200; 400 and 800 μg/l of Cypermethrin. The integration time (the delay to the first detection of the toxic) was concentration-dependent. This time was 110; 90; 50 and 40 minutes, respectively for the concentrations of 100; 200; 400 and 800 μg/l of Cypermethrin. These results were in concordance with those on valve movement with the Malathion (organophosphorus insecticide) although the integration time was relatively short with regard to the Cypermethrin and the detection becomes immediate for the high concentrations (unpublished data).

2499 SALINITY LEVELS AS A FACTOR OF PHYTOPLANKTONIC STRUCTURE AND DIVERSITY REMEDIATION IN A LAGOON IMPACTED BY HUMAN ACTIVITIES. (Bolmon Lagoon, Mediterranean Coast, France)

Fayolle S.[1], Charpy-Roubaud C.[2], Ollivier A.[1], Maasri A.[1], Franquet E.[1]
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The Bolmon lagoon (South-eastern France) is located in the coastal hinterland of the Mediterranean Sea. It is connected to the salty Berre pond and in the other hand receives fresh waters from the La Cadiere River. This hyper-shallow lagoon has been classified in the early 70's as β mixo-mesohaline. Bolmon lagoon is important for human activities (tourism, fishing) and for the preservation of the diversity of marine and birds species. In the last decades, the intensive urbanisation of its drainage basin induced a high eutrophication of its waters and, as a consequence, drastic modifications in the biodiversity and in the fonctionning of this ecosystem. The high growth of phytoplanktonic organisms, induced periods of anaerobic conditions. Blooms of cyanobacterias were recurrent, dominated by *Planktothrix agardhii* (which attempts 30.10^6 cells/l), in winter and in spring, and by *Pseudanabaena limnetica*, in summer; *Limnothrix planctonica* was present the whole year. During the year 2007, important hydraulic managements were made, resulting in a decrease of freshwater arrivals in Bolmon lagoon. The concentration in nutrients did not decrease strongly but the salinity increased drastically; Bolmon lagoon became classified as α mixo-mesohaline. The present work explored the influence of the salinity in terms of its impact on diversity and density phytoplankton changes, studied by microscopic observations and counts of cells (Fush-Rosenthal count cell). Results showed modifications in phytoplanktonic structure and in the cyanobacterial community. In particular, if the differents levels of salinity did not impact nor *Pseudanabaena limnetica*. nor *Limnothrix planctonica*, the dynamic of *Planktothrix agardhii* is particularly sensible to the salinity. This toxic species, which was predominant with a very high density when the waters were β mixo-mesohaline disappeared totally when the salinity was superior to 12 g/l. In the lagoonal Bolmon the salinity levels, because strongly contributing to diversity and development of indicating species of pollution such *Planktothix agardhii*, must be taken into account from the point of view of remediation of these types of ecosystems.

2217 RESPONSES AND STRUCTURAL RECOVERY OF PERIPHYTIC DIATOM COMMUNITIES AFTER SHORT-TERM DISTURBANCE IN SOME RIVERS (HANOI, VIETNAM)

Duong T. T.[1], Feurtet-Mazel A.[2], Coste M.[3], Dang Dinh Kim[1]
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Field transfer experiments of periphytic diatom assemblages developed on artificial substrates were set up to assess responses of periphytic diatom communities to environmental disturbances. Glass substrates were positioned for colonization in comparatively unpolluted site (Red, in Red River) and heavily polluted site (TL, in Tolich River) at the beginning of the experiment. After a period of two weeks colonized glass substrates were transferred from Red site to TL site and to a moderate polluted site (NT_2 in Nhue River) and conversely, from TL site to Red site, and to NT_2 site. Responses and capacity of periphytic diatom communities to adapt environmental changes were assessed by using cells density, diversity index, species richness, taxonomic composition and diatom indices after 2 and 4 weeks transfer periods and varied for each site. For transfers from Red to NT_2, TL to Red and TL to NT_2, diatoms density significantly increased till the end of experiment whereas growth inhibition of diatom cells was found in transferred biofilms from Red site to TL site. Thus, diatom communities have expressed their pollution tolerance or sensitivities by changing their composition to adapt themselves to changes of environment. In transferred biofilms, from Red to NT_2, characteristic species of Red site were replaced by *Nitzschia palea, Nitzschia umbonata, Aulacoseira granulata* species typical of NT_2 site. Relative abundances of typical diatoms species of Red site proliferated in biofilm transferred from TL site to Red site. Replacement of periphytic diatoms communities after transfer appeared from two weeks in the different sites. Slowly shift of Red species by typocal TL species could be in relation with the organized structure of the biofilm before transfer. Species richness and diversity index were not clearly reflecting responses of periphytic diatom to disturbance. Shifts in values of IPS and DAIPo indices, throughout the experiment indicated sensitivity of these indices to water quality changes.

2403. PRIMARY PRODUCTION AND ALGAE DIVERSITY vs. POLLUTION IN XOCHIMILCO WET-LANDS

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Xochimilco is an ancient endorheic lake located in the Valley of Mexico. Due to its shallow waters and the freshwater springs that lined the canals, they are surround raised agricultural fields called "chinampas". Since the Valley of Mexico was originally wetlands, the chinampas were the most productive means of agricultural production. Xochimilco are considered one of the most important "urban lungs" in Mexico City. However, it is not clear how the huge urbanization around the canals could affect the primary production with oxygen evolution. The main objective of this fundamental research is to know if there is a correlation between primary production (PP), algae diversity, BOD_5 and faecal coliforms. Sample collection was done every month over a year at six different canals named: Embarcadero Celada, Embarcadero Nuevo Nativitas, Canal Las Abejas, Canal Zacapa, Canal Santo Domingo y Canal Nacional. Temperature and pH were measured *in situ* and O_2 was measured asap with an oxymeter, as well as the BOD_5. Algae were grown in BG-11 agar at room temperature/15 days. Coliforms were evaluated by the most probable number (Official Mexican Norm NMX-AA-042-SCFI-2005). The results showed low PP during the year at the six sites of sampling. Two canals: Nacional y Santo Domingo basically did not have PP. The other four canals showed a picture where a PP increased according the effect of light intensity and temperature in spring and summer. We just found all together 16 genera of algae of which 14 were green algae and 2 were diatoms. The most abundant algae were: *Gloeocystis* spp, *Chlorella* spp, *Oocystis* spp, *Scenedesmus* spp, y *Kirchneriella* spp. Concerning domestic pollution data, BOD_5 had 13 of 72 samples over 30 mg O_2/l which showed a low organic contamination according the water quality. The results of feacal coliforms analysis behave almost like the BOD_5 where 6 of 72 samples were below 1000 MPN/100 ml coliforms. Despite lower coliforms abundance and lower BOD_5 determination, the average of PP was very low. Our conclusion is that Xochimilco wet lands are not working as urban lungs and perhaps they are strongly contaminated by chemicals which are not biodegraded.

2505 SELECTIVE GRAZING FROM PROTIST OVER ENTERIC BACTERIA IN AN AQUATIC SYSTEM

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It's very clear that the grazing from protozoan can be an important source of mortality for the suspended bacteria, both in marine and freshwater environments. Considering that the presence of fecal contamination its a frequent phenomenon in this environments, and that *Escherichia coli* and members of *Enterococcus* genera are indicators of microbiology water quality, we analyze the effect of grazing from protozoan over *E. coli* and *Enterococcus faecalis* in de Los Padres Lagoon waters (Buenos Aires, Argentina, 37° 56´30" S, 57° 44´30" W). We carry out microcosm tests, simulating lagoon's environments; confronting suspensions of autochthonous bacterivorous protozoan with suspensions of autochthonous and collection stocks of *E. coli* and *E. faecalis*, mixed and individually. Once a day we make a respective count for elaborate the survival line. The results that we obtain indicate that exist a preferential sequence for bacterial removal in the water, where *E. faecalis* is more grazing-resistant than *E. coli*. Moreover, we note that the source/origin of the bacterial stocks conditioned its sensibility for grazing, at least in a short time, because the collection stocks have less affected. For all of this, we conclude that in de Los Padres Lagoon, grazing can modify the relative abundance of fecal indicator microorganism, altering the results of water quality studies. Considering that the bacterial charge is one of the parameters that conditioning the water quality for human drink and recreational uses, the control that grazer's protozoan makes over the bacterial concentrations in water, it's a very interesting point of start for the development of bioremediation techniques. For the last, the enhance of *in situ* studies that allows weigh up the mortality induced by protozoan, makes possible the enlargement of data bases to consider when be necessary evaluates the risk of non autochthonous species introduction in a water body.

GUEST LECTURE

1001 SOIL THREATS AND SOIL PROTECTION: THE ROLE OF BIOTECHNOLOGY

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The concept of soil conservation/soil protection in its wider sense has undergone important changes through history. Perceptions of soil as a crucial base of life in ancient cultures progressively evolved to a more pragmatic vision, with close connection to food production for survival. For centuries, agrarian production and the provision of food for humankind remained the main and crucial vision of the interaction of societies with soil. However, there are also some other new and important concepts related to soil which have progressively developed.

Development of soil conservation is strongly connected to schemes of socio-economic expansion, territorial colonization and agricultural production. The global phenomenon of the 'Green Revolution,' implied new perspectives and adaptations of soil conservation schemes to the challenges of the demands for more soil productivity to cope with feeding an increasing world population. The recent developments on the world food shortage issues pose new demands for increased soil productivity, which impinges on soil conservation issues.

The Rio de Janeiro Summit of 1992 represented a landmark and a point 'of no return' in the consideration of the environmental dimension and implications of human economic activities, including agricultural and soil aspects. The subsequent UN Conventions on Loss of Biodiversity, Climate Change and Desertification globally institutionalized the need to incorporate new and more intelligent approaches in our interaction with our natural resources.

At present, globalization and new trade and market schemes are introducing new elements affecting agriculture and soil conservation. In these new global dimensions and more than before we still have the fundamental challenge to produce food for the unstoppable demographic growth. Nevertheless and slowly, new visions and perceptions of soil conservation are evolving.

Under the perspective of soil protection schemes society is considering new aspects, such as soil diversity, soil ecological functions, soil as a crucial environmental link, soil threats and new soil uses, besides the traditional role of soil for biomass production (agriculture, pasture and forest). The ongoing EU Strategy on Soil Protection includes some of these elements. The initiative of the EU was launched in April 2002 with the Communication of the EU Commission entitled 'Towards a Thematic Strategy for Soil Protection.' This document is a comprehensive analysis of the situation of Europe´s soils, highlighting the importance and dimension of soils as a threatened and non-renewable natural resource. The objective of the Soil Strategy is the "protection and sustainable use of soil, preventing further soil degradation and preserving its functions while restoring degraded soils" (COM 2006, 231). The European approach was innovative in the sense of incorporating this new and wider environmental perspective. The efforts of the EU are oriented to a wider consideration of the role of soil and its environmental functions. The main soil functions considered in the EU Strategy for Soil Protection are: food and biomass production; habitat and gene reservoirs; sources of raw materials; climate regulating system; hydrologic cycle regulator; buffer and filtration functions for toxic compounds; physical support of urbanization and infrastructures and cultural function and historical register.

Hopefully, the EU Strategy will open the door to conceptual and practical implications of soil as an important regulating system of both climate and the water cycle. The implications of the 4th IPPC Report and its alarming projections oblige us to consider the potential of every natural system to buffer the trend of global warming. The regulating capacity of soil in relation to gases with greenhouse effects (such CO_2, methane and nitrogen oxides) and its role as a source of dust and aerosols, deserves attention for its strong potential for both conserving soil and contributing to ameliorating the rapidity of climate change. Perspectives on water scarcity are also linked to soil. Evaluations and prospective analysis from international organizations (e.g. the UN, EEA, OECD, WRI and WWI) unfold a worrying future panorama. The three developments in aggravating water scarcity are: permanent and sustained increases in water demand (unstoppable since 1950); reduction in precipitation owing to climate change (drylands) and loss of the soil regulating capacity. This last aspect is clearly linked to the need to promote good soil stability and soil health to increase its important role as a regulating system of infiltration/runoff and as a water reservoir.

Soil conservation history shows important evolutionary stages, both in conceptual aspects and in technological-scientific developments. Some items of this evolution are mainly related to perception and to adaptation of new societal needs and demands. Progressively, farmers have been accumulating records and registers of performance of the land and plants in response to different practises. Therefore, over the centuries, basic adaptations to the potential and limitations of the natural environment underpinned successful approaches to the use of the land. The poor adaptation to the basic ecological conditions of the territory or the variability and impacts of extreme climatic events often represented failures in crop production and problems of famines or even survival. Food production is the 'leif motif' of agriculture and soil conservation. There is no need to insist in embracing this aspect, considering the exponential growth of world population, food shortages and increased prices of basic foods. However, in developed countries the success in increasing the agrarian productivity and some socio-economic trends, resulted in substantial changes in the proportion of the population dedicated to agrarian activities. Furthermore, during the last third of the 20th century, society began to be increasingly concerned with impacts and environmental deterioration. Agricultural activities started to be critically examined. High yields, high productivity and surplus of agricultural products reduced the need for agricultural surfaces. Because of these tendencies, much land will probably undergo important changes in land use, at least in the EU and other developed countries. Newly abandoned land will require the development of new conceptual approaches and management schemes to maintain ecological functions, landscape features and biodiversity. In this new framework, soil conservation will have room for substantial contributions.

Today's soil conservation frameworks should expand to include new perspectives and interlinkages in the context of the integrated functioning of terrestrial ecosystems. However, it is imperative that we maintain vital aspects from previous schemes, including maintaining soil fertility and improving soil-water interactions. Today we can see a steady trend in the pattern of soil use in Europe, with important future consequences for landscapes and the functioning of terrestrial ecosystems. This trend of change should be seriously evaluated for its implications on soil conservation and soil quality in the context of multiple uses and multi-functional approaches. Modern society places demands on scientific, academic and technological community to develop new ideas, new information schemes and new conceptual developments to deal with new perceptions of the role of soil in the global and local functioning of terrestrial ecosystems. The demands for biodiversity maintenance, carbon cycle regulation, combating desertification, food production, water resource regulation and landscape maintenance require new soil protection paradigms that should be developed under soil multifunctional and multiuse approaches. The emerging field of bio-engineering applied to soil conservation offers important options on soil restoration and stabilization, protection again erosion and in preserving the quality of the landscape in terms of biodiversity and esthetic values. The concept of soil as a crucial and menaced natural resource demands a general framework for sustainable use of soils that should be developed under the wider consideration of soil as a multifunctional medium, including new biotechnological developments in soil management in accordance with ecological principles.

2360 ASSESSMENT OF METAL CONCENTRATIONS AND TOXICITY IN THE GARDEN SOILS AND TAILING SAMPLED FROM TWO MINING AREAS IN SOUTH MOROCCO

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Anthropogenic activities such as phosphate fertilizers, industrial wastewater effluents derived from the mining process or wastewater sludge represent the main source of metal contamination in the environment (Adriano, 1986). Heavy metal toxicity disrupts natural ecosystems and affects the food chain, leading to health problems in humans and animals. Morocco, mainly the southern region, has a large number of metalliferrous sites, some of which are being exploited while others have been abandoned. These sites are concentrated in the region of Marrakech, Ouarzazate and in the North of the Anti-Atlas Mountains. The objectives of this work were to evaluate heavy metal concentrations and toxicity in the garden soils and tailings from tow mining sites located in the south of Morocco. This evaluation was obtained by running concurrently selective chemical extractions and the MetPPLATE™ biotest which detects the toxicity specifically due to the inhibition of the β-galactosidase enzyme of a mutant strain of *Escherichia coli* by the bioavailable fraction of the heavy metals. The pH varied between 6.8 in the tailing from mine B to basic values (7.6-8.0) in the most studied soil. In general, the soils contained high concentration of Cu (25.4-627.7 mg kg^{-1}), Mn (2179-4699 mg kg^{-1}) in the mine A and Co (35.5-1778 mg kg^{-1}), Cr (6.7-667.6 mg kg^{-1}) and Cu (26.8-166.8 mg kg-1) in the mine B. The results of the MetPLATE™ biotest showed a low percentage enzyme inhibition in the soils from the mine A and B (non toxic-32%), and contained low mobile fraction of metals except tailing of the mine B (51% inhibition). This toxicity was mainly due to the relatively concentration of soluble metals.The high metals concentrations and toxicity observed in soils at the two mining areas, present a potential health risk for the human populations residing in the vicinity of the mine. It has been suggested that Mn is associated with the onset of Parkinson's disease.

2434 IMPACT OF AMBIENT AND ELEVATED LEVELS OF OZONE ON GROWTH, REPRODUCTIVE DEVELOPMENT AND YIELD OF TWO CULTIVARS OF RICE (*Oryza sativa* L.)

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Two locally grown high yielding varieties of rice [*Oryza sativa* L. var *Malviya dhan 36* and var *shivani*] were transplanted within the open top chambers (OTCs) at the age of 20 days. Five different experimental plots were designed as open plot (OP), non-filtered chamber (NFCs), NFCs with 10 ppb ozone (O_3), NFCs with 20 ppb O_3 and filtered chamber (FCs) to asses the impact of ambient and elevated levels of O_3 on rice. Twelve hourly monitoring of O_3 and measurements of meteorological variables were done at experimental site during the growth period of rice plants. Morphological, reproductive, physiological and biochemical parameters were assessed to evaluate the responses of rice cultivars to ambient and elevated levels of O_3. In cv *Shivani*, an increasing trend was observed in case of shoot length, root length, total number of leaves in plants of FCs at all the sampling dates than other treatments and plants of NFC with 20ppb O_3 fumigation (NFCO2) were most affected. But the interesting results were obtained with plants growing in NFC with 10 ppb O_3 fumigation (NFCO1), where at initial two samplings the plants showed an increase in shoot height but this trend was not carried up to the last sampling. Total number of healthy leaves showed an increasing trend in FC, but in other set ups, it increased up to the 2nd sampling and thereafter it showed a reverse trend. Almost same trend was observed in case of shoot length, root length, total number of leaves and leaf area and in all the cases the plants of NFC with 20 ppb O_3 fumigation. Another variety, i.e. *Malviya dhan* 36, also showed more or less similar trend. Total biomass was higher in FCs as compared to NFCs, OP and NFCO1 and NFCO2 at 75 DAT. In case of plants of O_3 fumigated chambers, foliar injury appeared in the form of interveinal chlorotic stippling or flecking of leaves. The percentage of injury in leaves due to O_3 was directly proportional to the concentration and exposure durations of O_3. Higher Fv/Fm ratios were observed for plants growing in FCs in comparison to those in NFCs and OP at all the stages of observations. Plants of NFC with 20 ppb O_3 showed lowest Fv/Fm ratio but plants of NFC with 10 ppb O_3 fumigation showed a higher Fv/Fm ratio than plants of NFC and OP. Photosynthetic rate was also higher in plants of FC. In all the experimental setups, the maximum reductions in all the parameters were observed in NFC with 20 ppb O_3. Changes in photosynthetic pigments and various enzymatic (SOD, APX) and non-enzymatic antioxidants (ascorbic acid, phenol, total thiol, etc.) were also noticed in various treatments. In case of potent and viable flower development, the number was 15% - 20% higher in FCs than O_3 fumigated chambers. The viable flowers of OPs and NFCs were nearly 5 to 10% less than the FCs. The highest % of non-viable pollens was observed in NFC with 20 ppb O_3 for both the varieties followed by the NFC with 10 ppb O_3 fumigation. Changes in total protein profile of both cultivars rice plants were studied and standardized through SDS PAGE in all the experimental setups.

2691 EFFECT OF METHYLPARATHION ON NITROUS OXIDE PRODUCTION IN "CHINAMPA" SOIL OF XOCHIMILCO, MEXICO

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Contamination of soil from pesticides is a result of their bulk handling at the farmyard or following application in the field. Synthetic organophosphorus compounds like chlorpyrifos and methylparathion have been used in the particulate cultivated areas, the chosen "chinampa" (from Náhuatl or Aztec, chinamitl, bulrush or cattail stalks lattice for hydroponics cultivation) from the Xochimilco zone in Mexico City, Mexico (CICLOPLAFEST, 2006). The effects of the pesticides in the soil microbial communities and the activity of them has been studied a little, although it has been observed that in chemically degraded soil of tempered zones, the microbial diversity diminishes (Girvan et al., 2005). Nevertheless, other studies have observed the degradation of the pesticide after their repeated use in soil (Spokas et al., 2006). These studies of degradation indicate that the modification of microbiota seems to potentially alter the dynamics of the soil microbial functionality after the fumigation, as could be the alteration in the greenhouse gas balance. Recently, it has been disclosed that the use of chloropicrin increased the production of the gas nitrous oxide (N_2O) in fumigated soils (Spokas et al., 2005). On the other hand, the fertilization provides nitrogen in agriculture. The microbial transformations of nitrogen (N_2) via nitrification and denitrification contribute near 70% of the annual emission of N_2O at world-wide level (Rochette et al., 2004). Therefore, the investigation of pesticides in the production of N_2O in agricultural soil represents a great interest in the theme of climatic change. In tropical soil of Mexico, the studies related to the transformations of N_2 in contaminated agricultural soils with pesticides are poor. In this article, the objective is to study the diversity of a denitrifying gene (nirK) and the production of N_2O, in sites contaminated by pesticides. Each aerobic microcosm by triplicate contains 500 g of soil to 40% of water retention capacity (WRC) with three concentrations of methylparathion, within a 1600 ml-glass bottle covered hermetically with valve allowing the gas interchange to maintain the aerobic condition. The three studied concentrations of pesticide are 0.7, 1.4 and 2.8 g/kg dry soil. The following of experiment was executed to times 1, 14, 30, 60 and 90 days. The increase of methylparathion concentration was observed along with an increase in production of N_2O. Actually, the study of the denitrifying diversity by TGGE, (Thermal Gradient Gel Electrophoresis) is in sequence process.

2514 USE OF RADIONUCLIDE TECHNIQUES (^{137}Cs, $^{210}Pb_{EX}$ AND ^{7}Be) TO ASSESS SOIL EROSION IN MOROCCAN AGRICULTURE LANDS

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Water erosion is one of the major environmental and agricultural problems causing serious socio-economic impairment for not only developed countries but also developing countries. It reduces the fertility and productivity of agricultural lands and it is besides a major source of surface water pollution. On the other hand, Intensive or improper farming accelerates greatly this phenomenon. In Morocco, where agriculture is of about 12 to 20% of GDP, in 22 watersheds over 15 million hectares, according to the report of "Plan National d'Aménagement des Bassins Versants", about 11 million hectares are highly threatened by water erosion. For several years Moroccan scientists and managers were focused on this problematic. Several techniques were developed but have shown a number of important limitations. The use of environmental radionuclides (^{137}Cs, excess ^{210}Pb and ^{7}Be) as tracers is an excellent and innovator tool for documenting rates and patterns of soil redistribution within the landscape on long, medium and short term. This work was focused on the use of radionuclide techniques (^{137}Cs, excess ^{210}Pb and ^{7}Be) to study soil erosion in agricultural lands, located nearby Casablanca, Azrou and Rabat, in Morocco. The ^{137}Cs measurements showed spatial particle soil behavior. The major soil erosion occurred in upslope while deposition at the bottom of slope. The net erosion rates were found of about 32, 16.5 and 14 $tha^{-1}yr^{-1}$, respectively. The $^{210}Pbex$ and ^{7}Be measurements were, only, tested in Zaer region. Soil loss assessments resulting from $^{210}Pbex$ measurements were found slightly similar to those obtained from ^{137}Cs. ^{7}Be technique was tested for the last three years in order to study the efficiency of no-till technique. The results showed that mean and net erosion for soil cultivated with "no-till" technique are found significantly lower than those estimated for land under cover crop.

2549 EFFECTS OF ENVIRONMENTAL CONDITIONS ON SOIL SALINITY IN ARID REGION IN TUNISIA

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The shortage of water resources of good water quality is becoming an issue in the arid and semi arid regions. For this reason, the use of water resources of marginal quality such as treated wastewater and saline groundwater has become an important consideration, particularly in arid region in Tunisia, where large quantities of saline water are used for irrigation. Nevertheless, the use of these waters in irrigated lands requires the control of soil salinity and a comprehensive analysis even beyond the area where water is applied. The aim of this study was to investigate the effects of environmental conditions on soil salinity distribution and soil stability after long term irrigation with saline water. The study was carried out in the experimental site of the Olive Tree Institute of Sfax, Tunisia. Two olive orchards were subjected to two drip irrigated treatments: control field irrigated with fresh water (CF, EC = 1.2 dS m^{-1}) and stressed field irrigated with saline water (SF, EC = 7.5 dS m^{-1}). During two successive crop seasons (2004 - 2006), soil samples were taken from the surface until a depth of 1.2 m with a layer of 0.3 m. On these samples, the soil moisture (%) and the electrical conductivity (ECe) of the saturated phase were determined. For the ECe, it was determined also at different distance (0, 0.3 and 0.6 m) from the irrigation source. The soil samples were collected in different seasons (spring, summer, autumn and winter) during both crop seasons 2004/2005 and 2005/2006. The long term saline water irrigation has induced the accumulation of salt ions in SF at higher levels than that at CF. The soil salinity distribution (EC) showed its dependence on environmental conditions characterizing the experimental field. The highest levels of soil salinity, accompanied with the lowest levels of soil moisture, were recorded during summer time and to a lower extent during spring season. The rainfall occurring during autumn and winter maintain the salts leaching and thus low salinity values, in comparison to summer season. The horizontal distribution of soil salinity showed that the more moist layers (root zone) have low level of soil salinity. Despite, the soil salinization induced by saline water irrigation, the drip irrigation system has allowed the upholding of the soil structure stable (sandy) and the soil permeability was not too damaged.

2510 HEAVY METAL CONTAMINATION IN THE ENVIRONS OF THE Zn-Pb MINE IN NORTH-WEST OF TUNISIA

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The impact of industrial heavy metals (HM) pollution on soil quality and plant growth has become a public concern. To evaluate heavy metals concentration a Zn-Pb mine site was selected, as source of pollution, localized in BouGrine (BG) region at 120km North-west of Tunis characterized by calcareous soils. Soils of the mine site are occupied by forest pine. From all sides of the mine site soils are occupied by cereal culture, olive grove and pine. Both soils receive atmospheric and water pollution emitted by Zn-Pb mine. The aim of this research is to determine the evolution of HM (Zn, Pb, Cu and Cd) concentration with increasing distance from the source of pollution to prospect the possibility of existence of eventual pollution in these regions. To test this hypothesis, a sequence of 7 soils were sampled in the forest in the mine and 4 transects around the mine for each one 3 samples are collected. The sampling interested both the litter in the forest, the topsoil (0-20cm) and the subsoil (20-40cm) for all soils. Samples of the fine earth fraction (<2mm) were analyzed for total concentrations of HM, using aqua regia method (HNO_3+HCl), and bio available HM using the method of ammonia-acetate pH4, 65 to mimic metal mobilization in a soil contaminated by these pollutants. Top soils in the mine were found to have significantly higher concentration of HM in top soils exceeding AFNOR norm especially for the Zn (1320 ppm) and Pb (349 ppm). Around the mine, contents were 1300 ppm for the Zn and 311 ppm for the Pb. These results can be explained by the pollution due to the miner activity but also to the geogenic origin. The highest total concentration of the suspected pollutants was enregistrated for the litter with 2035ppm Zn; 5,8ppm Cd and 549ppm Pb. For available forms the concentration of Zn, Cu, Cd and Pb in top soils in the mine increased respectively from 4.12 ppm to 43.5 ppm; 0.8 ppm to 1.3 ppm; 0.4 ppm to 1.2 ppm and 4.4 ppm to 76.4 ppm. Around the mine, we found 1300 ppm for the Zn and 311 ppm for Pb. Concerning the subsoil, a decrease of the concentration of HM was observed regarding to the topsoil. According to the values of HM found, the decrease of the concentration of these pollutants is in relation to the increasing distance from the source of pollution and also to the depth.

GUEST LECTURE

1002 CLIMATE CHANGES AROUND THE WORLD

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This presentation addresses several important aspects of the climate changes that are occurring around the globe. The causes of climate change are first reviewed, with illustrations of orbital oscillations, the atmospheric greenhouse effect, and aerosol effects. Observed changes in climate are next reviewed, both throught many millenia and during the past century. Distinctions are made between global warming and regional changes in temperature and precipitation. Changes in the frequency of weather extremes, including heat waves and tropical storms, are also discussed. An examination of different climate feedbacks follows, illustrating the complex interactions between different components of the climate system. Feedbacks involving clouds and aerosols are shown to be among the major uncertainties in the prediction of future climates. The presentation concludes with an overview of climate prediction, including the role of parameterizations, deterministic chaos and computational limitations.

2610 **INDOOR-OUTDOOR RELATIONSHIP OF FUNGAL AEROSOLS IN DOMESTIC HOMES SITUATED IN HUMID-WARM CLIMATE**

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Among the different kinds of bioaerosols, fungi represent a heterogeneous group, which plays an important role in human pathology. These microorganisms can be the cause of a variety of infectious diseases as well as allergic and toxic effects. Therefore, it is necessary to assess their composition and concentrations indoors, outdoors and in domestic environments. The study of indoor-air quality is a relatively new activity in the world, and very recent in Mexico. The aim of this study was to establish the relation between indoor and outdoor fungal aerosols in domestic homes. Air samples were collected, using the 6-stage Andersen impactor, inside and outside thirty domestic homes of Merida city, in Yucatan, Mexico. Sixteen fungal species were isolated from these samples. The main species found were *Cladosporium* spp. *Fusarium* spp., *Acremonium* spp., *Alternaria* spp. and *Bipolaris* spp. *Cladosporium* was present in 53% of the homes studied and 70% at their outdoors. The second most common fungal species was *Fusarium*, present 43% indoors and 14% outdoors. It was found that indoors and outdoor fungal concentrations ranged from 268 to 7757 CFU/m^3 and from 705-4257 CFU/ m^3, respectively. Respirable fungal particles amounted to approximately 79-98% of the total fungi concentration. The percentage of respirable fungi is generally not dependent on the type of home (1 or 2 stories) or their orientation. Also, the water and the temperature are factors that affect the development of fungi. Therefore, these factors were monitored during 5 days, and the results showed that the relative humidity varied from 32 to 93 % outside and from 40 to 70% inside and the temperature, from 14 to 34°C and from 18 to 29°C, respectively. The relative humidity during the monitored period was below the ideal conditions (relative humidity above 70% and temperatures above 20°C) for the development and growth of fungal spores.

2320 **A MODELLING APPROACH FOR TROPICAL PIG MANURE NITROGEN UTILISATION AND EVALUATION AT THE LANDSCAPE SCALE**

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Intensive pig production units in the humid tropics excrete large and excessive amounts of manure daily. This manure is poorly handled in pond systems and under-utilised as a fertiliser resource. This may result in becoming an environmental hazard that contributes to greenhouse gas emissions and waterway pollution. The application manure as an organic substitute for mineral fertiliser in neighbouring annual or perennial cropping systems may provide a viable alternative to the problem. Optimising application rate and distribution of manure in neighbouring cropping systems would minimise environmental pollution loading and contribute to improvement of farm economics. Managing large amounts of manure and in consequence their nutrient load, in particular N, onto fields could be assisted by using a Soil N Cycle and Plant Uptake biophysical model and spatially resolved modelling system. This study aims at developing a landscape level biophysical and GIS modelling system for nitrogen cycling that evaluates the potential and the effect of different pig manure type applications on the surrounding cropping systems in terms of environmental pollution aspects. It further would assist determining the maximum nutrient loading capacity of pig manure in a cropping system (e.g. oil palm plantation) that meets plant nutrient demand whilst minimizing environmental pollution. The biophysical model incorporates the interaction of N cycling in soil and plant uptake via N losses pathways. As a result, this model could evaluate the environment pollutant rate (N losses) for processes such as ammonia and denitrification emission, leaching and surface runoff at household and field level. The integrated modeling system allows assessment of trade-off analysis and minimization of N pollution with different types of manure (fresh, liquid and compost manure) at landscape level. This should contribute to a better understanding of pig manure utilization and management in humid tropical countries. The results of the developed modelling system enable users to assess alternative pig waste management plans for various performance criteria such as loading trends of nitrogen in the environment. It is designed as an additional tool for answering queries related to environmental impact assessment and cost evaluation of manure management.

1892 FAST DEVELOPMENT AND IMPROVED PERFORMANCE OF ENVIRONMENTAL IMMUNOASSAYS USING PHAGE BORNE PEPTIDES

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The need for simple and high-throughput analysis of small molecules such as pesticides, drugs and persistent chemicals in the environment is rapidly growing. Immunoassays, which are simple, robust and fast techniques, are widely used for this purpose in a broad range of applications. Their simplicity and low cost make them suitable for large scale studies, particularly in the developing world were the lack of resources always works as a major obstacle for environmental studies. In order to speed up the development, and improve the performance of immunoassays we have introduce the use of phage borne peptides as assay reagents. Most immunoassays have a competitive format, and once the anti-analyte antibodies are produced, the same hapten (analyte-related molecule) is conjugated to a tracer enzyme or coating protein to set up the assay. We have explored the use of analyte peptidomimetics selected from phage display libraries as convenient substitutes of competing haptens, and the concept has been proven using molinate, atrazine and a pyrethroid metabolite as model systems. Interestingly, the phage particles, which can be conveniently isolated from phage display libraries, perform as robust and highly standardized assay reagents, and due to their filamentous repetitive structure, they function as sensitive multi-enzymatic tracers. We have also shown that short peptide loops can be isolated that specifically bind to the analyte-antibody complex and can thus be used to detect small analytes in a non-competitive assay format. This is of great significance since non-competitive assays are superior to competitive ones in terms of sensitivity, precision and kinetics, but to the present there is no general method for their development. This technology, which we term PHAIA (Phage Anti Immunocomplex Assay), yields higher sensitive assays and is of general application; the principle was demonstrate with five model analytes. Due to the non-competitive nature of the PHAIA format it can be more easily adapted into rapid on-site test, as we showed in the case of the herbicide molinate and the metabolite phenoxy-benzoic acid.

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2492 MODELLING OF AN INDUSTRIAL NGL-RECOVERY UNIT CONSIDERING ENVIRONMENTAL AND ECONOMIC IMPACTS

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In this work, an integrated model is presented that identifies key areas in the operation of a cryogenic NGL-recovery unit. This methodology sets out to provide deep understanding of various interrelationships across multiple plant operating factors including reliability, which could be essential for substantial improvement of process performance. The integrated model has been developed to predict the economic and environmental impacts of a real cryogenic unit (600 MMCUF/D) during normal operation, and has been built in Aspen TM. Further models are required to test the complete control and reliability systems. The model agrees satisfactorily with the measured performance of the unit. It is used to evaluate the benefits and losses from improvements made to the unit. Model-based simulation reveals considerable opportunities to improve efficiency and control of bypassing, which can be used to improve the existing maintenance programs and to get a more realistic quantification of environmental impacts.

2245 AEROBIC GRANULAR SBR SYSTEMS APPLIED TO THE TREATMENT OF INDUSTRIAL EFFLUENTS

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Systems based on aerobic granular biomass are an alternative to the conventional activated sludge plants for wastewater treatment. Large organic and nitrogen loads are treated in these systems where biomass grown as granules, easy to separate by settling, make unnecessary the construction of secondary settler reducing the surface requirements for the treatment system construction. Furthermore, in aerobic granular reactors simultaneously carbon and nitrogen removal is feasible.These systems have been already applied at laboratory scale for the treatment of different types of industrial and urban wastewater [1], [2].This study compares the performance of four sequencing batch reactors (SBR) based on aerobic granulation in terms of efficiency of organic matter and nitrogen removal and the physical properties of granular biomass (Table 1). Different industrial effluents were fed to each reactor from: a laboratory for analysis of dairy products (R1), a fish canning industry with 10 g/L of salts (R2), a processing of marine products plant (R3) and swine wastewater (R4). Reactors used have a working volume of 1.5 L. They were operated in 3-hour cycles distributed in: filling (3 min), aeration (171 min), settling (1 min) and effluent withdrawal (5 min). The exchange ratio was of 50% with hydraulic retention time (HRT) of 0.25 days.

Table 1. **Operational conditions and properties when biomass concentrations was 5-6 g VSS/L.**

Reactor	COD/N	COD rem. (g/L·d)	N rem. (g/L·d)	Average Diameter (mm)	SVI (mL/g VSS)	Density (g VSS/$L_{granule}$)
R1	10	5.2	0.56	3.5	60.0	15
R2	10	2.4	0.08	2.7	28.7	67
R3	10	1.5	0.09	2.8	35.2	60
R4	7	3.7	0.38	3.8	73.3	30

From the obtained results it can be concluded that production and operation of granular biomass is possible with industrial effluents containing readily biodegradable organic matter. In all the reactors combined removals of organic matter and nitrogen were achieved. Similar results were obtained in R2 and R3 in terms of properties of granular biomass, since in both cases is wastewater from seafood industry. In R1 and R4 the best values for organic matter and nitrogen removal were obtained, but the properties of the biomass were not so good, which may be because of the size of the granules is greater.

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2448 OPTIMIZATION OF THE DO CONCENTRATION IN ACTIVATED SLUDGE SYSTEMS TREATING PETROCHEMICAL EFFLUENTS.

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Electric energy is normally one of the important cost factors of activated sludge systems. If the aeration intensity is reduced, energy consumption will be lower, but the resulting reduction of the dissolved oxygen concentration in the mixed liquor may affect the treatment system in three ways: (1) reduction of the efficiency of organic material removal and nitrification, resulting in higher residual BOD, COD and NH_3 concentrations in the effluent, (2) reduction of the metabolic activity of heterotrophic or autotrophic bacteria and (3) deterioration of the sludge settleability which may reduce the separation efficiency in the final settler. The optimal DO concentration is the minimum value that allows stable operation without affecting significantly the performance of the system in terms of substrates removal efficiency or the biological and mechanical properties of the sludge. In this paper an experimental investigation was carried out to establish the optimal DO concentration for an activated sludge system treating the waste waters of a large petrochemical complex at Camaçari-Brazil. Pilot scale activated sludge systems were operated under the same operational conditions as the full scale unit, but at different DO concentrations. Respirometry was used not only to control the DO in the pilot scale systems, but also to evaluate the metabolic capacity of heterotrophic and autotrophic bacterial masses. The results indicated that DO could be reduced to very low values without affecting the removal efficiency of organic material. As for nitrification, it was established that the half saturation constant of Monod kinetics was Ko = 1.0 mg O_2/l, because at a DO concentration of 1 mg/l the nitrification rate was reduced to half of the maximum rate. However this was still enough to get efficient nitrification. The maximum specific growth rate constants for autotrophic and heterotrophic bacteria were not affected by the DO concentration. Sludge settleability was evaluated from the standard SVI test and remained very good throughout the experimental investigation (SVI< 50 ml/g). It was concluded that it is possible to operate the pilot scale active sludge systems at low DO concentrations (< 1 mgO/l) and yet obtain stable performance and high treatment efficiency. The same should be possible for the full scale system, but in that case it must be assured that the aeration capacity is sufficient ensure that the entire reactor is in an aerobic environment i.e. that no anoxic regions will be formed due to stratification of the DO profile.

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2406 EVALUATION OF THE VINYL ACETATE ELIMINATION PROCESS IN METHANOGENIC SLUDGE WITH OXYGEN

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The vinyl acetate (AV) is a volatile toxic used in the painting manufacture, causing serious problems of contamination in grounds, air and natural bodies of water. Under methanogenic conditions the complete mineralization of the AV has not been obtained, but evidences exist suggesting that with the addition of low oxygen concentrations to methanogenic sludge the elimination of this compound is possible. In this work was studied the respiratory process of elimination of the AV in methanogenesis and methanogenesis with oxygen (1 mg/L·d). Two reactors UASB of 1.5 L were used, with the following operation conditions: T = 32 ± 2 ºC, HRT = 24hrs, [Inoculum] = 35 g VSS/L and C/N = 50. The reactors were fed with two loading rates of AV: 60 mg/L·d and 150 mg/L·d, in the stationary regime. The response variables evaluated were: COD removal efficiency, consumption rates, formation rates and product yields. The methanogenic reactor had an AV consumption of 100%, being mineralized until CH_4 and CO_2 with the first loading rate of AV, and when increasing it to 150 mg/L·d, the consumed AV was accumulated as acetate and increase significantly its yield from 0.04 to 0.47. In this reactor, the increase of the loading rate of AV caused diminution of the removal efficiency of 65 ± 3 to 32 ± 9 %.The other reactor also had 100% of AV consumption, but when O_2 was fed it showed a significant diminution in the acetate yield from 0.43 to 0.21, and an increase of the CO_2 yield of 0.22 to 0.49. Therefore, the results of both reactors show evidence of which the AV does not inhibit the methanogenesis. The results of the methanogenic reactor show evidence of which the increase in the loading rate of AV affected the elimination process negatively and possibly the acetate accumulation must to the low affinity of inoculum by this substrate. The results of the reactor with oxygen suggesting that the addition of 1 mg/L·d of this electron donor promoted the oxidation of accumulated acetate.

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2303 THE EFFECT OF OPERATIONAL CONDITIONS ON THE SLUDGE SPECIFIC METHANOGENIC ACTIVITY AND SLUDGE BIODEGRADABILITY

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The Specific Methanogenic Activity (SMA) and sludge biodegradability of an anaerobic sludge depends on various operational and environmental conditions imposed to the anaerobic reactor. However, the effects of hydraulic retention time (HRT), influent COD concentration (COD_{Inf}) and sludge retention time (SRT) on those two parameters need to be elucidated. This knowledge about SMA can provide insights about the capacity of the UASB reactors to withstand organic and hydraulic shock loads, whereas the biodegradability gives information necessary for final disposal of the sludge. In this work, eight pilot-scale Upflow Anaerobic Sludge Blanket (UASB) were fed with municipal wastewater, and operated with different sets of HRT (2, 4 and 6 hours) and COD_{Inf} (approximately 200, 300, 350, 550, and 800 mgCOD/L). When a "steady state" had been established, sludge samples were withdrawn from taps located at four different heights of each reactor to assemble eight composite samples, which were then used for the SMA and biodegradability tests. The results show that at a lower HRT, and consequently a higher upflow velocity, sludge with relatively higher SMA develops. The effect of COD_{Inf} on the SMA is still not clear. A slight trend of declining SMA at increasing COD_{Inf} was found for reactors operated at longer HRTs. During the treatment of domestic sewage, a short hydraulic retention time leads to short SRT and a high SMA. The sludge from reactors operated at long HRTs and with low COD_{Inf} resulted in low biodegradability. The biodegradability of sludge has an inverse relationship with the SRT, i.e. the shorter the SRT the higher the biodegradability of the sludge. This is because the biodegradability is higher in reactors operated at a shorter HRT, which are also submitted to high OLR, and in the case of sewage, also generally to a high suspended solids loading rate. Finally, results of the methanogenic potential, calculated based on the SMA and the mass of sludge in the reactor, show that it is worthless to design an UASB reactor with a longer HRT to cope with organic shock loads.

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2246 START UP AND OPERATION OF A PILOT SCALE AEROBIC GRANULAR SBR.

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The development of biomass in the form of aerobic granules is being recently under study as an improvement of conventional activated sludge system [1]. Aerobic granules have been formed treating synthetic, municipal and industrial wastewaters in lab scale reactors, achieving good performances [2], [3]. The aim of this work is the study of the development and operation of aerobic granules in a pilot scale sequencing batch reactor (SBR). A SBR with a working volume of 100 L, an internal diameter of 30 cm and a useful height of 150 cm was used. The reactor was operated in cycles of 3 hours distributed in: 7 min of feeding, 165 min of aeration, 6 min of settling and 2 min of effluent withdrawal. The volumetric exchange ratio was 50% and the hydraulic retention time (HRT) of 6 h. A ceramic dome fine bubble diffuser (19 cm in diameter) was used to supply 30 L/min air, and it was placed at the bottom of the reactor. Temperature, pH and dissolved oxygen were measured and registered on-line during the entire experiment. The reactor was operated at room temperature (16-20 ºC), at dissolved oxygen concentrations between 0 and 10 mg O_2/L. A synthetic feeding solution containing sodium acetate, as carbon source, ammonia, phosphate and trace solution was used. The reactor was operated in different stages: Stage 1 at an organic loading rate (OLR) of 3.0-3.5 kg COD·m^{-3}·d^{-1} and under nitrogen limiting conditions; in stage 2 the reactor was operated at 2 kg COD·m^{-3}·d^{-1} and under no nitrogen limiting conditions. Removal efficiencies in terms of organic matter were around 95% and 90% during stages 1 and 2, respectively. Ammonia was fully depleted in stage 1 as a consequence of biomass growth; however the ammonia removal efficiency was totally lost when the inlet nitrogen concentration was increased. Aerobic granules were formed in the reactor after 50 days of operation. Based on the experience from previous works the formation of granules in a pilot plant is slower than the granulation in laboratory scale reactors due to the large amount of biomass involved.

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2482 KRAFT PULP AND PAPER MILL WASTEWATER TREATMENT USING FIXED BED ANAEROBIC REACTORS

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The effluents of pulp mills contain a myriad of toxic compounds, biodegradable organic matter and sulfur compounds. To decrease the amount of fresh water required for pulp and paper production closed circuits are in use, however, higher concentrations of salts, as oxidized sulfur compounds, are encountered in the wastewaters. Energy costs and new environmental concerns are motivating the use of anaerobic pretreatment as a way to decrease energy expenditure in the treatment plant together with lower sludge production. In anaerobic environment, the organic matter removal can follow methanogenic or sulfidogenic paths and with the latter simultaneous reduction of the oxidized sulfur compounds also occurs. The objective of this work was to compare the performance of two horizontal fixed bed anaerobic reactors (HFBAR), one kept at methanogenic conditions and the other under sulfetogenic/methanogenic conditions for the treatment of pulp mill wastewaters. Two HFBAR reactors (R1 and R2) with a volume of 2 L (diameter = 5.0 cm, length = 1.0 m) were used for the experiments. Both were inoculated with sludge from a UASB reactor used to treat poultry slaughterhouse wastewater. The sludge was immobilized in polyurethane foam cubes with 0.5 cm of edge length. The influent pH was kept close to 7.0, the hydraulic detention time at 18 h and the temperature at 30 (±1) ºC. Sulfidogenesis was established in R2 prior to the beginning of this work. The characteristics of the industrial wastewater used in R1 were COD = 1190+ 87 mg/L; BOD = 350 + 98 mg/L and AOX = 23.0 + 4.2 mg/L. R2 was fed with the diluted wastewater (1:2) and the reactors performances were followed during 120 and 90 days respectively for R1 and R2. During the start-up period of reactor R1, the COD removal efficiency ranged between 30 and 40% and became stable close to 60% from the 20th day of operation on. When the industrial wastewater was fed to R2, the COD removal dropped (99% to 46%) and this can be explained by the decrease of sulfidogenesis, an important organic matter degradation path that was previously established in the reactor. Sulfate removal dropped from 97% to 25% although the COD/sulfate ratio indicated that electrons were available for sulfidogenesis. AOX removal was higher in R2 (63%) than in R1 (55%) which may be associated with the action of sulfatereducing bacteria (SRB) that are associated with toxic organic compounds. The presence of toxic compounds in the wastewater and the composition of the organic fraction were of primary importance for the performance of the processes involved in this treatment. The sulfidogenic/methanogenic system has a potential for pulp mill wastewater treatment, including the removal of sulfate and AOX however, the methanogenic system showed better performance concerning organic matter removal.

2307 AEROBIC BIOFILTERS WITH SMALL SYNTHETIC GRAINS FOR DOMESTIC WASTEWATER TREATMENT: KINETICS, EFFICIENCY AND MICROBIAL DIVERSITY

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The aerobic biofilters with submerged packed bed are able to retain a large biomass quantity, which makes them compact and suitable for small wastewater treatment plants. The characteristics of the packed bed determine the structure of the formed biofilms, the operation mode and the effectiveness. The objective of this work was to compare the biofilm kinetics and the microbial diversity in three aerobic biofilters packed with grains (3.0-4.5 mm) made of high and of low density polyethylene (HDPE and LDPE), and of polypropylene (PP). The experimental work was carried out using three 20 L reactors, operated in downflow mode. Different organic loads were applied between 0.5 and 6.0 g COD.m^{-2}.d^{-1}. The dissolved oxygen (DO) was varied at two levels for each organic load. The performance was determined based on: COD, NH_4-N, NO_3-N, TN, PO_4-P, fecal coliforms and helminth ova. Micrographs of samples of the packed beds were obtained using a scanning electron microscope JSM-6400 Noran. The biomass in the reactors was measured and the biofilm ecological structure was characterized at the end of each experimental stage. The reactor with PP was the easiest for colonization. The obtained specific COD removal rates were similar in all the reactors at loads up to 2.0 g COD.m-^{2}.d^{-1}. The differences appeared when applying greater loads and the best results were obtained in the reactor with LDPE. The reactor with PP had the best performances during its operation with the greatest load. The NH_3-N and TN removals were dependant on the DO concentration and the removals at DO of 5 mg/L were 84-99% and 61-74% respectively. The best removals were determined in the reactor with LDPE. The cell retention times were 10-12d, the biomass quantity was 10-11 kg/m^3. The biofilms had a heterogeneous morphology, variable thickness, high predominance and diversity of filaments, as well as fungi. They form nets where coccus, bacillus, spiral, nitrifying bacteria and yeast, some protozoa and rotifers species are located. Amoebas increased substantially during the last two experimental stages. The aerobic submerged packed bed bioreactors with synthetic grains of specific area of 760-1,200 m^2/m^3 allow high biodegradation rates and high nitrogen removal.

2325 EFFECT OF APPLICATION RATES AND MEDIA TYPES ON NITROGEN AND SURFACTANT REMOVAL IN TRICKLING FILTERS APPLIED TO THE POSTTREATMENT OF EFFLUENTS FROM UASB REACTORS

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Trickling filters are a very promising alternative for the post treatment of effluents from UASB reactors treating domestic sewage, especially in developing countries. Although a fair amount of information is already available regarding organic matter removal in this combined system, very little is known in relation to nitrogen and surfactant removal in trickling filters post-UASB reactors. Therefore, the purpose of this study was to evaluate and compare the effect of different application rates and packing media types on trickling filters applied to the post-treatment of effluents from UASB reactors, regarding the removal of ammonia nitrogen and surfactants. The experimental apparatus fed with domestic sewage consisted of a UASB reactor, which effluent was distributed to four trickling filters (TF) operating in parallel, at the same operational conditions, each one filled with a different type of packing media, as follows: blast furnace slag, random plastic rings, Downflow Hanging Sponge (DHS), and pieces of plastic tubing. The experiment was conducted in two phases: in the first phase the TFs were operated with surface loading rate (SLR) of 20 m^3/m^2.d, and organic loading rate (OLR) of 0.43 kg BOD/m^3.d. In the second phase, the SLR and OLR were 10 m^3/m^2.d and 0.24 kg BOD/m^3.d, respectively. The differences in performance for surfactants and ammonia nitrogen removal among the packing media have been evaluated by means of the non-parametric statistical Kruskal-Wallis test. Regarding ammonia removal, it seems that OLR lower than 0.30 kg DBO/m^3.d should be used to increase the nitrification rates in order to obtain the ammonia removal efficiency above 40%. Effluent concentrations of ammonia presented statistical differences only in 2nd phase, when the bests packing media for ammonia removal was blast furnace slag and DHS. The full manuscript will contain more details about nitrification in TFs tested. Surfactant removal was high in both phases, reaching efficiencies varying from 78 to 91%, for the packing materials tested. Effluent concentrations of surfactants presented no statistical differences among all packing media tested. Additionally, in the 2nd phase, analysis of surfactant present in the sludge showed that the removal of the liquid phase may have been performed predominantly by biodegradation in contrast to adsorption. This issue will be better discussed in the full manuscript.

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2392 HETEROTROPHIC, NITRIFYING AND DENITRIFYING ACTIVITY OF BIOMASS FROM FLUIDIZED BED REACTOR OPERATED WITH AERATION CYCLES.

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Biomass activity can be defined as the mass of substrate metabolised per unit of biomass and time. This parameter have a great importance to know the metabolic conditions of the microorganisms in a biological process, and can be use for an adequate operation and control of a wastewater treatment system. There are different methods to determine biomass activity, but the more useful are those based on the determination of the rate of substrate consumption or products generation. In this work the nitrifying, denitrifying and heterotrophic activity of the attached and suspended biomass from two fluidised bed reactors that operate with aeration cycles was determined. The reactors used sepiolite and Granular activated carbon (GAC) as media. For the determination of aerobic activities (nitrifiers and heterotrophic microorganisms) a respirometric methods, based on the rate of oxygen consumption of the endogenous and exogenous processes was used, whereas denitrifying activity was determined following chromatographically the nitrogen produced. The initial substrate concentration was determined in preliminary tests to assure that the process kinetic were of order zero related to substrate removal. The reactor were fed with a synthetically prepared wastewater with a COD of 600 mg/L, 100 mg $N\text{-}NH_4^+$ /L and 15 mg P/L, taking place the removal of organic matter, nitrification and denitrification. The reactors were operated under three different conditions with aeration cycles changed from 15 to 25 minutes on and 15 minutes off. The organic loading rate varied between 1.3 and 2.2 g COD/L d and the nitrogen loading rate applied was of 0.20-0.33 g $N\text{-}NH_4^+$/L d. The obtained results indicate that there is biomass segregation in the reactors. Heterotrophic activity is higher in suspended biomass (400-530 mg DQO/g VS.d) than in attached one (130-200 mg DQO/g VS.d); however nitrifying activity is higher in the biomass attached to the media (19-39 mg $N\text{-}NH_4^+$/g VS.d) than in the suspended biomass (12-17 mg $N\text{-}NH_4^+$/g VS.d) for both reactors. Denitrifying activity was slightly higher for the attached biomass than for suspended one (0,8- 1,7 $N\text{-}NO_3^-$/g VS d). There is no significant difference between denitrifying activity when nitrate or nitrites were used as substrate, although that is slightly higher when nitrate was the substrate. It was checked that nitrification and denitrification rates in the system operated with aeration cycles were lower than those obtained in systems operated with separated biomass, but the values obtained in this study are higher than those obtained in other systems without sludge separation.

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2327 SUNLIGHT INACTIVATION OF FAECAL BACTERIA IN WASTE STABILIZATION PONDS IN A SAHELIAN REGION (OUAGADOUGOU, BURKINA FASO).

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Waste stabilization ponds (WSPs) are an appropriate sewage treatment system for developing countries in sahelian regions. Several studies on wastewater treatment in WSPs have shown that solar radiation is a major factor in the inactivation of faecal indicator, and that sunlight acts on interaction with other factors including dissolved oxygen (DO) and pH. However, the inactivation by sunlight is limited by the reduction of light penetration in ponds. Experiments on sunlight inactivation of *Escherichia coli* were conducted outdoors in 8 microcosms with different depths (0.1 – 0.9 m) filled with maturation pond wastewater in order to determine pond depth influence on sunlight inactivation of *Escherichia coli*. The long-term aim was to maximize sunlight inactivation of waterborne pathogens in WSPs in sahelian regions where number of sunny days enable longer exposure of wastewater to sunlight. The inactivation was followed during daylight from 8.00 h to 17.00 h and during the night. Sunlight inactivation (KS) rates, as a function of cumulative global solar radiation (insolation), were 16 and 24 times higher than the corresponding dark inactivation (KD) rates respectively in cold and warm season. In warm season, *E. coli* was inactivated far more rapidly. This suggests that they may be unsuitable as indicators of WSP effluent quality during warm season in sahelian areas. Inactivation of *E. coli* follows the evolution of radiation during the day. In shallow depth microcosms, *E. coli* was inactivated far more rapidly than in high depth microcosms. The physical chemical parameters (pH, DO) of microcosm's water were higher in shallow depth microcosms than in high depth microcosms suggesting a synergistic effect of sunlight and these parameters to damage *E. coli*. To increase the efficiency of the elimination of waterborne bacteria, the use of maturation ponds with intermediate depths (0.4 m) would be advisable in view of the high temperatures and thus evaporation recorded in sahelian regions.

1841 LIGNIN DEGRADATION IN WASTE WATER OF PAPER MILL INDUSTRY BY OZONE

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The objective of the present work is to investigate the lignin degradation in residual water of the paper industry by an outline treatment that includes two stages: chemical coagulation and simple ozonation. In the previous coagulation we use sulfuric acid as coagulant to promote the sulfolignin formation, which is broadly used in the industry of additives and of cement. Two different coagulations were made, taking the pH value as parameter of the same one; those were pH 1 with a concentration of acid of 2.25% vol. and pH 3 with a concentration of acid of 1.0125% vol. In this first treatment stage, we achieved to reduce the initial chemical oxygen demand (COD) in 77%, (up 7000 to 1599 mg/L). In the second stage we carried out the decomposition with ozone (33 mg/L), with a gas flow of 0.5 L/min during 60 minutes. The coagulation is demonstrated a significant effect in the time reduction of treatment with ozone, and the sulfolignin formation. At pH 1 in ozonation the color in 100% is reduced during 15 minutes. The lignin decomposition in ozonation is controlled by the UV-VIS technique at 254 nm, and by the HPLC technique to determine the final products of the lignin reaction with ozone. In ozonation no toxic and biodegradable organic acids are formed (formic acid, maleic acid, and traces of fumaric acid, malonic acid and catechol). For the characterization of the structures of the sludge obtained in the coagulation the infrared spectroscopy technique (FTIR) is used. The biodegradability of the treated residual water (BOD_5/COD) increases up 0.11 to 0.38 in ozonation. The kinetics of lignin decomposition at different pHs as the decolorization constants calculation also is studied with the help of a software (MATLAB 7.0) and of the Differential Neuronal Networks technique.

2352 ANTIBIOTIC ABATEMENT IN DIFFERENT ADVANCED OXIDATION PROCESSES COUPLED WITH A BIOLOGICAL SEQUENCING BATCH BIOFILM REACTOR

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During the last decade, the lack of fresh water is becoming a major concern. Recently, the presence of recalcitrant products such as pharmaceuticals has caused a special interest due to their undefined environmental impact. Among these, antibiotics are one of the numerous recalcitrant pollutants present in surface waters that might not be completely removed in the biological stage of sewage treatment plants because of their antibacterial nature. Advanced Oxidation Processes (AOPs) have proved to be highly efficient for the degradation of most organic pollutants in wastewaters. AOPs are usually combined with biological treatments in order to optimize operational costs. Concretely, the chemical phase is used to improve the biodegradability of the wastewater so that it can be treated biologically. The use of Sequencing Batch Biofilm Reactors (SBBRs) with fixed biomass improves bioreactor activity by means of higher biomass concentration. Moreover, the use of bacterial community analysis by means of DNA sequencing allows the identification of the microorganism population. The goal of this work is to evaluate how different advanced oxidation pre-treatments affect the SBBR performance and its microbial population. These oxidative processes compared are photo-Fenton and ozonation for the removal of 200 mg L^{-1} of sulfamethoxazole. The photochemical reactor used is a Pyrex jacketed thermostatic 2 L stirred vessel equipped with black-light blue lamps. The temperature was kept at 25±0.8 ºC. The ozonation reactor is a 1.2 L cylindrical stirred vessel equipped, working at room temperature, with 2 diffusers that provide ozone generated in a Sander Labor Ozonator using oxygen as a feeding gas. The ozone outlet was led to a killer solution prepared of saturated KI, were the ozone was destroyed. The biological reactor is a 2.5 L glass jacketed column filled with volcanic stones which serves as support for the micro-organisms. Air is fed continuously and the temperature is maintained constant at 23.5 ºC. The biomass used as inoculum comes from an urban wastewater treatment plant. The SBBR was fed with photo-Fenton pre-treatment effluents during the first stage and with ozonation products in the second one. As a result, comparative pre-treatment conditions are obtained for optimal SBBR performance, with total antibiotic degradation and overall TOC removals higher than 85%.

1835 ELECTROCHEMICAL TREATMENT OF PHARMACEUTICAL AND INDUSTRIAL WASTEWATER BY ANODIC OXIDATION

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In modern medicine pharmaceuticals play a decisive role. Because of an increased life expectancy and intensive care medicine an increasing amount of pharmaceuticals is produced. Thus these substances are consumed in a mass of tons per year in industrialized countries. Wastewater effluents from sewage treatment plants (STP) are important point sources for residues of pharmaceuticals and complexing agents in the aquatic environment. For this reason a research project, which started in December 2006, was established to eliminate pharmaceutical substances and complexing agents found in wastewater as micropollutants. For the treatment process a combination of anodic oxidation by boron-doped diamond (BDD) electrodes and ozonation is examined and presented. In the anodic oxidation step OH-radicals and other oxidants will be directly produced and used for the oxidation of the substances (pharmaceuticals and complexing agents). For the ozone production a non-conventional, separate reactor was used, in which ozone was generated by electrolysis with diamond electrodes. For the determination of the achievable remediation rates four complexing agents (e.g. EDTA, NTA) and eight pharmaceutical substances (e.g. diazepam, carbamazepin) were analyzed in several test runs under different conditions (varied flux, varied current density for the diamond electrode and the ozone producing electrode of the ozone generator, different packing materials for the column in the ozone injection system). The flow rates of the treated water samples were varied from 3 L/h up to 26 L/h. For the anodic oxidation the influence of the current density was examined in the range between 22.7 and 45.5 mA/cm^2, for the ozone producing reactor two densities (1.8 a/cm^2 and 2.0 A/cm^2) were tested. Matrix effects were investigated by test runs with samples from the effluent of an STP and synthetic waste water. Therefore the impact of the organic material in the samples could be determined by the comparison of the redox potential and the achievable elimination rates of the investigated substances. Comparing both technologies anodic oxidation seems to be superior to ozonation in each investigated area. With the used technology of anodic oxidation elimination rates up to 99% were reached for the investigated pharmaceutical substances at a current density of 45.5 mA/cm^2 and a maximum sample flux of 26 L/h. After these first investigations in the laboratory scale, a pilot plant was installed on the area of an STP. In a bypass a partial flow of the effluent (up to 900 L/h) will now be treated under realistic conditions by anodic oxidation. After further tests in the laboratory, an additional reactor for ozone treatment should be built on the facility area within the next weeks.

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2144 PHOTOCATALYTIC DEGRADATION OF METHYL TERT-BUTYL ETHER (MTBE) FROM CONTAMINATED WATER: COMPLETE MINERALIZATION

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Methyl tert-butyl ether (MTBE) has been commercially used as an octane enhancer to replace tetraethyl lead in gasoline since 1979. The high mobility, water solubility, and resistance to natural attenuation associated with MTBE may result in contamination of ground and surface waters. In this investigation the degradation of aqueous MTBE at relatively high concentrations was studied by UV-vis/TiO_2/O_2 photocatalytic process. The effect of important operational parameters such as pH, oxygen flow, catalyst loading, and irradiation time were also studied. Concentration of MTBE and intermediates such as tert-butyl formate (TBF) and tert-butyl alcohol (TBA) were measured using a gas chromatograph equipped with flame ionization detector and combined with headspace sampler. The results showed that the time required for complete degradation increased from 15 to 150 min, when the initial concentration was increased from 10 to 500 mg/L. The first order rate constant for degradation of MTBE from the hydroxyl radical was estimated to be 0.266 to 0.033 min^{-1} as the concentration increased from 10 to 500 mg/L. Study on the overall mineralization monitored by total organic carbon (TOC) analysis showed that in the initial concentration of 100 mg/L MTBE, complete mineralization was obtained after 110 min under UV-vis/TiO_2/O_2 photocatalytic process. The data presented in this paper clearly indicate that UV/TiO_2/O_2 advanced oxidation process provides an efficient treatment alternative for the remediation of MTBE contaminated water.

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2152 REMOVAL OF TEXTILE DYES WITH BIOPOLYMERS XANTHAN AND ALGINIC ACID

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Textile industry is an important activity that provides considerable benefits to people, but unfortunately dyeing of yarn and cloth produces pollution of water, a resource that is valuable and scarce. Dyeing of textiles fibers is an inefficient process, in view of the fact that approximately ten percent of total dye is thrown to municipal sewage. Although different treatment systems are applied to wastewater, dyes are resistant to physical, chemical and biological factors because of the way they are designed. This work consists in the utilization of two biodegradable biopolymers, xanthan (XANT) and alginic acid (ALG) for the removal of the textile dyes disperse yellow 54 (DY54) and direct black 22 (DB22). DY54 is used to dye synthetic substrates such as polyester, nylon, acrylic acetate and plastics. DB22 is applied in electrolyte solution to cotton, cellulose, paper and leather. Both compounds have different physicochemical properties and a sorption study of these chemicals using XANT and ALG will give us insight of the manner that both DY54 and DB22 are removed of aqueous solution by these biopolymers. Aliquots of dye solution were put in contact with constant mass of biopolymer (powder) in order to obtain sorption isotherms of the following systems: XANT-DY54, ALGDY54, XANTDB22 and ALGDB22. Langmuir and Freundlich models were applied to data obtained from sorption isotherms. Neither Langmuir nor Freündlich described satisfactorily experimental results. Zimm Bragg model proved to be the best model that describes sorption isotherms of all systems. This is the first report of the application of Zimm Bragg model to polysaccharide-dye systems. Structure and functional groups of both molecules, dyes and biopolymers are key factors that influence on the removal of DY54 and DB22 by XANT and ALG. Main interaction between the polysaccharides XANT and ALG with DY54 are hydrogen bonding and hydrophobic interactions while DB22 interacts with them through electrostatic interactions. Additionally, hydrogen bonding and hydrophobic interactions contribute to increase removal of this dye when using XANT and ALG biopolymers.

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2262 REMOVAL OF MICROPOLLUTANTS DURING PHYSICOCHEMICAL PRETREATMENT OF HOSPITAL WASTEWATER

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The fate and occurrence of micro-pollutants, such as pharmaceuticals, hormones or cosmetic ingredients, has attracted an increasing attention in environmental research. The main sources for such compounds in the environment include domestic sewage, hospital effluents and discharges from the pharmaceutical manufacturing industry. The aim of the present work was to analyse the efficiency of coagulation-flocculation and flotation processes for the pre-treatment of hospital wastewaters, focusing on the removal of 12 Pharmaceutical and Personal Care Products (PPCPs), including musk fragrances, anti-epileptics, tranquilisers, anti-inflammatory drugs, antibiotics and one iodinated contrast media. In the first part of the work batch coagulation-flocculation assays have been performed in a Jar-Test device, which where afterwards complemented with the set-up of a continuous coagulation-flocculation pilot-scale plant. Additionally raw hospital wastewater as well as the effluent from this continuous coagulation plant has been treated in a flotation cell. In general, flotation of raw wastewater led to slightly worse results compared to batch coagulation regarding both, Total Suspended Solids (TSS) and PPCPs removal, although when applied to the effluent obtained from the coagulation pilot plant the overall efficiency of the process was positively affected. Removal of TSS during pre-treatment was very effective reaching maximum efficiencies of 88%, 72% and 97% for batch coagulation, raw wastewater flotation and combined coagulation-flotation, respectively. In the case of total Chemical Oxygen Demand (COD) the efficiency of the processes was dependant on the fraction of particulate organic matter, which was the fraction that was considerably removed, whereas soluble organic matter was normally not liminated. From the selected PPCPs, iopromide, carbamazepine and diazepam were the most persistent compounds, whereas fragrances and diclofenac were eliminated to a high degree. For naproxen and ibuprofen the decrease in concentration was in between the previous substances. Finally, for antibiotics negative removals have been generally measured.

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2155 COAGULATION-FLOCCULATION PROCESS APPLIED TO WASTEWATERS GENERATED IN HYDROCARBON-CONTAMINATED SOIL WASHING

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A wastewater produced in the contaminated soil washing was treated by means of coagulation-flocculation (CF) process. The wastewater treated in this work contained petroleum hydrocarbons, a surfactant, i.e., sodium dodecyl sulphate (SDS) as well as salts, humic acids and other constituents that were lixiviated from the soil during the washing process. The aim of this work was to develop a process for treating the wastewaters generated when washing hydrocarbon-contaminated soils in such a way that it could be recycled to the washing process, and at the end of the cleaning up, the waters could be disposed properly. A second objective was to study the relation among the coagulant and flocculants doses and the pH at which the CF process is developed, for systems where methylene-blue active substances (MBAS) as well as oil and greases were present. The results for the selection of the right coagulant and flocculant type and dose, the optimum pH value for the CF process and the interactions among the three parameters are included in this work. It was concluded that it is feasible to treat the wastewaters generated in the contaminated soil washing process through CF process, and therefore, wastewaters could be recycled to the washing process or disposed to drainage. The best coagulant and flocculants were alum sulfate and Tecnifloc 998 at doses of 4,000 and 1 mg/l, correspondingly at pH of 5. These conditions gave color, turbidity, and chemical oxygen demand (COD) and conductivity removals of 99.8, 99.6, 97.1 and 35%, respectively.

2592 DETERMINATION OF THE HYDRAULIC CHARACTERISTICS BY MEANS OF INTEGRAL PARAMETERS IN A MODEL OF WETLAND WITH SUBSUPERFICIAL FLOW

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The main objective of this study was to assess the portions of plug flow and death zones using tracer tests by empiric models as Wolf-Resnick and Dispersion in evaluate bed-packed reactors with horizontal subsurface flow, as a model of a constructed wetland. In order to assess the hydraulic behavior of systems such as packed-bed reactors and constructed wetlands both of subsurface flow, it is necessary to study and evaluate them modifying some variables while others remain constant. As well it is important to use mathematical models to describe, as precise as possible, the different phenomenon inside the systems, in such a way that these models bring information in an integral way to predict the behavior of the systems. Experiments were carried out in three lined beds, every bed received three different controlled flow of primary affluent (0.3 L/min, 0.6 L/min, and 1.5 L/min), and that had an almost equal surface area with the following aspect ratios: 1:1, 1.5:1, and 3:1. The size of the granular medium (gravel) varied with three different sizes; the diameters were 4.7 mm, 9.2 mm and 12.7 mm. The water depth of all the beds was set to 0.3 m. Tracer tests were conducted by a single-shot injection of dissolution of NaCl into the inlet tubes of the beds. In that way with the combination of three different flows, three aspect radios and three different sizes of granular medium, twenty seven tests were made. The results indicate that one increase in the aspect ratio caused an increase in the dispersion number and the portion of plug flow, and the Actual Retention Time tens to be equal to the Nominal Retention Time. The effect of the size of the medium on the hydraulic behavior was that one reduction to the size of the granular medium increases the portion of plug flow and reduces the dispersion number. Finally the increment of the flowrate reduces the dispersion number and increases the portion of plug flow in the system. Therefore, the main conclusion of this study is that the construction of a packed-bed reactor with a higher aspect ratio and flowrate, and a finer medium improves the hydraulic behavior of the system by reducing the internal dispersion, at least in the ranges tested in these experiments.

2430 ELECTROKINETIC REMEDIATION OF COPPER MINE TAILINGS

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The heavy metal contamination from mining industry has become a growing problem both in Chile and worldwide. This contamination includes large areas with soil pollution, contaminated rivers and continuous generation of mining waste deposits. The solid waste that will be analysed is mine tailings, which are the residual products after the flotation process in conventional sulphide copper mining. These tailings are known to contain considerable amounts of different heavy metals. Today the mine tailings are deposited directly on areas close to the mine without any controlled pre-treatment or ground and surface water protection. This work consists of series of electrochemical remediation experiments using an electric field as remediation agent in order to remove/concentrate heavy metals (specially copper) in a way that minimises the volume of contaminated matter significantly. During the experiments several parameters are monitored such as current density, voltage drop, pH in the soil/waste, metal mobility and removal rate. Adjustable parameters are current density, water content, distance between electrodes and remediation time. Furthermore, the remediation process can be optimised with the use of ion exchange membranes, which also is included in the experimental part of this project. Other parameters that will be discussed in this work are: the use of pulsed direct current, addition of dissolving or complexing agents, and use of different electrolyte solutions.It was found that electrochemical remediation of mine tailings and soil contaminated from mining industry could be an alternative to depositing the waste. To positive aspects are generated by this remediation technology: 1) an environmental friendly solid waste without elevated concentrations of metals, 2) a profit consisting of dissolved copper that can be used directly in the copper refinery process meaning that natural copper containing minerals can be saved, and 3) a possible tool to recover copper from minerals with low grade.

2268 AIR FILLED POROSITY IN COMPOSTING PROCESSES

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As it is widely known, the composting process consists in the aerobic decomposition of the biodegradable organic matter present in different types of solid wastes. Water and oxygen are necessary for the biological activity of microorganisms involved in the composting process and their availability is directly related to the total and the air filled porosity (AFP). Maintaining adequate AFP level satisfies the oxygen content requirement to achieve the desired composting conditions and thus, to enhance biological activity. Among the different methods used to measure AFP in composting materials, air pycnometry is considered as the most suitable methodology by a wide number of researchers. The objective of the present work is to study the suitability of air pycnometry for measuring AFP in different composting materials at different process scales (laboratory and full-scale) using different custom-made air pycnometers. Three different pycnometers have been used, each of them designed to accommodate increasing quantities of material (1.65 L, 15 L and 52 L). The 52 L pycnometer is in fact a composting reactor coupled with a pycnometer that allows for the in situ AFP determination without affecting the material structure. Optimal AFP levels have been determined by correlating this parameter to the global aerobic biological activity expressed as Oxygen Uptake Rate (OUR). An optimum value of initial AFP for composting processes at laboratory scale has been established around 40% and this parameter has been used in the preparation of the initial mixtures instead of indirect measures such as weight or volume ratios between organic wastes and bulking agents typically used in composting processes. Also the evolution of AFP values through the composting process has been studied both at laboratory and industrial scale. In general AFP evolution in all the processes studied was as follows: a reduction in AFP is observed because of compaction and settlement of the material in the first stage of the process; afterwards AFP gradually increases due to the biodegradation of the organic matter. Mechanical resistance of the material to be composted should be considered together with AFP in order to optimize initial mixtures for an enhanced biological activity especially in full-scale facilities.

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2334 EXCESS SLUDGE REDUCTION IN ACTIVATED SLUDGE PROCESSES BY INTEGRATING ULTRASOUND TREATMENT

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Biological sludge produced in the activated sludge process can be minimised modifying the water line, the sludge line or the final disposal strategy. Selecting the water line the general idea is to reduce the sludge production reducing the yield coefficient by means of the called *"lysis criptic growth process"*. The main techniques referenced in literature are ozonation, chlorination and chemical and heat treatment. Ultrasounds are widely used to increase anaerobic biodegradability but are not reported as system to control excess sludge production. The aim of this paper is to quantify experimentally the effect of ultrasound over the sludge production measured as yield coefficient. Domestic sewage pre-treated in a primary settler was aerobically treated in four continuous reactors (6 L volume), working in parallel with HRT = 6 h and SRT = 6 d. To test the sonication effect, four series of experiments were performed in duplicate, treating with ultrasounds different volumes of mixed liquor (0, 1/12, 1/6 and 1/4 of the total reactor volume per day) and applying 370 J/mL. Each pseudo-stationary state was maintained 20 days. The main average results for the duplicate experiments appear in the table. (Each value is the average for two reactors operating in parallel 20 days)

$V_{sonicated}$ (L/d)	0	0.5	1	1.5
$COD_{removed}$ (%)	80	75	72	68
Coefficient yield (g SSV / g DQO_{rem})	0.34	0.17	0.11	0.06
Excess sludge reduction (%)	0	50	68	82
Energy applied (kwh/$m^3_{wastewater}$)	0	7.5	15	22.5

The main conclusions are: Biological sludge excess can be controlled and dramatically reduced applying ultrasounds. The coefficient yield decrease is directly related to the applied energy.
The input of energy consumed limits the economic feasibility of this technology.

2365 STABILITY MEASUREMENTS OF COMPOST TROUGH ELECTROLYTIC RESPIROMETRY.

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An experimental technique for compost stability measurements based on electrolytic respirometry was optimized and subsequently applied to a composting process. Anaerobically digested sewage sludge mixed with reed was composted during 90 days in a pilot scale rotary drum with forced aeration. Periodic solid samples were taken, and a previously optimized respirometric procedure was applied to them in order to measure the oxygen consumption. The respirometric experiments were made directly with a few grams of solid samples, optimum moisture and 37 ºC during 96h. The SOUR instant values from the oxygen consumption curves were obtained and two commonly used respirometric indexes (IR24 and AT4) were calculated for all samples. Both IR24 (a mean of the SOUR values during the 24h maximum activity period) and AT4 (total oxygen consumption after 4 days) were the recommended parameters for the estimation of compost stability by the European Union in the second draft of the Working Document on the Biological Treatment of Biowaste in 2001. Both indexes exponentially decreased with the composting time, and a good linear correlation between them was observed. Taking into account the obtained IR24 and AT4 curves, the compost obtained after 90 days seemed to be stable. A discussion have been considered in order to consider if this technique could be classified as a Dynamic or Static method, the two main groups of respirometric techniques for compost stability measurements. Supposing that the proposed procedure could be considered as dymanic method (no limitations in the amounts of oxygen supply) the final IR24 obtained was compared with the DRI proposed by the EU (1 mg O_2 gVS^{-1} h^{-1}). Our result (0.6 mg O_2 gVS^{-1} h^{-1}) indicated that stable compost was obtained after 90 d. However, if a static limit should be considered (AT4 lower than 10 mg O_2 gTS^{-1} as proposed by the EU) our result (26 mg O_2 gTS^{-1}) indicated that more residence composting time would be needed. Taking into account these results, the validity of the proposed method was discussed.

2368 MICROBIAL ACTIVITIES IN A VERTICAL- FLOW WETLAND SYSTEM TREATING SEWAGE SLUDGE WITH HIGH ORGANIC LOADS

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The rhizosphere is the most active zone in treatment wetlands where take place physicochemical and biological processes between the substrate, plants, microorganisms, and contaminants. Microorganisms play the key role in the mineralisation of organic matter. Substrate respiration and phosphatase activities (acid and alkaline) were chosen as indicators of microbial activities, and studied in a vertical-flow wetland system receiving sewage sludge with high organic loads under the Mediterranean climate. Main investigation was focused on plant species influence and seasonal effects. Three helophyte species, common reed (*Phragmites australis* Cav.), broadleaf cattail (*Typha latifolia* L.), and yellow flag (*Iris pseudacorus* L.), were planted as monoculture in an organic substrate (pine bark / peat: 1/1) and irrigated with a liquid sewage sludge from a local food industry, characterised by high concentrations of organic matter (e.g. COD>8000mg/L). Unplanted mesocosms were also set up to study plant benefits. Measurements were carried out on the surface (0-4 cm) substrate, where microbial activities are the highest. Samplings were performed just before and after sludge application, every one, two or three days and lasted two weeks, in November 2007 and May 2008. First results (November 2007) showed that substrate respiration and phosphatase activities were always higher in planted than unplanted mesocosms. This was consistent with overall removal efficiency. Phosphatase activities increased after sludge application and attained a peak within two days in mesocosms planted with Phragmites or Typha and within three days in those planted with Iris. Afterwards, they decreased and then stayed stable in mesocosms planted with Iris or Typha, though showed fluctuations in those with Phragmites. These activities were always higher than before sludge application. In contrast, sludge effects were minor in unplanted mesocosms and phosphatase activities were relatively stable before and after sludge application. Substrate respiration showed similar trends to phosphatase activities. These results confirm that the presence of plants can enhance microbial activities, with different change patterns according to plant species. Measurements in May 2008 will allow revealing seasonal effects on microbial activities in this system for sludge treatment.

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2386 A DOUBLE STAGE DRY-WET-FERMENTATION PROCESS FOR A FAST AND SAFE DIGESTION OF DIFFERENT KINDS OF ORGANIC MATERIAL

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The fermentation of organic material is a four-step-process. It is admissible to merge the first two steps (hydrolysis and acidification) to hydrolysis in general and the last two steps (acetogenesis and methanogenesis) to methanogenesis. The Brandenburg University of Technology in Cottbus has devised a double stage dry-wet-fermentation process for fast and safe anaerobic degradation. Using these processes, it is possible to decompose different kinds of organic material like renewable material (e.g. maize silage), waste (e.g. household-waste) and industrial material (e.g. glycerine). With this process, it is possible to optimize the different steps of biogas generation separately. As a consequence of the optimisation, the process runs extremely stable, it is controllable and allows a demand-oriented biogas generation. There is a high optimization potential of the hydrolysis. A fast and continuous hydrolysis is the basis for a reliable and constant biogas generation. The hydrolysis depends on different parameters e.g. pH-value, COD-concentration, VOA-concentration and temperature, which were optimized. After optimisation, the retention time of food and market waste in the biogas process could be reduced from over 40 days to 6 days, for maize silage from over 100 days to 21 days. The open hydrolysis releases CO_2 and allows oxidation of sulphur. Consequently, the biogas has high methane (> 72 %) and a low H_2S (< 100 ppm) concentration. Stirrers or other agitation equipment are not necessary, because only liquids are pumped. In the year 2007, a pilot and an industrial plant, both based on this technology, were built. The paper is meant to give an overview of the technology and its special features as well as of the pros and cons.

2504 SEPARATION OF INERTS BY DIFFERENTIAL SEDIMENTATION AS PREVIOUS STAGE TO ANAEROBIC DIGESTION OF ORGANIC FRACTION FROM MUNICIPAL SOLID WASTE

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Municipal solid waste (MSW) treatment plants have as main function the valuation of municipal waste by means of materials recovery and valuation of organic fraction. In this type of facilities, the anaerobic digestion is a biological treatment of the organic matter collected in origin or mechanically separated from the mixed MSW. The objective is its valuation under the form of biogas and organic compost. Anaerobic digestion has high energy efficiency and a good range of organic matter elimination. Nevertheless, treatment of organic matter recovered from mixed MSW presents serious operation problems due to sedimentation of heavy improper materials (sands, glasses, metals) and flotation of light materials inside the digestors and piping. These facts produce a decrease on the availability of equipment and an increase on maintenance costs. These kinds of problems do not happen in case of collected in origin organic matter digestion, which can be treated without important problems. PAYMACotas jointly with the EMSHTR has developed and registered a patent for a separation system of inerts from organic fraction aimed to feed digestion. This separation system is based on the differential sedimentation of improper materials in the organic fraction through an upward flow of water, separating a clean fraction of heavy and floating materials, and recovering the organic fraction free of these improper ones. This treatment system appears as a solution for the problem of accumulation of improper materials inside the digestors, facilitating the use of organic fraction free of most of inerts. Therefore the above mentioned problems are solved and the heavy metal content in the obtained compost is decreased. The obtained results in the developed pilot plant and their state of evolution to make this system applicable by the MSW treatment plants are presented in this Congress Paper.

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2355 USE OF PETRI NETS FOR THE DEVELOPMENT OF NEW CONTROL SOFTWARE FOR THE IN-VESSEL COMPOSTING PROCESS

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The composting process has been consolidated as one of the most accurate techniques for the treatment and recycling of the organic wastes. Along such process three different stages can be recognized taking into account temperature evolution, so the automation equipments used to control the process at industrial scale must work on the control elements (fans, valves, sprinklers, etc.) with the objective that each of the stages is developed in a correct way. However, the organic waste's high variability, and the great amount of variables which can influence the microbial activity (responsible of the process), makes difficult to predict the behaviour a specific waste will develop once inside the bioreactor. As a result, often the initial control parameters (PID control, ventilation air direction, etc.) are not properly set, thus not being able the system to control such unexpected process evolution (i.e., PID variables set to such a low level that the fan is unable to control the temperature rise, appearance of gradients inside the composting matrix, etc.). This prompts that continuous supervision of the system by a person with process knowledge is usually needed, so the control parameters can be adjusted as soon as possible in case of an anomalous evolution of the process.The present work shows a new control software for in-vessel composting which, unlike the linearity of the actual control software, is based on a Petri Net philosophy. Thus, instead of treating the composting process as a linear evolution of stages, the process is regarded as a succession of different conditions, connected among all of them, so the PLC will be situated on each moment on the most suitable state for the process evolution. This changes the present linear evolution of the composting control systems into a variable evolution, prevailing a correct control of the microbiological process, and choosing the PLC automatically which is the most suitable state evolution. This software has been developed and tested on the Composting Mobile Pilot Plant (PPM), property of Ros Roca IMA S.L., which possess a composting bioreactor with a capacity of 20 cubic meters. The PLC used was a Schneider Modicom TSX Momentum. The software development was made with Concept 2.5 XL SR2 and the Scada HMI interface was developed on Citect 6.1. The results obtained after the trials with this software on the PPM showed that this facility can adapt the control elements in a much better way after an unexpected evolution of the composting process, readjusting automatically the control parameters to the new process conditions, and keeping the composting process evolution within the optimal limits. This new software can be a first step towards more technically advanced and versatile composting facilities, capable of adapt themselves automatically to several types of organic wastes, and thus maintaining the process evolution and the compost production stable and at the optimal levels.

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2540 BIO-GAS PRODUCTION FROM MUNICIPAL SLUDGE WASTE USING ANAEROBIC MEMBRANE BIOREACTOR

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A laboratory scale anaerobic membrane bioreactor (AnMBR) system for the bio-methane gas production was operated for 60 days with municipal sludge wastes as a sole carbon source. The AnMBR system utilized the external cross-flow membrane module and was equipped with on-line data acquisition which enables continuous monitoring of the performance of both bioreactor and membrane through the analyses of pH, temperature, gas production; permeate flow rate, and trans-membrane pressure (TMP). Such a configuration also provides an efficient tool to study rapid variations of monitoring parameters in the system, which would be impossible to explore in general. The AnMBR resulted in a gradual increase of methane gas production from 63 to 71% which was normalized to the total chemical oxygen demand (COD) processed (i.e., VFAs- and methane-COD) from sludge waste along with the operation time. The residual COD in the effluent was attributed to acetic acid mainly and propionic acid. Based on the resistance in-series model ($J = \Delta P/[\mu(R_m+R_{if}+\theta\Delta P)]$), the permeate flux is a function of TMP and there are two distinct pressure-dependent and pressure-independent regimes. The viscosity of permeate measured using a falling ball-type viscometer was 0.82 cp at 35 ℃ and the biomass concentration in the reactor was 5.8 ± 0.3 g VS/L. Each resistance value (R_m and R_{if}) was determined from a flux profile on a clean and a fouled membrane after the surface rinsing. For the PVDF MF (0.07 μm) membrane, resistance values of Rm and Rif were $1.2*10^{12}$ and $3.5*10^{12}$ m^{-1}, respectively at the end of 60-d operation period. The obtained regression parameter value (θ) from a non-linear regression of the membrane performance data was $6.82*10^{11}$ (r^2=0.96) $psi^{-1}\cdot m^{-1}$. The close fit between the model-estimated and experimentally measured permeate flux as a function of TMP confirms that the applicability of the resistance in-series model for the AnMBR system for the methane gas production from municipal sludge waste.

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2618 OCCURRENCE OF HIGH-TONNAGE ANIONIC SURFACTANTS INTO SPANISH SEWAGE SLUDGE

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The Sewage Sludge Directive 86/278/EEC seeks to encourage the disposal of sewage sludge in agriculture applications and regulate its use to prevent harmful effects on the soil environment. Currently, the sewage sludge Directive is under revision and a possible cut-off limit for some organic chemicals (including linear alkylbenzene sulphonates – LAS, the main synthetic anionic surfactant) is to be implemented. This legal limit is based on monitoring studies carried out in Scandinavian countries, being strongly rejected by most EU countries since the Nordic situation was regarded as not representative. Besides, the proposed maximum value does not take into account the vast monitoring information and positive risk assessment of LAS in agricultural soils (and other environmental compartments). In this research work, the concentration of LAS in sewage sludge samples from all regions of Spain has been determined by HPLC with fluorescence detector. The data collected allow us to make a map of sludge in Spain showing that, similarly to other European countries, LAS concentration in sewage sludge samples is above the proposed cut-off criterion.
The calculated average concentration of LAS considering all samples is 6.3 g / kg dm. Only 5 % of the anaerobic sludge samples are below 2.6 g LAS / kg dm. This value would make the use of sewage sludge in agriculture unviable in many cases (more than 800000 tons are currently used for agricultural purposes in Spain). Moreover, this concentration level of LAS poses no environmental impact on soil when sludge is applied to land even at the highest concentration. Given the well proved safety of LAS in all the environmental compartments (water, sediments, soils, etc.) the removal of LAS from the list of organic contaminants regulated by the future Sludge Directive is completely justified. For comparison purposes, soap has been also monitored in sewage sludge by HPLC-DAD. Regarding the concentration of soap, the measured concentration of this surfactant is generally much higher than that of LAS despite soap is known to be biodegradable under anaerobic conditions. These results confirm that the physical-chemical properties of surfactants (precipitation and adsorption phenomena) rule the presence of surfactants in environmental solid matrices rather than anaerobic biodegradation.

2391 COMPARISON BETWEEN CHEMICAL AND MECHANICAL DISINTEGRATION ON SLUDGE ANAEROBIC DIGESTION PERFORMANCE

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The sustainable management of sewage sludge, as regards economical and environmental aspects, is one of the critical issues of the last decades. The concern is related to the very fast increase in sludge production coupled with increased difficulties in properly locating disposal works and complying even more stringent environmental quality requirements imposed by legislation. It is therefore important to consider the full range of alternatives for sludge handling and disposal when planning sewage management strategies. An efficient organic stabilization is the preliminary condition for sludge agricultural use or landfill disposal. Anaerobic digestion was always considered in the past years the principal process to stabilize sewage sludge, at least for the big plants. Nevertheless, municipal sludge, particularly waste activated sludge, is very difficult to digest due to the rate-limiting cell lysis. This paper deals with the comparison of ultrasound (mechanical) and ozone (chemical) pre-treatment on the performances of excess sludge digestion. Sludge solubilisation has been investigated by varying specific energy input for ultrasound and ozone dose for the chemical disintegration. For each pre-treatment, semi-continuous digestion tests were carried out by two parallel digesters: one reactor, as control unit, was fed with untreated waste activated sludge, and the other one was fed with disintegrated sludge. To evaluate and compare the efficacy of both pre-treatments the disintegration degree was maintained the same. The digestion tests were carried out to investigate the feasibility on anaerobic digestion performance (total biogas production, volatile solids removal) and to assess the heat balance for each digestion test. Results obtained from the digestion of sonicated sludge at 3% disintegration degree (~2500 kJ/kg TS) showed that the ultrasound pre-treatment may be effective both in increasing VS destruction (+20%) and cumulative biogas production (+30%). Preliminary results from the digestion test with ozonized sludge always at 3% disintegrated sludge (ozone dose of 0.05 g O_3/g total solids corresponding to ~2000 kJ/kg TS) do not indicate a significant improvement on the digestion performances. Work is in progress to investigate the effects of ultrasound and ozone on the floc structure and on the nature of the solubilised material.

2248 ANAEROBIC CO-DIGESTION OF ANIMAL WASTE: SWINE MANURE AND TUNA FISH WASTE

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Anaerobic digestion has become an established and proven technology for the treatment of solid wastes. Co-digestion offers several possible ecological, technology and economical advantages. Anaerobic co-digestion can increase CH_4 production of manure digesters in a 50-200% according to the operation conditions and the co-substrates used. Last September 2007, PROBIOGAS project started up with the objective of improving the production and use of biogas from co-digestion of farming, agricultural and industrial waste. Our research group takes part in the study of co-digestion of swine manure firstly with tuna fish waste and secondly with glycerine (biodiesel production waste). The study consists on three sections: Waste characterization, batch co-digestion assays and continuous co-digestion in complete mixed reactors. In this moment, we are performing batch co-digestion assays with manure and tuna fish wastes. Pig manure was collected in a 150 swine farm sited in the surroundings of Santiago de Compostela. Manure waste characterization is the following: 1678 g TCOD/kg_{dry}; 886 g SCOD/kg_{dry}; 17.5 g TS/kg_{dry}; 189 g TKN/kg_{dry}; 182 g NH_4^-N/kg_{dry} and 89 g PO_4^{-3}/kg_{dry}. Tuna fish waste was collected in a fish meal factory, which uses canning industry waste as input material. This fish meal factory treats 28000 ton/y of canning industry fish waste, which is constituted by a 99% of tuna fish. So, tuna fish waste consists on offal, heads, fish bones, etc. Tuna fish waste was triturated until obtained a homogeneous paste, after this, the following characterization was realised: 999 gTS/kg_{dry}; 910 gTKN/kg_{dry} and 1.5 gTCOD/gTS_{dry}. Batch assays were carried out in triplicate using 500 ml glass flasks with the following operation conditions: 35 ºC, mixed at 120 rpm, 5 gVSS/l of inoculum (using biomass from a manure digester) and 5 gTCOD/l of substrate, which was divided in the following manure/fish waste ratios: 70/30; 80/20 and 90/10. Biogas production is measured by gas chromatography and SCOD, VFA and NH_4-N of liquid fraction are analysed. Blank assays were also performed with inoculum, manure and fish waste, separately. After 40 days, the $gCOD_{CH4}$ produced at only manure, only tuna fish, 70/30 co-digestion; 80/20 co-digestion and 90/10 co-digestion assays were 1.69; 1.06; 1.75; 1.50; 2.64 g, respectively. Preliminary results of this work indicate that 90/10 ratio batch co-digestion increases CH_4 production in 56% and 149% respect to individual manure digestion and tuna fish digestion, respectively. VFA and NH_4-N data of batch assays will permit us to know if it is possible to improve the co-digestion as well as informing about possible inhibition of the process.

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THE THIRD INTERNATIONAL MEETING
ON ENVIRONMENTAL BIOTECHNOLOGY
AND ENGINEERING

TUESDAY 23 SEPTEMBER

ORAL COMMUNICATIONS

II.- Wastewater and solid waste management, treatment and reuse

III.-Strategies for the protection and remediation of natural environments

TUESDAY 23 SEPTEMBER 2008 ORAL COMMUNICATION

Emerging pollutants (endocrine disruptors, micropollutants, etc), recalcitrant compounds and new metabolism

Agronomic Valorization of Crude or Treated Wastewater

Organic Waste Management

Landfills

III STRATEGIES FOR THE PROTECTION AND REMEDIATION OF NATURAL ENVIRONMENTS

GUEST LECURE

1003 ANAEROBICS FOR WASTEWATER TREATMENT, ENERGY RECOVERY, AND GREEN HOUSE GAS EMISSION REDUCTION

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High-quality process water and drinking water is becoming a scarce commodity at various locations of the world. Environmental technologies, designed for treating and reclaiming water for subsequent usage in a productive chain are becoming crucial in modern water strategies. In principle, all water can be turned into drinking water but financial constraints limit the applicability of proper (waste) water treatment technologies. Anaerobic treatment technologies are considered the most powerful tool in treating organically polluted waste streams, with the objective to close water cycles in productive chains.

GUEST LECTURE

2902 PILOT SCALE HYBRID PROCESSES FOR OLIVE MILL WASTEWATER TREATMENT, ENERGY PRODUCTION AND WATER REUSE: COMPARISON BETWEEN FUNGAL AND ELECTRO-COAGULATION PRE-TREATMENTS

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Olive oil mill wastewaters (OMW) cause disposal problems because they contain powerful pollutants such as phenolic compounds. Complete biodegradation or removal of these compounds is hardly achieved by a single treatment method. In this work, we investigated 2 integrated technologies for the treatment of the recalcitrant contaminants of OMW, allowing water recovery and reuse for agricultural purposes. Firstly, we have developed a pilot plant based on fungal pre-treatment using *Phanerochaete chrysosporium* followed by anaerobic digestion. *P. chrysosporium* DSM 6909 able to depolymerize high molecular-mass polyphenols, was cultivated on OMW as sole carbon and energy sources in a 120 l air lift reactor (ALR) in a semi-continuous feed of OMW at a Hydraulic Retention Time (HRT) of 3 to 5 days. The *P. chrysosporium* DSM 6909 pre-treated OMW was fed in a 300 l anaerobic filter after a decantation step. The anaerobic filter was loaded continuously with undiluted pre-treated OMW for 6 months at loading rates reaching 7 $gl^{-1}d^{-1}$ of COD without any apparent toxicity. A second process has been developed at pilot scale combining electro-coagulation and anaerobic digestion. Application of electro-coagulation procedure in a semi-continuous mode permitted, by contrast to the fungal process, the copolymerization of the phenolic compounds leading to high removal efficiencies of COD (50%) and monophenolic compounds (95%). This pre-treatment was found to enhance the anaerobic activity of an up-flow anaerobic filter (300 l) significantly. In the bioreactor, COD removal efficiency of 75% was reached at a hydraulic retention time of 4.5 days and an organic loading rate of 10 g COD $l^{-1}d^{-1}$. An economic calculation of both integrated treatment processes revealed that electro-coagulation followed by anaerobic digestion was more advantageous providing significantly more surplus energy than the fungal pre-treatment process

2357 EFFECT OF FEED STRATEGY AND COD/SULFATE RATIO ON THE REMOVAL OF SULFATE IN AN ANSBBR WITH RECIRCULATION OF THE LIQUID PHASE

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The objective of this work was to analyze the effect of the interaction between feed strategy and COD/[SO_4^{2-}] ratio on the efficiency of sulfate removal from a synthetic wastewater. To this end, an anaerobic sequencing batch reactor, with recirculation of the liquid phase, containing immobilized biomass on polyurethane foam (AnSBBR) was used. The reactor, with a working volume of 2.7 L, treated 2.0 L synthetic wastewater in 8-h cycles. The system was inoculated with anaerobic biomass from a UASB reactor and was maintained at 30 ± 1 ºC in a chamber with temperature control. Two feed strategies were assessed: (a) batch an (b) batch followed by fed-batch. In strategy (a) the reactor was fed in 10 minutes with 2 L wastewater containing sulfate and carbon sources. The carbon source acts as an electron donor in the reduction of sulfate to sulfide. From this point the react stage initiated. The cycle ended at reactor discharge (10 minutes). In strategy (b) 1.2 L wastewater (containing only the sulfate source) was fed during the first 10 minutes of the cycle and the remaining 0.8 L containing only the carbon source in 240 min. COD/[SO_4^{2-}] ratios assessed were 1 and 3 and, based on these values and on the concentrations of 500-11250 mg COD/L and 500-2500 mg SO_4^{2-}/L, the organic matter and sulfate loading rates varied between 1.5 and 13.5 g COD/L.d, and 1.5 and 4.5 g SO_4^{2-}/L.d, respectively. After stabilization of the system time profiles were run of the several monitored parameters (like COD, sulfate, sulfide, and sulfite). In general, the reactor showed to be robust for use in the anaerobic treatment of wastewaters containing sulfate. Gradual feeding (fed-batch) of the carbon source favored sulfide formation, which implies improved removal efficiency of the added sulfate (> 80%). In all tests residues of sulfite (< 10 mg/L) and sulfide (< 100 mg/L) were observed. These did not seem to inhibit the active biomass in the reactor. Minor formation of elemental sulfur was also seen at the top of the reactor, due to micro aeration.

2241 ANAEROBIC TRANFORMATION OF 1,4-TYROSOL TO 4-HYDOXYPHENYLACETATE BY *Desulfovibrio* SPECIES

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1,4-Tyrosol (4-hydroxyphenylethanol) is a phenolic compound that is typically found in olive oil, olive brine, and olive oil mill wastewaters. Its anaerobic transformation was investigated in *Desulfovibrio* strain EMSSDQT (Chamkh *et al.*, 2008) and *Desulfovibrio alcoholivorans* (Qatibi *et al.*, 1991) using high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (13C-NMR) as analysis technics. To our knowledge, this is the first report showing the transformation of 1,4-tyrosol to 4-hydroxyphenylacetate (PHPA) by *Desulfovibrio* sp in anoxic conditions. The phenolic compound was transformed by both strains into PHPA at 100% in sulphate-reducing condition, leaving the aromatic ring intact. PHPA was not further metabolized by both strains even after more than one month of incubation. When replacing sulphate by fumarate, the transformation rate of 1,4-tyrosol to PHPA by D. alcoholivorans and strain EMSS-DQ4T was 100 % and only 50 % respectively. Under fermentation conditions, both strains were unable to use the phenolic compound. These observations suggest that the transformation of 1,4-tyrosol to PHPA seems to depend on the electron acceptor presence under anoxic conditions. Moreover, Both strains were unable to metabolize other tested aromatic compounds such as, unsubstituted phenol, 2-hydroxyphenol (catechol), 3-hydroxyphenol (resorcinol), 4-hydroxycinamate (p-coumarate) and 4-hydroxybenzoate (“4-carboxyphenol”) indicating that the composition and the position of the side chain was determinant for substrate specificity. A primary alcohol side chain in the para position was required by both strains for the transformation of the aromatic compounds. On the other hand, in order to evaluate toxicity of 1,4-tyrosol towards both strains, growth at increasing concentrations of the phenolic compound were tested under sulphate-reduction condition. The experimental results indicate that 40 mM of 1,4-tyrosol were needed to inhibit the growth and production of sulfides by strain EMSS DQ4T while *D. alcoholivorans* seems to be more sensitive to 1,4-tyrosol which inhibits the growth and production of PHPA at concentrations greater than 25 mM. However, for both strains, the maximum PHPA accumulation was only 20 mM.

2151 IDENTIFYING ANAEROBIC DIGESTION MODELS USING SIMULTANEOUS BATCH EXPERIMENTS

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As in other wastewater treatment processes, anaerobic digestion models have become a valuable tool to increase the understanding of complex biodegradation processes, to teach and to communicate using a common language, to optimize design plants and operating strategies and for trying operators and process engineers. Models require accurate and significant parameter values for being useful. Although the calibration problem is an issue of concern, often is neglected. A method for calibration is the use of simultaneous batch experiments (SBE), which requires simple and usually available equipment, and is based on the analysis of replications and the responses obtained when starting from different initial conditions. The objective of the present work is to systematize the SBE method, illustrating and discussing the applicable steps.

The main steps of the method are: 1.) Set up the mathematical model and define the calibration problem. Namely, define exactly the problem to be solved; 2.) Preliminary experimental design approach. Set-up different SBE characterized by different initial conditions, define the output function, characterized by the variables that can be measured during the experiments, and define variables which initial values can be known; 3.) Perform a structural identifiability study, consisting of a theoretical analysis to find out whether the designed experiment can lead to a unique set of parameter values and unknown initial conditions (i.e., initial microorganisms concentrations); 4.) Perform the experimental design leading to parameters and initial biomass identification, based on previous step results; 5.) Perform the practical identification or estimation of the unknowns, as a result of optimizing an objective function, i.e. maximizing the sum of multiple determination coefficients for all measured variables and for all simultaneous experiments performed; 6.) Study the statistical characterization of estimated parameter values, calculating its confidence intervals, its statistical significance and the correlation matrix; and 7.) Return to step 4) or 1) if step 6) provides low significance levels for the calculated parameters or if the model structure cannot explain experimental behavior.

Taking into account that appropriate experimental design depends on kinetic parameters values, which determination is, actually, the objective of the work, results of previous experiments or results from literature are required to orientate initial substrate and biomass concentrations to be used, in order to obtain an experimental design allowing unique and significant parameter values. The explanation of every step is done providing the theoretical basis, discussing applicable methods and equations, and illustrating with examples, some of them previously published*.

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2530 **MODELING A FULL SCALE OXIDATION DITCH SYSTEM, COUPLING HYDRODYNAMICS AND BIOLOGICAL KINETICS USING ASM1 MODEL.**

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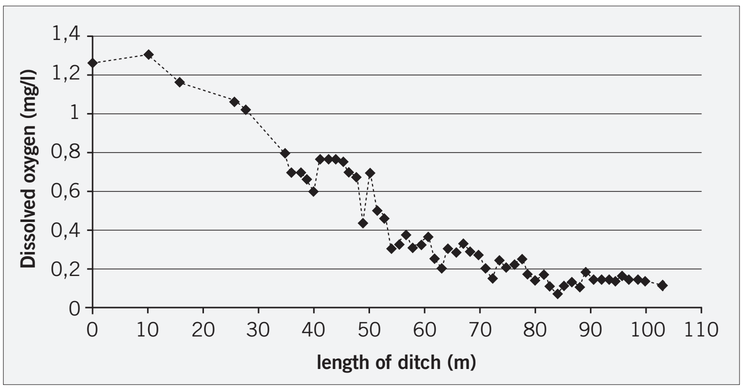
Optimising the aeration in oxidation ditch aims on one hand, a better wastewater quality and on the other hand, a reduction of the energy expenses of the treatment. Given that the energy expenses relative to the aeration represents 60 to 80 % of the operating costs of a wastewater treatment plant and given that the biological activity is strictly dependent on dissolved oxygen, the transfer of oxygen is considered as one of the key parameters of the process. Moreover nitrogen removal is directly influenced by the ratio between aerobic and anoxic zone which determines nitrification and denitrification. In several studies of biological reactors, the hydrodynamic and the biological activity were separately handled. In the literature, the studies concerned essentially the interaction between hydrodynamic and oxygen transfer in oxidation ditch (Simon, 2000; Gillot and Héduit, 2000; Tanguy P, 2003; Potier, 2005). Few authors (Stamou, 1994, 1997, 1999; Lesage N, 2003) adopted an approach coupling hydrodynamic and biological kinetics. Based on these works, the coupling of the hydrodynamic and the biological activity for the modeling seems to be necessary as much as a better understanding of the phenomena and the optimization are aimed. The objective of the present study is the integration of the hydraulic phenomena, oxygen transfer and the biological kinetics to develop a model allowing to predict spatial and temporal evolution of concentrations (oxygen, substrate and COD) within an oxidation ditch. The heterogeneity in this kind of treatment system should be represented by the model. Consequently, Hydrodynamic in the ditch was described by plug flow model with axial dispersion. Hydrodynamic effect is represented in the model by average circulating velocity and dispersion coefficient. To represent the biological activity, we used the model ASM1 (Henze, 1987), taking into account the biodegradation of the organic matter, the nitrification and the denitrification. A mathematical model is presented to predict the concentrations in heterotrophic biomass, soluble substrate and dissolved oxygen in a partially aerated ditch. The equations are solved with an optimisation tool programmed in Matlab because of non linearity of equation. First part of this study deals with a presentation of equations system and the dimensionless parameters that represent different scale time in the system. Second part consists of the calibration and application of the model to a full scale plant. The plant was equipped with surface aerator. The DO concentration was measured in the ditch when the rotor working and when it is off to determine respectively DO concentration along the ditch and biological consumption. These measures at a full scale plant which the volume is 1300 m^3 allowed to see important concentration gradients existing in the ditch. The DO profile measured confirms the plug flow behaviour (fig.1).

Figure1: Dissolved oxygen measured in the full scale plant.



The present model was performed to take into account the heterogeneity in this kind of treatment system. Predicted and measured concentrations was confronted.

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1875 SOIL MICROBIAL ACTIVITIES AND SOLID-STATE ^{13}C NMR TO ASSESS ORGANIC MATTER TRANSFORMATION IN A REED–BED UNDER CHEESE-DAIRY FARM EFFLUENTS

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In this study, we described the soil microbial activities involved in a small dairy effluent purification using a reed filter under a Mediterranean climate. We tested whether certain enzyme activities used as bioindicators of dairy waste degradation (β-galactosidase and protease), vary through time which might influence organic matter degradation and to test whether specific microbial communities were selected at the contact of the effluent using community level catabolic profiles (CLCPs). β-galactosidase and protease activities were followed in a fourteen-month monitoring experiment. These enzyme activities were strongly expressed during whey-discarding period from February to May. CLCPs using Biolog Ecoplate showed a great microbial diversity, as described by the Shannon-Weaver index, and no difference was observed in microbial diversity between the areas at the top of the reed filter (where the effluent is discarded) and at the end. This may be explained by successive environmental factors which make enzyme activities vary: whey discarded from February to May and Mediterranean climate conditions (drying-rewetting effects). Microbial enumeration using epifluorescence microscopy also showed a pattern linked to Mediterranean conditions with a drastic decrease in biomass during summer drought. These results about functional biodiversity were associated with high purification yields: the minimum decrease in Biological Demand in Oxygen was 84% and that in matter in suspension was 75%.

2376 RECOVERY AND CONCENTRATION OF AMMONIA FROM SWINE MANURE USING ELECTRODIALYSIS COUPLED WITH AIR STRIPPING.

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This project aimed at producing a concentrated nitrogen fertilizer from liquid swine manure using electrodialysis (ED) coupled with an acid trap, as a mean to help resolve the excess nutrient problem faced by swine producers, and offer an alternative to commercial nitrogen fertilizer. Raw swine manure was first treated by solid-liquid separation. The liquid fraction used as feed for the ED process, had 3200 and 14000 mg/L of NH_4-N and alkalinity, respectively, and a pH of 8.5. ED was carried out as a batch process, in a dilution-concentration configuration using 10 primary units of AR204SZRA anionic membranes and CR67HMR cationic membranes (Ionics, USA). Four manure batches of 8 litres were electrodialyzed at 5 different voltages until the manure conductivity was reduced by 80%. Concentrate reached an average concentration of 11000 mg/L of NH_4-N. Energy used for ammonium transfer varied from 0.20 kWh to 0.47 kWh for an applied voltage of 7.5 and 17.5 volts respectively. However when considering pumping energy, values of 0.07 and 0.13 kWh/kg of ammonia–N were needed for 17.5 and 7.5 volts, respectively, over the four batches. Electro-osmosis resulted in the transfer of an average of 840 ml of water per batch toward the concentrate. Recovery of ammonia was carried out at a voltage of 17.5 volts using batches of 8 litres of swine manure. The air from the concentrate compartment was recirculated in a cylinder containing 1 litre of HNO_3 2N to trap volatilized ammonia. Ammonia-N concentration reached 21000 and 1220 mg/l in the concentrate and the acid trap, respectively. Results showed that ammonia recovery from swine manure using ED coupled with air stripping was technically feasible. Process improvement would include pH control as well as a reduction in electro-osmosis and operational pressure.

1834 N-REMOVAL FROM THE LIQUID FRACTION OF PIG SLURRY IN A LABSCALE SEQUENCING BATCH REACTOR CONSIDERING DIFFERENT OPERATIONAL STRATEGIES

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Nitrogen is one of the main limiting factors to be considered when treatment of livestock manure is required. Biological N-removal is an alternative applicable in those situations in which arable lands availability, or transportation costs, do not enable recycling manure as fertilizer. Specifically, when dealing with slurries processing, an initial solid-liquid separation allows obtaining a liquid fraction which could be subsequently treated through nitrification-denitrification (NDN). The availability of a calibrated model for a particular system makes it possible to predict its behaviour. Thus, modeling can be used as a tool for developing treatment strategies based on N-removal. The objective of this work was to corroborate the validity of a mathematical model for predicting the behaviour of a sequencing batch reactor (SBR) operated aiming N-removal under different conditions when treating the liquid fraction of pig slurry (LFPS). A model initially proposed to simulate partial nitrification in an aerobic chemostat was modified in order to predict the behaviour of a discontinuous and intermittently aerated reactor. This development was extended by adding processes related to the anoxic step. Nitrification and denitrification were both regarded as two-step processes, and pH was included as state variable. Raw LFPS collected from a sow-herd farm was used to calibrate the model experimentally. A labscale SBR (20 l) was operated at room temperature (~20 ºC) considering three different step-feed strategies (Nitrogen loading rate = 0.13 g TKN l^{-1} d^{-1}; Cycle length = 24 h), as shown below: Parameter Strategy 1 Strategy 2 Strategy 3 A/O periods, nº per cycle 2 3 4 A/O periods, length (h) 14/9 8 6 tanox/treac (h/h) 0.48 0.43 - Aeration, controller timer PID real-time Aeration, O2 mass transfer coefficient (d-1) 450 ≤ 300 85 Aeration, set-point (mg O_2 l^{-1}) - 3.0 max. 2.5 BCOD/TKN, LFPS used as fed 6.5 4.9 3.9 A/O: Anoxic/Oxic; BCOD: Biodegradable Chemical Oxygen Demand; TKN: Total Kjeldahl Nitrogen In relation to nitrogenous forms trends, good agreements were obtained between simulated and Experimental data in the three cases. Compositional aspects of the LFPS such as the BCOD/TKN ratio were pointed out as one of the most influential factors for an adequate NDN performance. Low biodegradability of the organic compounds limited denitrification, although treating raw waste. Operational aspects, i.e. number of A/O periods per cycle and aeration controls during nitrification, also affected decisively the SBR behaviour. Thus, low aeration intensity during oxic periods allowed to mainly conduct the process along the nitrite route and thereby to reduce the organic and oxygen requirements of the process. Finally, numerical optimization of the SBR cycle performance was conducted accounting for model-based simulations.

2193 SHORT- AND LONG-TERM EFFECTS OF AMMONIA AND NITRITE ON THE ANAMMOX PROCESS

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Autotrophic anaerobic ammonium oxidation (Anammox) process is a feasible alternative to treat industrial wastewater with high ammonia concentration but low content of organic matter. In this process ammonium and nitrite are used by Planctomycete-type bacteria under anoxic conditions to generate nitrogen gas. Both substrates can exert inhibitory effects on the process, causing the decrease of the specific activity of the biomass and the loss of the performance and stability of reactors. The aim of the present work is to evaluate the effects on the stability of this process of ammonia and nitrite presence in short- and long-term experiments. The short-term effects were determined using batch activity tests, which indicated a decrease of the Specific Anammox Activity (SAA) of 45% at concentrations about 30 mg NH_3-N/L, while 100 mg NH_3-N/L caused an inhibition of 80%. The SAA was not affected at concentrations up to 6.6 µg HNO_2-N/L but it suffered a decrease over 70% in the presence of 0.013 mg HNO_2-N/L. The study of the long-term effects of ammonia and nitrite on the Anammox process was carried out in lab-scale Sequencing Batch Reactors (SBR). An Anammox SBR was operated under nitrite limiting conditions at a constant nitrite loading rate of 0.25 g NO_2—N/(L·d) and the ammonia inlet concentration was stepwise increased. Concentrations up to 20 mg NH_3-N/L in the reactor showed no effects on either efficiency or biomass activity. Nevertheless, when free ammonia concentrations reached values between 35-40 mg N/L, the operation of the reactor turned unstable and the efficiency was totally lost. To test the effect of nitrite, a second Anammox SBR was operated under ammonia limiting conditions at a constant ammonia loading rate of 0.15 g NH_4^+-N/(L·d). The nitrite was initially fed in a stoichiometric ratio and, later, its inlet concentration was stepwise increased to achieve steady nitrite accumulations inside the reactor. A nitrous acid concentration of 0.12 µg HNO_2-N/L caused a decrease over 40% in the SAA. However, the remaining capacity of the reactor was enough to maintain a full efficiency. A total restoration of the SAA was observed along the operational period in spite of the accumulation of nitrous acid was increased up to 2.5 µg HNO_2-N/L.

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2267 FROM PARTIAL NITRIFICATION TO CANON IN AN AEROBIC GRANULAR SBR

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Nitrogen removal via nitrification-denitrification processes is commonly used in biological wastewater treatment plants to remove nitrogen compounds. In recent years new technologies emerged bringing solutions to remove nitrogen from wastewaters with not enough COD content to complete the denitrification process. An alternative strategy to the conventional nitrification-denitrification processes has been developed in the nineties consisting of a combination of the oxidation of half of the ammonium from the wastewater to nitrite via partial nitrification and the removal of both, ammonium and formed nitrite by the Anammox process. This new process requires less oxygen supply and avoids the necessity of organic matter as electron donor, lowering the operational costs. The partial nitrification-Anammox combination can be carried out in two reactors or in a single step, the so-called Completely Autotrophic Nitrogen removal Over Nitrite (CANON). In this research, the partial nitrification process was stably operated in an aerobic granular SBR. For 500 days, partial nitrification was performed in the reactor treating firstly an autotrophic synthetic wastewater and then the supernatant from an anaerobic sludge digester. Treated ammonium loads ranged from 0.6 g to 1.6 g NH_4^+-N/(L·d) with a NO_2^-/NH_4^+ ratio ranging from 0.6 to 1.5. Nitrate concentrations measured in the effluent were lower than 10 mg NO_3^--N/L. Dissolved oxygen concentration ranged from 1.5 to 4.0 mg O_2/L and the temperature was controlled at 20 ºC. The granular biomass with a mean diameter of 3 mm presented good settling properties: Sludge Volume Index of 31 mL/g VSS and settling velocities larger than 100 m/h. After 500 days of operation at these conditions, nitrogen losses were registered from the calculated balances which were associated to the occurrence of the Anammox process together with the partial nitrification. The CANON process was established in the reactor operated at low temperatures, high dissolved oxygen concentrations and without inoculation of Anammox bacteria. Stable CANON operation was registered for more than three months at these conditions. The maximal nitrogen removal rate attained was 1.1 g NH_4^+-N/(L·d), the highest value ever reported for a CANON system at temperatures below 30 ºC. FISH analyses of the biomass populations confirmed the presence of Nitrosomonas genus as ammonia oxidizing bacteria placed in the external layers of the granules and Anammox bacteria in deeper layers where oxygen was not present. The large nitrogen removal rates registered in the granular SBR operated at 20 ºC open the possibility for new fields of application for the CANON process.

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2150 KINETIC MODELS FOR AMMONIUM-OXIDISING BACTERIA INHIBITION BY FREE AMMONIA AND FREE NITROUS ACID

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In order to fulfil the new legal requirements with regards to the sewage sludge produced in the WWTPs an on-going EU research project is devoted to the Reduction, Modification and VALorisation of Sludge (REMOVALS, no 018525, www.etseq.urv.es/removals/). One of the objectives in this project is the development of a specific treatment for the high-strength ammonium wastewater produced in the dewatering process of the digested sludge, the so-called reject water. This specific treatment is the Biological Nitrogen Removal (BNR) via nitrite and its first step is the partial nitrification or biological oxidation of ammonium to nitrite by ammonium-oxidising biomass (AOB). The main objective is to achieve a stable partial nitrification process in an activated sludge system removing all the nitrite-oxidising biomass (NOB) but affecting the less as possible the AOB. Both biomasses are inhibited by their substrates and products (free ammonia (FA) and free nitrous acid (FNA)) and the utilisation of specific inhibitory conditions can produce the selective elimination of NOB. However, it is very important to determine the effect of these inhibitions over the AOB to optimise the partial nitrification process. This work is devoted to determine the kinetic models for AOB inhibition by FA and FNA. Respirometric experiments with continuous aeration and constant pH and temperature were used to measure the activity of AOB at different FA and FNA concentrations. The respirometric experiments were repeated twice to ensure the reproducibility and off-line measurements of ammonium, nitrite and nitrate were carried out to know the FA and FNA concentrations and confirm the lack of NOB activity. The results showed that AOB inhibition by FA was well described by a common kinetic model for substrate inhibition (Haldane model) until a FA concentration of 300 mg L-1 but other kinetic model was needed for higher FA concentrations. On the other hand, the AOB inhibition by FNA was described by a non-competitive kinetic model. Finally, the activity of AOB in absence of CO_2 was determined with the same procedure but using CO_2-free air in the respirometer. The AOB activity with and without CO_2 permitted the comparison of the FA and FNA inhibition over the anabolism and the catabolism of AOB.

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1868 START-UP OF A BIOFILM AIRLIFT SYSTEM TO OBTAIN PARTIAL NITRIFICATION OF A HIGH-STRENGTH AMMONIUM WASTEWATER

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In order to fulfil the new legal requirements with regards to the sewage sludge produced in the WWTPs an on-going EU research project is devoted to the REduction, MOdification and VALorisation of Sludge (REMOVALS, nº 018525, www.etseq.urv.es/removals/). Among many other initiatives inside this project, a possible valorisation of sludge is the production of activated carbon (AC) which could be used, among other applications, as carrier for biofilm development. One of the possible applications of biofilm reactors in the current process diagram of a WWTP is the specific treatment of a high-strength ammonium wastewater produced in the anaerobic digestion of the sludge, the so-called reject water. The use of AC as carrier has been investigated for the design of a compact biofilm airlift reactor to obtain partial nitrification. Partial nitrification of the reject water is an intermediate process in the Biological Nitrogen Removal (BNR) via nitrite. This is an interesting process because it allows a 25% reduction of oxygen requirements, reducing considerably the amount of sludge produced compared with the conventional BNR process. The manual start-up of a pilot-scale biofilm airlift reactor of 120 L to achieve partial nitrification with a reject water of about 1200 g N-NH_4^+ L^{-1} was carried out. The airlift reactor was loaded with 4.5 kg of AC (4% of total reactor volume) and was inoculated with 25 L of activated sludge from the biological reactor of a municipal WWTP. Operating conditions were pH 8, 30 ºC and dissolved oxygen between 5 - 8 g mL-1. Total ammonia nitrogen (TAN), total nitrite nitrogen (TNN) and nitrate concentrations in the effluent were analyzed and volumetric nitrogen loading rate (NLRv) was increased progressively. NLRv was increased if TAN in the effluent was lower than 100 g N NH_4^+ L^{-1}. After a period of 4 months of continuous operation the volumetric nitrification rate was about 0.6 g N L-1 d-1 and the percentage of TNN accumulation was about 70%. Fluorescence in situ hybridization coupled with Confocal Laser Scanning Microscopy was used to confirm the biofilm development and to assess the ratio between ammonia-oxidizers, nitrite-oxidizers and heterotrophic microorganisms during the start-up of the airlift pilot plant.

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1836 BIOLOGICAL ANOXIC PHOSPHORUS REMOVAL IN A CONTINUOUS-FLOW EXTERNAL NITRIFICATION ACTIVATED SLUDGE SYSTEM

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Application of Biological Nutrient Removal (BNR) processes in wastewater treatment is necessitated for the protection of water bodies from eutrophication. An alternative BNR method is tested for simultaneous Carbon (C), Nitrogen (N) and Phosphorus (P) removal in a continuous-flow bench scale plant for municipal wastewater treatment. The plant operation is based on the activity of two microbial populations which grow under different operational conditions (two sludge system). Denitrifying Phosphorus Accumulating Organisms (DPAOs) are enriched under alternate anaerobic and anoxic conditions, whereas nitrifying biomass grows under aerobic conditions. Potential benefits of the two sludge system over a conventional BNR plant are: i) minimized competition between Ordinary Heterotrophs and PAOs for organic substrate, ii) separate optimization for nitrification and phosphorus removal, iii) minimal utilization of oxygen for phosphorus removal (lower aeration demands), iii) utilization of intracellular carbon storage as electron donor for denitrification by DPAOs, iv) minimized nitrate effluent concentration with no recycling of nitrifying mixed liquor (post-denitrification system) and v) reduced production of surplus sludge. Results of a 300 operational days continuous monitoring campaign for an operational mean Hydraulic Residence Time (HRT) of 10h could be summarized as following:
1. Complete separation of nitrifiers and DPAOs is practically feasible due to the great sludge setlleability characteristics that are favoured due to the system configuration (Sludge Volume Index less than 60 ml/g for both sludges).
2. Mean total COD and BOD_5 removal efficiencies were determined up to 84% and 91%, resulting to mean effluent values as high as 93 mg/L and 27 mg/L, respectively.
3. NH_4^+-N and TKN effluent residues did not exceed 3 mg/L and 10 mg/L, respectively, resulting to a 94% and 85% removal efficiency. 4. Complete and stable PO_4^{3-}- P removal was observed for the two sludge system proving the practical applicability and exceptional efficiency of the denitrifying phosphorus removal process. Furthermore, complete denitrification is practically possible without any internal recirculation stream of nitrified mixed liquor, because of denitrification following the nitrification stage. 5. Influent COD/TKN ratio seems to be the main factor governing the denitrification efficiency of the plant operation. Replicate results show complete NO_3^--N removal when an influent COD/TKN ratio more than 8 is assured. On the other hand, denitrification is severely affected when this ratio drops below 5. However, the same phenomenon is not observed for the system phosphorus removal efficiency, which remains almost complete, irrespective to the denitrification decrease. 6. Surplus sludge production was minimized by maintaining a high enough sludge age for the DPAO biomass equal to 20d, without any detrimental effect to the plant phosphorus removal efficiency.

1848 PERLITE AS A CARRIER OF PHOSPHATE-ACCUMULATING BACTERIA

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The phosphate (P)-accumulating bacteria are important for biological P removal from wastewater. Currently, attention is being drawn to the immobilisation of desired bacteria on different carriers in order to achieve a better efficiency of the wastewater treatment. In this study, two size fractions (0.1-1 and 0.1-2 mm) of different forms of expanded perlite (original, autoclaved and magnesium-exchanged) were investigated as possible carriers of P accumulating bacterium A. *junii*. After 24h of incubation, most of the cells in the reactors were immobilised onto the perlite particles by means of adsorptive growth, while the rest of the biomass remained as free cells in the supernatant. The highest immobilisation rate of 3.27 ± 0.13x109 CFU/g along with the highest removal of P (49.05 ± 0.97 mg/g of perlite) was in the reactors containing the magnesium-exchanged perlite of particle size 0.1-1 mm. Considering the very high bacterial biomass accompanied by high P removal and satisfactory reactor performance, the magnesium-exchanged perlite proved to be the most suitable carrier of bacteria among the materials tested in this study.

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2438 ENRICHMENT OF DENITRIFYING PHOSPHORUS ACCUMULATING ORGANISMS (DPAOs) IN A CONTINUOUS FLOW LABORATORY SCALE PLANT

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Enhanced Biological Phosphate Removal (EBPR) is a well established method for efficient removal of phosphate during wastewater treatment by using biological instead of chemical phenomena. EBPR is currently implemented at a number of Waste Water Treatment Plants (WWTPs) and usually phosphate removal occurs by recirculating activated sludge between an anaerobic and an aerobic tank. However biomass recirculation between an anaerobic and an anoxic tank, where phosphorus removal and denitrification occur simultaneously is also an interesting alternative.
The present work focuses on the operation and the optimization of a continuous flow laboratory scale plant for the enrichment of denitrifying bacteria responsible for phosphorus removal from synthetic wastewater. Recirculation of the biomass between an anaerobic and an anoxic tank enhances the growth of bacteria capable to denitrify and remove phosphorus simultaneously. Extracellurar carbon is uptaken during anaerobiosis and stored in the form of polyhydroxyalkanoates (PHAs) in the bacterial cells. At the following anoxic phase phosphorus is removed by bacteria using nitrate instead of oxygen as final electron acceptor.
After 60 days of stable operation, at a mean hydraulic residence time of 15hrs, biomass is enriched with Denitrifying Phosphate Accumulating Organisms (DPAOs). The denitrification rate reached 3,5mg N-NO_3/g VSS.h for a respective phosphorus removal rate of 6,12mg P-PO_4/g VSS.h resulting to a P/N removal ratio of 1,75 at the expense of 300 mg/L COD as acetic acid contained in the synthetic wastewater. The intracellular levels of poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV) were 125 and 25 mg/g VSS respectively, six times greater than that determined on day one, of glucogen 185 mg/g VSS, triplicated during the experimental period and of total biomass phosphate 70 mg/g VSS compared to 20 mg/g VSS of the not acclimatized inoculum. These variations clearly indicate the gradual enrichment of DPAOs in the consortium, successively achieved during the plant operation.
By monitoring the abovementioned parameters from the plant inoculation with activated sludge which did not have phosphate removal capability, until stable and efficient phosphate removal was established, we managed to gain insight on the stoichiometry and the biochemistry of the anoxic phosphate removal process.

2214 *p*-CRESOL MINERALIZATION BY A NITRIFYING CONSORTIUM

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Nitrification and denitrification processes are considered economically feasible technologies for nitrogen removal from wastewaters. Knowledge on the toxic or inhibitory effects of cresols on the nitrifying respiratory process is still insufficient. The aim of this study was to evaluate the kinetic behavior and oxidizing ability of a nitrifying consortium exposed to *p*-cresol in batch cultures. Biotransformation of *p*-cresol was investigated by identifying the different intermediates formed. The sludge used for inoculating batch reactors (136 ± 17 mg microbial protein/L) was obtained from a continuous reactor in steady-state nitrification. All experiments were performed in 160 mL serum bottles with a lithoautotrophic medium and an initial NH_4^+-N concentration of 100 ± 10 mg/L. *p*-Cresol was added at 25 mg/L. Headspace and liquid phases were saturated with oxygen. Cultures were shaken at 300 rpm at 30 ºC. *p*-Cresol inhibited the ammonia-oxidizing process with a decrease of 80% in the specific rate of ammonium consumption during the first 18 h. The little consumed ammonium was totally oxidized to nitrate with a yield of 0.97 ± 0.05 g NO_3^--N/g NH_4^+-N consumed. After 48 h, ammonium consumption efficiency was 96 ± 9% while nitrate production yield reached 0.95 ± 0.06 g NO_3^--N/g NH_4^+-N consumed. High values for yield showed that the nitrifying metabolic pathway was only affected at the specific rate level being nitrate the main end product. The consortium was able to totally oxidize *p*-cresol (0.16 ± 0.06 mg *p*-cresol-C/mg microbial protein-h) into *p*-hydroxybenzaldehyde (*p*-OHDO) and *p*-hydroxybenzoate, which were later completely mineralized. *p*-OHDO appeared to be the most recalcitrant of the transient intermediates with an accumulation of 7 h in the cultures. Allylthiourea (ATU) was used as specific inhibitor of ammonia monooxygenase (AMO), enzyme previously shown to be able of oxidizing various aromatic compounds. In presence of ATU (25 mg/L), *p*-cresol was oxidized to the same intermediates in a similar pattern than results obtained without the AMO inhibitor. AMO seemed not to be involved in the *p*-cresol oxidation process, suggesting that the inhibition of the ammonia-oxidizing process observed in the presence of *p*-cresol was not due to the oxidation of *p*-cresol by AMO.

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2551 CONTRIBUTION OF QUINONE-REDUCING MICROORGANISMS TO THE ANAEROBIC BIODEGRADATION OF ORGANIC COMPOUNDS UNDER DIFFERENT REDOX CONDITIONS

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Reduction of humic substances (HS) has recently been recognized as a microbial respiratory process supporting growth of several distinct microorganisms. Quinone moieties, which are very abundant in the humic acid fraction of humus, are the main functional groups conferring electron-accepting capacity to HS. The capacity to reduce HS has been reported in anaerobic consortia from a wide diversity of environments. The aim of this study was to evaluate the role of quinone-reducing microorganisms to the biodegradation of organic compounds in the presence of nitrate and sulfate. The capacity of an anaerobic sludge to oxidize different organic compounds, including acetate, propionate, lactate, phenol and p-cresol, in the presence of nitrate, sulfate and the humic model compound, anthraquinone-2,6-disulfonate (AQDS) as terminal electron acceptors, was evaluated. Denitrification showed the highest respiratory rates in the consortium studied and occurred exclusively during the first hours of incubation for most organic substrates degraded. Reduction of AQDS and sulfate generally started after complete denitrification, or even occurred at the same time during the biodegradation of p-cresol, in anaerobic sludge incubations; whereas methanogenesis did not significantly occur during the reduction of nitrate, sulfate, and AQDS. AQDS reduction was the preferred respiratory pathway over sulfate reduction and methanogenesis during the anaerobic oxidation of most organic substrates by the anaerobic sludge studied. Propionate was a poor electron donor to achieve AQDS reduction; however, denitrifying and sulfate-reducing activities carried out by the consortium promoted the reduction of AQDS via acetate accumulated from propionate oxidation. Our results suggest that microbial reduction of humic substances (HS) may play an important role during the anaerobic oxidation of organic pollutants in anaerobic environments despite the presence of alternative electron acceptors, such as sulfate and nitrate. Methane inhibition, imposed by the inclusion of AQDS as terminal electron acceptor, suggests that microbial reduction of HS may also have important implications on the global climate preservation, considering the green-house effects of methane.

2283 OCCURRENCE AND FATE OF SELECTED PPCPS IN ACONVENTIONAL SEWAGE TREATMENT PLANT LOCATED IN NORTH WEST UK

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Over the last decade, the occurrence of pharmaceutical and personal care products (PPCPs) in wastewater effluents has become an increasing concern. Most of these compounds are released into the environment through sewage treatment plant effluents, due to the fact that these plants are not able to remove many of them in a significant extension. Presently, studies regarding hazardous effects in the aquatic environment are emerging worldwide, but still there is little information available regarding their potential ecotoxicological effects. In this work, the occurrence and fate of eight PPCPs during sewage treatment have been studied in a fully instrumented activated sludge pilot-scale plant placed in a wastewater treatment plant in the north west of the UK. This pilot-scale reactor was fed with both sewage and the liquid supernatant produced after sludge stabilisation. Selected compounds exhibited different physico-chemical properties, which are considered a key aspect concerning the diverse behaviour observed for these substances. Operational parameters in sewage treatment units like hydraulic retention time, solid retention time, mixed liquor suspended solids and redox conditions are also relevant in order to understand and maximize removal efficiencies for these substances. Five points corresponding to different locations along the water line of the pilot plant were sampled and analyzed during two days in order to detect pharmaceutically active compounds belonging to various therapeutic groups such as anti-inflammatory drugs (ibuprofen, naproxen and diclofenac), tranquillisers (diazepam) and antiepileptics (carbamazepine). Analyses for detection of three polycyclic musk fragrances (galaxolide, tonalide and celestolide) were carried out as well. Four of the selected compounds (ibuprofen, naproxen, galaxolide and tonalide) were detected and quantified in the influent with concentrations ranging from 9 to 0.1 μg/L. On the other hand, diazepam and tonalide were not detected. Diclofenac and carbamazepine were present in sewage, but below their limit of quantification. Concerning PPCPs removal along the different units of the pilot plant (primary settler, aeration tank and secondary settler), anti-inflammatories were almost completely removed from sewage during biological treatment and musk fragrances were only partially removed, most probably by sorption onto the suspended solids separated in the primary settler and on the biomass existing in the aeration tank, due to their strong lipophilic character.

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2531 THE USE OF TREATED WASTEWATER FOR CHEMLALI OLIVE TREE IRRIGATION: EFFECTS ON SOIL PROPERTIES, GROWTH AND OIL QUALITY

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Olive tree (*Olea europaea* L.) cultivation, the major tree crops in Mediterranean countries is being extended to irrigated lands. However, the limited water availability, the severe climatic conditions and the increased need for good water quality for urban and industrial sector uses are leading to the urgent use of less water qualities (brackish water and recycled wastewater) for olive tree irrigation. The aim of this work was to assess the effects of long term irrigation with treated waste water (TWW) on the soil chemical properties, on olive tree growth and on oil quality characteristics. The trial was carried out, during two crop seasons, on the Chemlali olive tree, characterizing the landscape of the south of Tunisia (34° 43N, 10° 41E). Eighteen year- old- olive tree, spaced 24 x 24 m, were used in a randomized complete block design with two different treatments: irrigation with well water and with wastewater. Each treatment consisted of 20 trees, with four replications of 5 trees each. All the plants were irrigated with the same amount of water (4000 m^3 ha^{-1} $year^{-1}$) and subjected to the same fertilization and common olive cultivation practices applied in Sfax area. Soil samples were characterized before and after irrigation. Olive tree growth, yield and oil quality were measured every year. Irrigation with TWW caused a slight decrease in plant growth and yield. This reduction was associated with higher accumulation of toxic salts in soil. On the other hand, it improved the available nutrients such as K and P. The same results showed that irrigation with TWW did not affect oil quality indexes (free acidity, specific ultraviolet absorbance K232 and K270). The mean values of these parameters are lower than the upper limits established for the best commercial olive oil quality designated as "extra virgin". However, a significant increase of palmitic, palmitoleic, linoleic, linolenic and stearic acid contents was found. In contrast, a decrease of oleic acid and polyphenol content was observed at the end of the experimental period.

2556 SOIL AMENDEMENT WITH OLIVE MILL WASTEWATER: IMPACT OF STORAGE BEFORE SPREADING

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The olive oil production performed by the traditional three-phase process generates considerable amounts of olive mill wastewater (OMW) that is a liquid effluent, red to darkcoloured depending on its level of oxidation. OMW is well known for the ecological problems it causes owing to the highly toxic polyphenolic compounds it contains.
A two-phase process has been adopted by some countries particularly Spain to avoid its production, but the three-phase system remains the dominant technology in the other countries especially in the southern part of the Mediterranean basin and there is no sign that this should change in the short term. Beyond other factors, this is probably due to the fact that the waste generated by the two-phase process and which is called "alpeorujo" (mixture of olive cake and OMW) also requires an adequate disposal and as a consequence does not really solve the problem.
Generally, freshly produced OMW (FOMW) is stored outdoor, in evaporation ponds, in an attempt to reduce its volume, after what it is widely released in soil. During storage, FOMW is subjected to auto-oxidation, after which occur a condensation of simple phenolic compounds in polymers of high molecular weight, responsible for the black colour of oxidized OMW (OOMW). Resulting black-stored OMW is theoretically less toxic than FOMW but also less biodegradable.
In this study, variable proportions of FOMW and OOMW (prepared in the lab from the same FOMW) were added to a sample of soil distributed in several plastic containers. The soil was selected to be representative of an important olive oil production area of Tunisia. Biological and chemical analyses were realised, so that biodegradability of fresh and stored OMW in soil, and effects of each kind of effluent, on soil microflora, could be settled. It appeared that OOMW greatly reduced the total number of aerobic bacteria and fungi that could be recovered from the soil by plate count. On the contrary, these numbers remained stable or even increased when the soil was amended with FOMW. In the same way, while soil respiration was doped for each dose of FOMW added, it was completely inhibited by the same dose of OOMW. The polyphenolic compounds of the soil were extracted immediately after OMW amendment and after 3 months of incubation and their molecular mass distribution was determined by filtration on Sephadex G-50 gel. It appeared that for the soils amended with FOMW, the permeation profile had shifted towards molecules of lower molecular weight whereas this was much less evident in the case of OOMW suggesting that the polymeric compounds present in OOMW were less biodegradable by the soil microorganisms than those of FOMW. These results suggest that in order to avoid any negative impact on soil microbial activity and on the contrary to favour the degradation of polyphenols, it should be recommended to perform OMW spreading at the same time it is produced or if this is not possible to avoid contact with air during storage. This could be obtained by storing OMW in closed vessels under vacuum or under an atmosphere of inert gas.

2559 SOIL DEGRADATION UNDER RECLAIMED WASTEWATER IN "ARENADOS" (LANZAROTE, CANARY ISLANDS, SPAIN)

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In the Island of Lanzarote, one of the most arid zones of the EU, a traditional farming system based on the soil mulching with basaltic tephra, locally known as arenados. was developed since the 18th century This dry-framing system accounts currently for more than 21% of the island surface. However, in the last decades, this system has been transformed with the incorporation of irrigation, due to the availability of new non-conventional water resources (desalinized and reclaimed municipal wastewater). Desalinized water is used for municipal uses. Wastewater destined for irrigation after a tertiary treatment is pumped to a water-tank from which it is distributed to the culture zones. The irrigation area is about 400 ha and the main crop is sweet potato (Ipomoea batatas). Drip irrigation is generally used, with doses varying with the season and crop development. Reclaimed water quality is not well suited for irrigation due to salinity, boron, sodium and chloride hazards which pose short-term risks according to different guides. In addition, water quality shows a great variability. The aim of this work was to evaluate the impact of irrigation on soil properties, plant nutrition and tuber quality. To this end, twelve field plots were selected in order to have both irrigated and adjacent non-irrigated mulched fields. Soils were sampled at two depths (0 - 10 and 10 - 30 cm). The results showed an increase of salinity in irrigated soils (0.3 - 1 dSm^{-1} in non-irrigated versus 0.5 - 4 dSm^{-1} in irrigated ones, saturated paste). The same trend was observed for SAR values. Plant N, P and K tended to increase in irrigated soils which we attributed to the contribution of reclaimed water to these nutrients; the increase, however, was significant only for P. Among the micronutrients, only boron (B) showed significant differences, both in soil solution saturated paste and hot water soluble B values, increasing in irrigated soils. Although these values exceeded the recommended phytotoxicity thresholds, no effect was observed in the plants. Tuber dry-matter contents were significantly lower in irrigated soils.With irrigation, a yield increase is obtained; however, the results suggest the sustainability of the system, which is a model of conservation agriculture against desertification, is not warranted.

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GUEST LECTURE

1004 CHARACTERIZATION PROGRAM IN THE FRAMEWORK OF THE NATIONAL SEWAGE SLUDGE PLAN IN SPAIN

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Sewage Sludge is the waste originated from the process of treatment of waste water. Due to the physical-chemical processes involved in the treatment, the sludge tends to concentrate heavy metals and poorly biodegradable trace organic compounds as well as potentially pathogenic organisms (viruses, bacteria, etc.). However, sludge is rich in nutrients such as nitrogen and phosphorous and contains valuable organic matter that is useful when soils are depleted or subject to erosion. The organic matter and nutrients are the two main elements that make the spreading of this kind of waste on land as a fertiliser or an organic soil improver suitable.
As a waste, the Sewage Sludge is regulated by the Waste Law 10/1998 but also by specific regulations about agricultural applications. These regulations and the National Plan seek to encourage the use of sewage sludge in agriculture.

The objectives of the Waste Law 10/98 are:

- Apply the hierarchy principle (Agricultural soils > energy recovery > landfill)
- Establish the obligations of management of the WWTPs
- Set out the conditions to get License for disposal and recovery facilities
- **Make a National Plan to ensure the correct management of the Sewage Sludge protecting the environment and specially the soil.**

In the last Sewage Sludge National Plan, which covered the period from 2001 to 2006, it was proposed the realization of different studies in order to know the usual contents of nutrients but also the contaminants of these wastes in Spain. One of these studies is the "Characterization Program of the Sewage Sludge generated in Spain".
The study began on 2005 and was designed and made by the Ministry of Environment in collaboration with the Autonomous Communities and the Spanish Association of Water and Sewerage Supply (AEAS), the coordination was carried out by the Centre for Studies and Experimentation of Public Works (CEDEX). The chemical and microbiological analyses were performed by the laboratories of Centre for Studies and Experimentation of Public Works (CEDEX), the Research Centre for Energy, Environment and Technology (CIEMAT) and the Research Institute for Rural and Agricultural Development of Madrid (IMIDRA). And the results were statistically analyzed by Geologic and Mining Research Centre.
In this study 66 WWTPs which supply 22.959.251 equivalent inhabitants were selected depending on the population of the Autonomous Communities. Most the WWTPs are located in Cataluña, Madrid and Andalucia where the population is higher than in the others Communities.

For this study representative samples corresponding to different sort of Sewage Sludge treatments were included and the parameters analyzed were agronomics, organic contaminants and pathogens.

- Agronomic parameters: pH, conductivity, TOC, oxidable organic matter, total N, total and available P, total and available Ca, Mg, K, Fe
- Heavy metals: Cd, Cr, Ni, Hg, Pb, Zn, Cu
- Organic parameters:
  - AOX (adsorbable organically bound halogens)
  - LAS (linear alkylbenzene sulphonates)
  - PHTHALATES
  - NONYLPHENOLS
  - PAH (Polycyclic aromatic hydrocarbons)
  - PCB (polychlorinated biphenyls)
  - PCDD/PCDF (dioxins, furans)
  - PBDE (Polybrominated diphenyl ethers)
- Microbiological parameters:
- *E. Coli*
- *Salmonella spp*
- Total and fecal coniformes

The results were statistically analyzed to assess the distribution and ensure their reliability.
The results were compared with the legal and proposed limits, and with regard to:

- Agronomic parameters, although every Sewage Sludges have suitable nutrient contents, the results show a wide variability between installations as well as in each installation.
- Heavy metal results, although the majority of the values are below to the established legal limits, some installations present values above them which are in relation with the industrial contributions to the sewer system.
- Most of the organic compounds values are below the proposed limits in the Sewage Sludge 3th Directive Draft and its proposal. The values above these limits are in relation with the industrial contributions.

- The LAS values are above the proposed limits in the 50% of the samples, but most of the WWTPs wich exceed the proposed limits treat the Sewage Sludge by anaerobic digestion and other installations receive waste water from agrofood and cleaning products industries, etc.
- The PBDs values are below to 1000ng/g, except some punctual cases where the values are above to 10.000 ng/g. In general these figures are in relation with the textile industrial contributions. Although their toxicity is less than the dioxins toxicity, the concentration is one thousand bigger. These compounds are not included in the legislation neither the Drafts, but they are in the list of the The Stockholm Agreement about the persistent organic pollutants, for this reason they have to be taken into account specially in the future legislation about sewage sludge agricultural applications.
- The microbiological results show the presence of pathogens except in the thermal dried, composted and chemical stabilized samples.

The conclusions for the study are the following:

- The quality of the Sewage Sludge is not constant and depends on the composition of waste water and their further treatments.
- Although most of the Sewage Sludges are excellent sources of nutrients, it is fundamental to take into account their concentrations in the Sludge and in the receiving soils, as well as the soil physical characteristics, to calculate the correct doses of the Sewage Sludge applied in prevention of groundwater contamination.
- As well as the nutrients, the conductivity is another parameter that has to be taken into account to calculate the doses of the Sewage Sludge in order to avoid the negative effects of the osmotic pressure into the roots.
- The low heavy metals values observed allow establishing lower legal limits for agricultural Sewage Sludge applications.
- The values of heavy metals and organic compounds above the limits show the necessity of stress the prevention of the contamination by the industries to the sewer system.
- In general the organic compounds values are bellow the proposed for the agricultural applications, except for the LAS.
- The presence of pathogens indicates the importance of the treatments before apply the Sewage Sludge on the soil.

GUEST LECTURE

2903 THE IMPORTANCE OF TEMPERATURE IN THE EFFECTIVE SANITIZATION OF AQUEOUS EFFLUENTS: A REVIEW OF THE CASE FOR A THERMAL TREATMENT THAT IS ENVIRONMENTALLY AND ECONOMICALLY CREDIBLE

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The survival time of pathogens in the environment is normally longest in cold conditions. A large number of published studies have demonstrated a fall in this period from months or years down to a matter of weeks as the ambient temperature rises into the twenties. The effect of temperature is thus not surprisingly a key actor in the sanitizing efficiency of any biological treatment process as conditions move through the mesophilic range (30 to 45 ºC) towards thermophilic conditions (over 50 ºC) – again, many publications report a substantial positive impact of such elevated temperatures on many harmful organisms. The potential de-contamination effect of these conditions is increasingly recognised by prescribed treatments based on a minimal temperature-time exposure, with 1 hour / 70 ºC often cited as a bench-mark. The drawback of this criterion is that 70 ºC is at the limit of most biological processes and that even then, some pathogenic organisms can still survive such conditions by the formation of spores or oocysts. Temperatures above 70 ºC are necessary to ensure the reliable elimination or inactivation of many pathogens implying the external supply of some heat within a pasteurizing or sterilizing process. In many cases, the implied energy costs discourage this approach; where sanitization is crucial, the reliance is rather on chemicals despite the potential harmful effect on the wider environment and some doubts on the efficiency of the process. In this review, the application of heat is demonstrated as a reliable process in that it is readily possible to ensure that all material is exposed to the raised temperature based on established heat transfer principles. Furthermore, following subsequent cooling, there remains little long term impact on the effluent other than the inactivation of any pathogenic organisms present. The actual energy requirement is demonstrated as substantially less than a simple thermal calculation might suggest because (a) the starting point can follow a biological treatment that has already raised the effluent temperature to 40 or 50 ºC and (b), 80 to 90 % of the applied energy can be recovered via a heat exchanger. For this example, the net effect would be an implied energy cost of 0.3 to 0.5 euro/tonne of effluent heated to 80 ºC (energy supplied at 0.07 euro per kWh). The availability of biogas from the anaerobic digestion of many effluents could meet this demand to produce an energy-neutral process for all except the most dilute of wastewaters. A relatively simple process can thus be envisaged that can be readily controlled and monitored by virtue of the ease of temperature monitoring. There is no current argument for the wide spread thermal treatment of effluents but a case can be made for its use in a series of important situations requiring a thermally efficient process. The first application is its use to treat contaminated wastes on livestock farms in the event of outbreak of notifiable diseases – this would enable a reduction in the normal consumption of chemical sanitizer used. A second scenario is to enable the reduction of water consumption within the food industry by enabling the safe recycling of sterile process water for specific cleaning duties. Finally, thermal treatment may be applied to liquid animal manures (slurries) to permit their safe land application to certain crops as an organic fertiliser where the health risk would be otherwise be considered to be unacceptable. In this last case, the wider need for environmental protection is met by utilising the nutrient content of the waste which may otherwise end up contributing to water or air pollution.

2354 COMPARISON OF THREE DISTINCT MANAGEMENT STRATEGIES FOR PIG SLURRY APPLIED TO THREE GROUPS OF FARMS

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Poor management of pig slurry can lead to the contamination of the soil, water and air, which is mostly of the result of surplus nutrients. Such environmental impacts from pig farming are common in areas with intensive livestock farming. The project's primary objective is to demonstrate at farm scale the application of the three main manure management technologies deployed within structured local schemes to minimize the environmental impact. This will be achieved by the development of system planning that can produce a strategy to respond to a wide range of different regional scenarios.
This approach will be applied to three selected study areas in the Aragón province of Spain each with different needs resulting with the three examples of manure management scenarios: Tauste (sufficient local crop land - targeted spreading to meet local crop needs), Maestrazgo (insufficient local land but option of transport to farmland in nearby regions) and Peñarroya (insufficient local land – requirement of treatment to remove nutrient excesses). The demonstration scheme in each case will be applied at a realistic and credible scale incorporating more than 30 participating farms in every area. The advantage of working at such a scale is that the true practicalities of the implementation of the three options can be more reliably evaluated – an option rarely possible in most current research.
The implementation of the scheme is based on the creation of local group referred to as Swine Waste Management Enterprises (SWMEs). Each SWME will act as an intermediary between pig farmers and crop land farmers or the treatment plant. To implement the strategy the SWMEs will be supplied with a Global Waste Management Tool (GWMT), a computer software package that will be developed to enable the design and implementation of the most appropriate system.
The paper reports early results on the combined volume of slurry produced by the farms involved in the management scheme, the implementation of the infrastructure and application technique in the three study areas including, control analysis of pig slurry, soil and water samples, ammonia volatilization, as well as the initial steps of the treatment plant.
Experience gathered in this project shows that social acceptance of the central management approach is crucial for its success.

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2223 INFLUENCE OF ORGANIC MATTER TRANSFORMATIONS ON THE BIOAVAILABILITY OF HEAVY METALS IN A SLUDGE BASE COMPOST

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The agricultural use of anaerobically digested sewage sludge (ADSS) as stable, mature compost implies knowing its total content in heavy metals and their bioavailability. Since the chemical form of the metal in the sewage sludge-based compost depends on the effect of stabilization and maturation of the organic material during composting, the objective of this work was to examine the relationships between the changes in the organic matter content and humus fractions, and the bioavailability of heavy metals in a mixture of ADSS and wood chips (70:30 on wet basis) with an initial C/N ratio of 30.4, during its aerobic batch composting at 30 ºC of external temperature in an open type lab-scale reactor without lixiviation. A detailed sampling at 0, 14, 84, and 140 days of the composting process was performed to measure humic (HA) and fulvic FA) acids, and Humin contents, the total content of Zn, Pb, Cu, Ni, Cd, and Hg, and also their distribution into mobile and mobilisable (MB), and low bioavailability (LB) forms, which were determined by a sequential extraction procedure. Significant changes of C contents in HA, FA, and Humin, and in the FA/HA, HA/Humin and Chumus/Ctot ratios were observed during composting. The MB and LB fractions of each metal also varied significantly during composting, with the MB fraction increasing for Zn, Cu, Ni, and Cd, the MB fraction decreasing for Hg, and the LB fraction increasing for Pb. Stepwise linear regressions and quadratic curve estimation conducted on the MB and LB fractions of each metal as dependent on the measured organic variables indicated that for the MB fractions, Zn increased with the %C in FA, Cu and Ni increased with the %C in HA, and Cd increased with both %C in FA and HA. For Hg, the MB forms negatively depended on the %C in FA. For Pb, the variations of the LB forms during composting followed a curvilinear function of the Chumus/Ctotal ratio. Our results suggest that apart from their initial concentration in the compost, the bioavailability of metals at the end of composting depends on the amount, type and relative proportions of humic substances because they influence the metal bonding affinity for organic fractions of different stabilization degree.

2688 SOLIDS AND NUTRIENT REMOVAL FROM FLUSHED SWINE MANURE USING POLYACRYLAMIDES

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Most of the organic nutrient elements (nitrogen and phosphorus) and carbon compounds (COD) in liquid swine manure are contained in fine suspended particles. Flocculation treatment with polyacrylamide (PAM) followed by screening is one the best methods to separate the liquid fraction from the solid fraction in swine manure, and thus to eliminate nutrient elements associated with solids.
In this study, we evaluated the efficiency of two different formulations of synthetic polyacrylamide polymers, one of them is a dry powder and the other is in the physical form of emulsion. After polymer treatment samples were screened through a 300 µm pore size screen and the filtrated were analysed for total suspended solids, volatile suspended solids, chemical oxygen demand and total Kjeldhal nitrogen. Optimum polymers rate were very similar: 40 mg/L for dry form and 37 mg/L for the emulsion form.
Removal rate values for the dry and emulsion polymers are respectively: 96 % and 90% for total suspended solids, 95 to 91% for volatile suspended solids, 80 to 65% for chemical oxygen demand and 44 to 31% for total Kjeldhal nitrogen.

2370 BACTERIAL BIODEGRADATION OF MODIFIED POLYURETHANE FOAMS: COMPARISON OF SINGLE AND MIXED CULTURE

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Polyurethanes (PUR) present widespread group of polymers which are involved in many aspects of modern life. They found use in medical, automotive and industrial fields. Live of modern industrial society is connected with accumulation of bad-degradable polymer waste including PUR materials. This situation leads to necessity to solve problems with negative effects of polymer solid waste on environment. In agreement with recent trends to avoid the accumulation of environmental pollution new modified PUR foams were synthesized. In this work, biodegradability of relatively resistant PUR foams was enhanced using biopolymer components (acetyl-, hydroxyethyl- and carboxymethyl cellulose, acetylated starch, glutein) as modifying agents. The biodegradation of modified PUR with mixed aerobic thermophilic bacterial culture *Thermophillus* sp. and single culture of common soil bacteria *Artrobacter globiformis* were tested. Dynamic cultivation of microorganisms was performed in the minimal media with PUR. Growth characteristics and the chemical oxygen demand were measured in regular intervals. At the end of cultivations PUR mass decreases and surface changes were analysed. In both bacterial systems, PUR biodegradation mechanism probably included two additional effects: the abiotic decomposition of PUR foams followed by consequent utilization of degradation products by the bacterial culture. Higher biodegradation activity was observed in thermophiles. This is probably caused by relationships of individual strains in mixed culture which facilitated utilization of PUR foams fragments. Thermal decomposition of PUR could play important role too. During experiments with limited nutrition factors mixed culture was able to use PUR foam modified by acetylated starch as the only carbon source and foams modified by carboxymethyl cellulose and acetylated starch as the only nitrogen source. *Artrobacter globiformis* was able to use abiotic fragments of PUR foams for growth with substantially less effectiveness as mixed culture. In this single culture PUR foams could not serve as the only carbon or nitrogen source. In conclusion, carboxymethyl cellulose and acetylated starch were found to be the best modification agents. Mixed thermophilic culture exhibited higher biodegradation activity than the single culture.

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2884 HIGH VALUE CO-PRODUCTS FROM WINE BYPRODUCTS (I): DIETARY FIBRE CONCENTRATES WITH ANTIOXIDANT PROPERTIES

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The concept of dietary fibre (DF) is well established and the nutritional benefits (protection against certain types of cancer, regulation of food transit through the digestive system, blood cholesterol lowering) of DF intake are generally accepted. Our DF intake comes primarily from cereal products, fruits and vegetables that are consumed in its traditional presentation. However, foods can also be supplemented with DF. The objective of this study was to evaluate the potential of wine by-products, mainly stems and grape pomace, from selected varieties widely used in the Balearic Islands, as sources of DF. Therefore, plant cell walls, as the main sources of DF, composed of polysaccharides (mainly cellulose, hemicelluloses and pectins) were extracted from those by-products and the main physico-chemical properties of the different DF extracts were analysed.
On dry matter basis, stems and pomace obtained from varieties used for red wine production (Merlot, Tempranillo, Sirah, Manto Negro, Cabernet Sauvignon and Callet) contained between 65 and 82% of DF. Further, stems and pomaces from white grape varieties (Chardonnay, Premsal Blanc and Macabeu) contained similar amounts of DF, ranging from 66 to 77%. Stems cell walls from both types of grape varieties contained around 50-53% of cellulose, 29-32% of pectins and 14-17% of hemicelluloses; whereas grape pomaces contained significantly lower amounts of pectins, ranging from 18 to 21%, and higher amounts of hemicelluloses (31 to 34%). No significant differences were found between red and white grape varieties. Degradation/solubilisation of pectins might have occurred during grape fermentation process. Stems from Cabernet Sauvignon and Merlot varieties contained the largest amounts of pectins, suggesting a larger proportion of soluble material in these DF extracts.
Overall, the results suggest that either stems or grape pomaces could be excellent sources of DF extracts with potential use in the preparation of functional food ingredients and nutraceuticals.

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1818 EVALUATION OF FRUIT AND VEGETABLE WASTE (FV) FROM THE MARKET PLACES: A POTENTIAL SOURCE FOR ANIMAL FEEDING?

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The generation of organic solid waste and its inappropriate management is considered one of the main environmental problems in the world associated with emissions of methane from landfill sites, with emission of dusts, odors and hazardous gases, and with contamination of water. There are different sources for the generation of solid wastes; the market places are considered one of them on a global scale. FV from a market place in Colombia was characterized in order to know its possible use for animal feeding. The characterization included the quantification of waste generated daily, the identification and quantification of the products with major presence in the waste, and its nutritional evaluation during 4 periods, of 7 days each one. Additionally, it was evaluated the differences in its nutritional composition between periods and days of the evaluation. FV were grouped by cluster analysis and by principal components. PRIMCOMP and FASTCLUS programs of the Statistical Analysis Software 2006 (SAS 2006) were used. FV was composed by 43% fruits, 30% vegetables and 27% stems, leaf, leaf wrappers, corncobs, roots, refuse and others. FV was defined in four main groups; groups 1 and 2 represented basic products included in a higher quantity and frequency while groups 2 and 3 represented products changing according different factors. FV had 10% protein, 36.6% FDN, 29.6% FDA, 87.8% ruminal degradability at 24h, 3657 Megacal/kg, 0.59% Ca, and 0.21% P. There were not statistical differences either between days or between periods of evaluation ($p > 0.05$) for the crude protein and for calcium. As for the detergent neutral fiber and for the detergent acid fiber there were statistical significant differences between periods but not between days. The microbiological parameters only increased when the moisture was up of 12%. FV represents a potential source for animal feeding, being necessary its characterization in each market place and in different periods of the year before its use in a great scale.

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1819 EVALUATION OF FRUIT- VEGETABLE WASTES (FV) FROM A MARKET PLACE AS ALTERNATIVE FEED STUFFS IN DAIRY CATTLE DIETS

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Solid food residues from market places represent about 10% to 20% of the total wastes of a city. A big proportion comes from the overproduction of fruits and vegetables in some production steps, its low price and abundance, turning them into potential contaminants, particularly since not always there is an appropriate knowledge and experience in its alternative use. The nutritional value found for FV in a previous work showed that this product might be considered as a potential alternative for animal feeding. However it needs to be demonstrated. The aim of this study was to evaluate the inclusion of FV as a component of concentrated dry food for dairy Holstein cattle and its effect on milk production and quality. FV was included in 6, 8, 12 and 18% of the dry food. A Latin square model 4 x 4 was used to analyze data (4 animal groups, 4 periods, 4 treatments). The differences between averages were analyzed with the Tukey test Statistical analysis were made using the software SAS 2006. No statistical differences were found in milk production; there was a significant effect of the treatment on Milk CLA (Cis 9 trans 11) and omega 3. FV can be used to balance dry food in high production dairy cattle including them between 6 and 18% in the food, as long as the animal requirements are covered, since the quantity of milk production is not altered and its quality improves in terms of Omega 3 and CLA. The main impact of these results is the alternative generated for the improvement of the environment.

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1832 AMMONIA RELEASE AND CONVERSION IN BIOREACTOR LANDFILL SIMULATORS

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Bioreactor landfills are considered to be an improvement to normal sanitary landfills, because the Municipal Solid Waste is stabilised faster and the biogas is produced in a shorter period of time (Valencia et al 2008a, b). In spite of these advantages, it is still difficult to reach –within 30 years– a safe status of the landfill due to the elevated NH_4^+ levels (up to 3 g/L) in the leachate. Nitrogen compounds are extremely important when defining the closure of landfill sites, due to their potential to pollute aquatic environments and the atmosphere. Nevertheless, limited information is known on NH_4^+ evolution and on in-situ removal methods.
This research paper presents the results of a series of experiments carried out to determine the evolution and fate of NH_4^+ in bioreactor landfill simulators. Experiments were carried out with different reactor sizes: from bench (1 L) to pilot scale (800 L). The effect of environmental conditions (temperature, pH, fresh versus old waste) on the release of NH_4^+, with special focus on the first 24 hours, was assessed.
The NH_4^+ release was compared to the release of other compounds (NO_2^-, NO_3^- SO_4^{2-}, PO_4^{3-}, Cl^-, heavy metals) and other parameters (TOC, conductivity) in the liquid phase. The different mechanisms (physical, chemical, biological) of ammonium release from the solid into the liquid phase are discussed. The NH_4^+ level in the liquid phase of the simulators starts decreasing after some time. This is in contrast with real-scale observations, where the NH_4^+ level stays constant. Based on the absence of oxygen in the simulators, the detectable levels of hydrazin and the presence of anammox bacteria, it is likely that anammox is actively involved in the conversion of NH_4^+ into N_2.
In conclusion, nitrogen release is governed by physical and biological mechanisms and anammox bacteria are serious candidates to contribute in the nitrogen removal process in bioreactor landfills. These results, when combined with carbon removal and improved hydraulics, will accelerate the achievement of environmental and economical sustainability in the landfilling of MSW.

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2147 INTERACTION OF ORGANIC CONTAMINANT WITH NATURAL CLAY TYPE GEOSORBENTS: POTENTIAL USE AS GEOLOGIC BARRIER IN URBAN LANDFILL.

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The great amount of municipal solid wastes generated by the cities can be processed in different ways such as incineration, derivation to composting plants or, simply, deposition in controlled landfills. One of the landfill characteristics is possess an adequate geological barrier for contaminant contention. The most important chemical processes affecting the behaviour and bioavailability of pollutants in soils are those concerned with the sorption from liquid phase (leachates) onto solid phase (clay). The aim of this work is to characterize the capability of several clay materials as preservative of organic pollution. For this purpose, we study the interaction of representative organic pollutants with different polarity and water solubility (toluene, atrazine, biphenyl, benzamide and paraquat) with several clay materials to establish the critical parameters to guarantee that the material is functional as geologic barrier in the design of the landfills multicarrier.
Five types of Spanish clay were selected with two premises: low cost and availability. These selected clays, were mineralogical and physicochemical characterized (X-ray diffraction, total organic and inorganic carbon, specific surface, etc) to fulfil the parameters established by Spanish and European Normative. To simulate typical conditions, a synthetic leachate was made with a high ionic strength and pH 5. These conditions correspond to young urban landfills and dangerous landfills. The clays were maintained in contact with synthetic leachates, containing the pollutants, in batch experiment. The obtained data of the analytes sorption were modelized by several sorption isotherm models. The best fitted data were got with Langmuir adsorption isotherm. All results confirm that all assayed clays fulfill the parameters established by Spanish and European Norms (mineralogical composition, structural stability, hydraulic conductivity, porosity, etc) and present excellent behaviour for organic retention. Therefore these clays could be used as components of the multicarrier in controlled urban landfills.

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2378 GEOPHYSICAL TECHNIQUE AND GROUNDWATER MONITORING IN THE SUROUNDING AREA OF A SANITARY LANDFILL – LONDRINA (PR – BRAZIL)

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The use of non-invasive techniques such as surface geophysics usually allows a preliminary mapping providing information that will guide the use of invasive techniques of soil investigation. Surface geophysics also helps the location of unconfined aquifer and monitoring wells (Mondelli 2004). However is necessary to point out that results obtained through surface geophysics are not conclusive, and their combination with data from monitoring wells is indicated in order to identify or confirm the behavior of the underground resistivity, which in turn may change according to some conditions such as: intrinsic porosity of rock and sediment, air and aqueous fluid content and variations of the groundwater chemical composition (Veja et al. 2003; Mota et al. 2004).
The present study was developed to define leachate plume using both techniques: geophysical and groundwater sampling in order to evaluate water contamination. After topographic survey and using geophysical - electroresistivity technique, the leachate plume was defined. Six underground soil profiles and five levels of investigation were defined. With those data it was located eleven sampling wells for groundwater monitoring. Determinations of: Chemical Oxygen Demand – COD, Biochemical Oxygen Demand – BOD, pH, turbidity alkalinity, condutivity, total Nitrogen and heavy metals are presented for surface and groundwater.
The geophysical technique of indirect prospection, allowed the determination of the leachate contamination plume and location of monitoring wells. The values of COD and BOD are high if compared to the values established by Brazilian environment regulations. Nitrogen concentrations were above drinking water standards wich indicates contamination of groundwater and surface water by organic matter, probably originating from the leachate. The thick layer of local soil had a filter effect for heavy metals, since groundwater did not present concentrations of heavy metals above established standards.

2506 AN INTEGRATED APPROACH TO NATURAL AND GEOSYNTHETIC CLAY BARRIERS PERFORMANCE AGAINST THE DIFFUSION OF A POLLUTION PLUME FROM MUNICIPAL SOLID WASTE (MSW) LANDFILLS

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Technical requirements for the landfills of municipal wastes in the European Union (EU) are given in the Council Directive 1999/31/EC. A geological barrier of at least 1m thickness with a hydraulic conductivity (HC) of $1x10^{-9}$ m/s is required. Where the geological barrier does not naturally present the above conditions, a geological barrier of at least 0.5 m thick must be artificially established. In addition, some geosynthetic clay materials, geobentonites, should be included as substitutes for natural clay barriers. The main objective of this research is to study the diffusion of the pollution plume (dissolved organic carbon (DOC) and heavy metals (HM) in an ammonium-sodium chloride pH 6-8 environment, affected by changing REDOX conditions), in both natural and geosynthetic clays. Typically, studies on this field are focused on natural attenuation of DOC plume in the watershed, usually without explicit mention of geochemical reactions and surface clay properties. The scope of the work is to achieve the calibration of several transport and reaction geochemical codes in order to predict contaminant transport throughout clay liners. These models are being feed by a three-fold experimental data: (1), micro-scale interaction and diffusion experiments performed on different natural Spanish clays (kaolinite or illite based with or without carbonates); (2), 1:1 UE Directive demonstration experiments (0.5 m thick clay barrier, 1-4 years synthetic pollution plume diffusion); and (3) analysis of drills performed in old landfills selected from locations without artificially implemented clay barriers or polyethylene membranes. Currently, a relevant process has been detected in relation to diffusion of salts (mainly chlorides), as far as their migration in response to concentration gradients is more effective than a pure advective permeability ruled transport. The study of the influence of this salt pulse on the transport of other constituents (i.e. HM) is important if we note that the unique landfill technical regulation affects just permeability requirements.

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2173 DESORPTION OF ORGANOPHOSPHOROUS PESTICIDES FROM SOIL WITH WASTEWATER AND SURFACTANT SOLUTIONS.

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Surfactants can be introduced in the environment by wastewater discharge, point-charge pollution or deliberate action, e.g. to remediate contaminated soil or groundwater. The irrigation of soil with wastewater containing surfactants may modify pesticide desorption from soil, thus affecting their environmental fate. Desorption from soil of the plain of Granada (South-eastern Spain) of two organophosphorous pesticides, diazinon and dimethoate, differing in solubility and hydrophobicity, has been evaluated in the presence of different surfactant aqueous solutions and municipal wastewater. Soil was added with both insecticides and aged during two weeks. Batch experiments were performed for seven surfactants: a cationic (HDTMA), three anionic (Aerosol 22, Biopower and SDS), and three non ionic (Tween 80, Triton X-100 and Glucopone 600), municipal wastewater (MWW) from a secondary treatment (Granada municipal plant) and MilliQ water as a control. Besides, non ionic/anionic mixed-surfactants solutions were assayed. The insecticides were extracted from the aqueous solutions using microwave assisted extraction and analysed by gas chromatography. For any given solution, the greater desorption rates corresponded to the most polar insecticide, dimethoate. Single surfactant solutions affected insecticide desorption mainly depending on the surfactant type. When compared with MilliQ water the cationic surfactant enhanced both insecticides retention on soil, while the anionic and non ionic ones increased desorption rates for the most polar insecticide, dimethoate, decreasing those for diazinon. Additionally, the maximum percentage of insecticide extracted to the aqueous phase for non ionic and anionic surfactants was determined through linear regression. Non ionic/anionic mixed-surfactant solutions assayed, at concentrations including those normally found in wastewater, enhanced desorption rates for dimethoate. Desorption performed with MWW did not largely modify the desorption rate for both pesticides with regard to control water and for the assayed soil:solution ratio. The data obtained will require further consideration before establishing the factors responsible for this behaviour, since MWW constitutes a complex matrix, which can induce several changes in soil solution apart from those concerning surfactant content. These results provide useful information to assess surfactant effects on pesticides desorption rates.

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2424 THE BIOTESTING OF OIL-OXIDIZING BACTERIA AND FUNGI ASSOCIATIONS FOR THE CERTIFICATION OF NEW BIOABSORBENTS AND WATER REMEDIATION CONTROL

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Application of various types of biopreparations possessing high adsorbing capacity in relation to oil-products is now widely used for handling of spilled oil. The control of non-toxicity of cultures and adsorbing materials is necessary for elaboration of bioaugmentation methods which are perspective for effective oil utilization. There are several biotest-systems used in the Ecotoxicological laboratory (*www.letap.ru*), which are officially recommended for natural ecosystems and waste biotic control in Russia. Two types of microbe combinations and fresh and sea oil polluted water were investigated in the laboratory experiments for purpose of certification of new methods. The study was carried out to test the toxicity of oil bioabsorbent with immobilized cultures of microorganisms (*Pseudomonas* sp., *Cellulomonas* sp., *Zoogloea* sp *Arthrobacter* sp., *Fusarium lateritium, Gliocladium deliquesce*, 10^4 - 10^5 KOE/g) using freshwater test-organisms *Daphnia magna, Scenedesmus quadricauda Paramecium caudatum*. The results obtained showed that samples of such kind oil biosorbent don't reveal any toxic properties. The cleaning effect of applying biosorbent on oil polluted surface fresh water after 30 days from initial 15 percent concentration was also verified using these test-systems. The remediation effect natural indigenous bacterial associations were investigated at the laboratory model at the high level of aeration in oil-polluted see-water of Baltic Sea. The water was collected previously from Baltic Sea near Svetlogorsk city. The following species were identified from the sea water: *Arthrobacter, Bacillus, Micrococcus, Nocardia, Rhodococcus, Corynebacterium; Pseudomonas, Cytophaga, Spirillum, Alteromonas, Myxococcus*. For preparing of biopreparation "Marine Snow" the enriched bacterial culture were accumulated and used for immobilization at the mineral porous absorbent, fungi mycelium and cotton floating material. The remediation effect by own sea bacterial associations and the bioabsorbent "Marine Snow" was controlled using of two species of tests-organisms which belong to salt biotope inhabitants - *Artemia salina* and marine algae *Chlorella minutissima*. Cells of Ch. minutissima were exposed in enriched bacterial cultures without dilution, *A. salina* – in suspension of diluted cultures (1:2, 1:10, 1:10^2, 1:10^3, 1:10^4). The survival of *A. salina* and growth reduction of algae cell population were compared at the following experiments: (i) intact Baltic Sea water (control), (ii) the sea water with 2.5% of oil, and (iii) enriched culture prepared at the sea water with 2.5% of oil. It was investigated that the growth of *Ch. minutissima* was even stimulated both at (ii) and (iii) variants of experiment. *A. salina* was more sensitive than the algae. The acute lethality LC_{50} of oil pollution sea water on *A. salina* induced by enriched culture as well by it's twofold dilution. But these high concentrations of bacterioplankton can exist only at the laboratory vessel and are never observed at the natural conditions. So, the inhibiting effect of 10^{-2}, 10^{-3}, and 10^{-4} diluted enriched culture was absent. The samples of the biosorbent "Marine Snow" don't reveal any toxic properties and it belongs to the V class of toxicity (according to Russian Environmental Standard Criteria of Toxicity., # 511). Thus it was shown that natural indigenous associations of hydrocarbon-oxidizing bacteria and the biopreparation at it's basis are effective remediation agents for oil-polluted water without toxic effect on the phytoplankton and zooplankton organisms.

2545 PHYTOBIOPESTICIDE: AN IMMUNORESTORER OF THE IMMUNOSUPPRESSED RESPONSE BY MALATHION. AN EXPERIMENTAL STUDY

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Recent advances in research of Environment, Biotechnology and health effects have led to the replacement of the chemicals with the biological materials. The increased use of chemical pesticides over the years in the past few decades and studies in this domain show that the chemical pesticides badly effect the various systems of the body but the literature reveals that only a few studies have been made to see the impact of chemical pesticides in vivo on the immune response. The present study was conducted experimentally in the swiss albino mice to see the effect of chemical pesticides and various biopesticidal plant extracts on the immune response and further based upon the results that the chemical pesticides are immunosuppressive, the study was extended to find out the immunorestorative effect of the best immunomodulatory biopesticidal plant extract i.e. aqueous leaves extract of Nyctanthes arbor tristis as immunorestorer of the suppressed immune response by malathion (Most immunosuppressive of the three chemical pesticides used). Our results revealed that biopesticidal plant extracts not only act as immunomodulators but also antagonize the suppressive immune response by the pesticide.It is suggested that a combined bio and chemical pesticides should be used to have the best pesticidal effect without effecting the health.

2585 SOIL STRUCTURE AND ORGANIC CARBON RELATIONSHIPS FOLLOWING 3 AND 7 YEARS OF CROP RESIDUE MANAGEMENT IN NO-TILL IN SEMIARID AREA: TUNISIA

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In Tunisia low and erratic annual rainfall together with the intensive agricultural use of soils have led to a depletion of soil organic matter (SOM) with increases in the degradation of their chemical, biological and physical fertility. In order to develop efficient soil management practices for rapid restoration of these seriously degraded soils, direct sowing mulch-based cropping systems have been adopted. This study was carried out to evaluate the effect of no-till management on the soil organic carbon (SOC) concentration and physical properties for the 0- to 50-cm soil depth. The field experiment, conducted in a semiarid area in north Tunisia, consisted of three treatments: conventional tillage (CT), 3 years no-tillage (NT3) within a wheat / broad bean rotation (1/1 year), and 7 years no-tillage (NT7) within a wheat /Sulla rotation (1/2 year). The restitutions are of 500 kg/ha of wheat, 700 kg/ha of sulla and 450 kg/ha of broad bean. This study also quantified relationships between soil properties and crop residue-induced changes in SOC concentration. Soil structural stability was determined by the Henin and Monnier method. Soil bulk density was determined by using the core method. Saturated hydraulic conductivity was estimated by percolation through a ground sample altered under a head of constant water. The total C and N contents of whole soil were determined by pyrolyse at 950°C with Carmhograph, and Kjeldhal procedure, respectively. Application of no-till management significantly increased levels of SOC for the first 30 centimetres. The highest contents are obtained with NT7 (1.8% from 0 to 10 cm, 1.4% from 10 to 20 cm and 1% from 20 to 30cm of depths). Below 30 cm, differences in SOC pool between mulched and unmulched soil were not significant. Within the upper 0 - 30cm soil depth, increased SOC content of the soil in turn improved its aggregation status, infiltration rate and decreased the bulk density.
The relationship between SOC and physical proprieties of soils shown a significant interaction between the bulk density and the instability index (log 10Is) of aggregates <2 mm and SOC. A good correlation is shown under NT3 soil with (R^2 = 0,97) for bulk density and (R^2 = 0,99) for soil structural stability. The relationship between the organic matter and hydraulic conductivity was more important under NT7 soil. Overall, the crop residue mulching increased SOC concentration and improved near-surface aggregate properties which depend of quality of the restitutions more then the period of no-tillage.

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2192 EVALUATION OF PHYTO-BIOREMEDIATION TECHNIQUES APPLIED TO DREDGED MARINE SEDIMENTS FOR THEIR REUSE AS TECHNO-SOILS

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Phytoremediation can improve metal-contaminated sites by the extraction (phytoextraction) and/or immobilisation (phytostabilisation) of contaminating metals. Phytoextraction has emerged as a novel approach to clean up metal-polluted soils: plants are used to transfer toxic metals from soils to shoots. However, for most trace elements, this technique needs significant improvements to become practically feasible. On the other hand phytostabilisation uses transpiration and root-growth of plants to immobilise contaminants in the soils by reducing leaching, controlling erosion, creating an aerobic environment in the root-zone and adding organic matter to the substrate that binds metals. However, phytostabilisation requires that the site be permanently vegetated, thus limiting future land use options. A multidisciplinary approach is necessary to make these techniques commercially feasible for applications to metal-polluted soils and sediments. Although never used until now in significant real-scale applications, the phyto-bioremediation techniques find a possible field of application also in the treatment of marine dredged sediments, where some extra problems should be solved as the presence of high salinity and the need to adapt the marine characteristics to the land environment. In this study we focused on the phyto-bio remediation technique applied to slightly contaminated marine sediments with the aim to reach a physical, chemical and biological amelioration of the solid matrix to be reused as a techno-soil. Sediments were dredged from the port of Livorno (central Italy) and found to be contaminated by low levels of heavy metals and hydrocarbons. The dredged sediments were pre-conditioned to obtain a substrate suitable for plant growth. The sediment was conditioned by mixing it with excavating calcareous sands (5:1 w/w) and green compost on the top. We also analyzed the mutual interaction between plants species (*Paspalum vaginatum* and *Tamarix gallica*), microorganisms in the rizosphere and other organisms, such as earthworms (*Eisenia foetida*), that were found to recreate conditions similar to a natural soil. This technique allowed plants to grow in a fine textured and salty medium, otherwise not suitable for plant development. Results after one year of experimentation showed that the presence of microorganisms in the rizosphere and earthworms produced a reduction of about 50% of the initial total hydrocarbons content. The use of a natural chelator with phyto-hormonal properties (humic substances - HS) showed the capability to form stable complexes especially with Pb and Ni, and their contribution as a green agronomic fertilizer. The matched effect of the *P. vaginatum* and *T. gallica* in the extraction of heavy metals from the sediment showed promising percentages of metal effectively extracted in comparison with its available fraction (e.g. available Ni = 6.2%; Ni extracted = 15.7%). Plants species and especially *T. gallica*, had shown good behaviour in the extraction of salt from the sediment (more than 60%), one of the most problems connected to the re-use of sediments for agronomic purposes. The study proposed and the results reached represent a suitable alternative option for the final disposal of the great amount of dredged sediments from rivers and marine harbours, since it can be reused for various purposes, such as agriculture, reforestation, reclamation of abandoned areas, etc.

2485 INCENTIVES AND MARKET DEVELOPMENT TO ESTABLISH SUSTAINABLE BIOMASS SYSTEMS

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Business-as-usual is not acceptable when it comes to the future for biomass-to-energy/product conversion industry. Incentives and market development need to be applied to guide the owners and operators towards the sustainable practices. Sustainability for biomass is defined to be future energy fuels and bioproducts that are secure, renewable, and accessible locally, affordable, and pollution free. Incentives are required to convert biomass-to-energy/product conversion systems that are not sustainable into sustainable formats. This paper reviews existing incentives and proposes new incentives that are to be imbedded in the biomass industries' selection and conversion of feedstocks. Market development is the design/implementation of new ways of providing goods and rendering the services. Designing the market for biomass involves the resource assessment of feedstock and the opportunity for sale of fuels which can be then be converted into electrical energy, heat, mechanical energy, along with specific products such as straw bales for construction or plant fiber for potting nursery plants. This paper assesses applicable laws and regulations, and financial climate while forecasting technological change. It develops a market for biomass using life cycle assessment of the biomass conversion system, while attracting investors with competitive pricing of biomass power and product. Market development is approached with a degree of caution for the biomass industry may not automatically provide the right type or quantity of energy or products. The desired outcome is a biomass conversion industry that is sustainable.

2158 POTENTIAL OF ORGANIC BIOPROCESSED MATERIALS FOR BIOREMEDIATION (BIOSTIMULATION) OF CHLORPYRIFOS CONTAMINATED SOIL FOR FOOD SAFETY

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Chlorpyrifos (CPF), a well-known neurotoxic agent, causes inhibition of some important enzymes such as acetylcholinesterase, ATPase, cholesterol ester-hydrolase. It can pass through the placenta in to the body of fetus and target more on developing brains and new born. Its metabolites are detected in the air, in amniotic fluid and in infant meconium. The inherent persistency of CPF may ensure long-term pest control, but enhances the risk of uptake (by edible plants) and subsequent bioaccumulation. Hence, remediation of CPF-contaminated soils is an important environmental concern. Objective: Main aim of study was to assess the impact of organic bioprocessed materials (BPMs) on CPF dissipation (10 ppm) in soil under controlled (37 ºC) & ambient conditions. Methodology: At first efficacy of the following four BPMs (1%): vermicompost (VC), farmyard manure (FM), biogas slurry (BS), mushroom spent compost (MS) in comparison to control (no BPM) for dissipation of CPF was investigated. Further, pot experiments were carried out with best performing BPM (VC) in ambient environment, and the effect of different doses (1%, 3% and 5%) was tested. The effect of CPF and different BPMs on microbial activity was estimated in terms of dehydrogenase activity (DHA) (Friedel *et al.*, 1994). Residual CPF in soil was extracted by column method and analyzed by GC-ECD. Uncontaminated soil showed almost double microbial activity as compared to CPF contaminated soil. When different BPM amendments (1%) were done to the contaminated soil, higher DHA was reported in all the treatments (BS, FM, MS and VC) as compared to the control, highest microbial activities were shown in VC and MS amendments. Regarding pesticide dissipation, only marginal difference was observed under the controlled (37 ºC) environment as all the BPMs stimulated 90-93% CPF dissipation. However, VC showed the highest dissipation. With BPM amendments under controlled condition, there is definitely a significant enhancement in microbial activity but that is not so reflected in CPF dissipation. Hence it is clear that apart from microbial activity, abiotic factors also play very significant role in CPF dissipation. Further, interesting trends emerged when data from different experiments under control and ambient conditions were compared. Although under controlled conditions, significant difference between amended (1% VC) and unamended soils was not observed, a clear superiority of the amended soil for pesticide dissipation was seen under ambient conditions. Faster (89%) dissipation was achieved on 24th day under control conditions with 1% VC, whereas the same was achieved on 38th day in ambient environment and this was not achieved even after 50th day in control (unamended) soil. When dose of applied VC amendments was increased to 1, 3 and 5%, there was relative enhancement (4.6, 11.1 and 22.2 %, respectively) in pesticide dissipation as compared to unamended soil even on 12th day. The experimental data clearly reveal the significance of VC amendments for CPF dissipation under ambient i.e. field conditions. It is interesting to note that although addition of organic manure has been an integral part of sustainable agriculture practices; the present findings give a new dimension of it's utilization for removal of persistent pesticides from soil leading to enhanced food safety. Present study clearly reveals that biostimulation through bioprocessed materials has a good potential as a cost-effective pragmatic tool for CPF dissipation in the sandy loamy soil.

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1888 ALMOND TREE FOR SOIL QUALITY IMPROVEMENT IN SOUTHERN ITALY

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Soil degradation is one of the most important environmental problems worldwide recognized. The Mediterranean region, characterized by long dry periods followed by heavy bursts of rainfall, is particularly prone to soil erosion. The main goal of this study is to demonstrate the efficacy and suitability of the cultivation of almond trees as an environmental approach to improve soil organic carbon and biological conditions in order to mitigate risks of soil degradation due to topographic, climatic, and unproper farming managements. The selected study area was located in Basilicata Region (South of Italy) in the "Pantanello farm". The orchard fields have been set up in March 2006 and considered two farming systems (traditional and organic amendment) and three slope gradients (0%, 2%, 6%). To evaluate the only effect of the plants, the soil in the interrows was also collected. Soil samples were taken at three levels along each slope: high, medium and low, at the surface layer 0-15 cm. After one year from the beginning of the experiments the results showed a higher stimulation of the enzyme activities involved in C (β-glucosidase), P (phosphatase) and N (protease and urease) cycles. The higher stimulation of these enzyme activities was often observed in the presence of plants and in 0 and 2% slopes, suggesting better conditions for soil microorganisms in carrying out their metabolic activity. In addition, the planted soil samples showed high content of total organic carbon, water soluble carbon, and humic acids, while low enzyme activity and humic carbon content characterized the interrow unplanted soil samples. In the 6% slope and without plants, soil metabolism was reduced and this seemed to be related to a reduction in the nutrient cycling and organic carbon metaboilsm, meaning that carbon sequestration has not yet taken place after one year.

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2377 ISOLATION, IDENTIFICATION AND SCREENING OF ECTOMYCORRHIZAL FUNGI FOR REFORESTATION PURPOSES

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Pinus pinaster occupies almost 30% of Portuguese forest area and fire is one of its major threats. *Pinus pinaster* is resistant to low fire intensities, however, the frequency and intensity of the current fire regime causes a disturbing reduction of its area of distribution. Ectomycorrhizal fungi can form symbiotic associations with *P. pinaster* improving, amongst other factors, plant growth and resistance to biotic and abiotic stresses, which can be a useful tool for an efficient reforestation of burned areas. The aim of this work was to isolate and identify native fungi from Northern Portugal and to study their compatibility with *P. pinaster*. Carpophores were collected in Mogadouro and Porto and isolated in two different media, PDA and MMN. For their identification, extraction of DNA was performed and the ITS region was amplified by PCR with the fungal specific primer pair ITS1F/ ITS4. Sequencing showed that 12 species belonging to 9 genera were obtained: *Suillus, Pisolithus, Scleroderma, Lactarius, Amanita, Rhizopogon, Hypholoma, Leccinum* and *Tricholoma*. The isolated fungi were screened for compatibility with *P. pinaster*. Seedlings from P. pinaster were inoculated with individual isolated fungi in a mixture of peat and vermiculite. These tests were performed on P. pinaster plus, which are trees carefully selected taking into account parameters such as higher growth rate, increased wood quality and better log morphology. Results showed fungal growth and formation of mycorrhizal structures. Selected native ectomycorrhizal fungi can be a valuable biotechnological tool for reforestation of soils with fire history.

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2242 MEASURING BIODEGRADATION OF OIL PRODUCTS BY MEANS OF ENVIRONMENTAL FORENSIC METHODS

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Bioremediation technologies are focused to the biodegradation of organic pollutants. This approach is particularly helpful when soils and/or groundwater are affected by oil or oil products spills, given the satisfactory biodegradability of most hydrocarbons. However, during a “bio-treatment” the decreasing in pollutants concentration may be due to both biotic and abiotic processes, whose distinction is very important, albeit difficult, in order to evaluate if bioremediation is being properly applied. Within the available analytical possibilities helpful to measure biodegradation (isotopes, respirometry, microbial growth, etc.), the use of “non-biodegradable” compounds as a standard reference presents some advantages, specifically if we are dealing with a mixture of contaminants as happens with oil and its derivatives. In this sense, chemical-fingerprinting of source-characteristic and environmentally persistent biomarkers generates information in terms of determining the source of the spilled product, monitoring the weathering state of hydrocarbons under a wide variety of conditions, and specifically their biodegradation. For instance, in crude oil spills, hopane normalization has been reported as the best method to quantify hydrocarbon biodegradation, specifically the use of 17α (H), 21β (H) hopane. Using this or other compound as an internal conserved standard, it is possible to obtain biotransformation indices, defined as percentages of degradation for the different families of hydrocarbons. In this work, a set of diverse cases of biodegradation monitoring using chemical biomarkers after GC-MS analysis is presented. This includes examples taken from sites affected by marine oil spills, industrial spills, leaks/spillages from underground storage tanks, and also microcosm’s studies previous to scale-up bioremediation. Consequently, not only examples of crude oil spills, but also others including light derivatives such as gasoline, kerosene or gasoil will be described.

2496 SLURRY BIOREACTORS WITH SIMULTANEOUS ELECTRON ACCEPTORS FOR BIO REMEDIATION OF AN AGRICULTURAL SOIL POLLUTED WITH LINDANE

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Lindane or γ-hexachloro-cyclohexane (HCH) is a toxic and recalcitrant chlorinated insecticide widely used in developing countries, particularly in Mexico. So far, most applications of SB have been focused on aerobic operation. More recently, a few promising lab scale applications of anaerobic or anoxic SB for removal of lindane have been described. However, little is known on the operation of SB with simultaneous electron acceptors (also known as SB with combined environments), particularly partially-aerated methanogenic (PAM) and methanogenic-sulfate reducing (M/SR) SB. Given the success of bioreactors with simultaneous electron acceptors in wastewater treatment, we hypothetized that this approach could be also beneficial in SB operation. So, the purpose of our work was to evaluate the effect of the type of combined environments SBs (PAM and M/SR) and supplementation of degradable organic co-substrate sucrose on lindane removal from a clayish agricultural soil with high levels of organic matter. A simple 2 x 2 factorial experiment was carried out with two types of 'combined environments'(simultaneous electron acceptors, i.e., PAM and M/SR) and factor initial sucrose concentration at 0 a 1 g/L. Initial lindane concentration in soil was 100 mg/kg dry soil. Average removals of lindane in SBs without sucrose followed the order M/SR> PAM (34 and 10%, respectively). On the other hand, the order of lindane removals in SBs with supplementation of sucrose was M/SR ~ PAM (20 and 16%, respectively). All overall removals of lindane were poor. Performances of SB with combined environments were significantly poorer (half or lower lindane removals) than those of SB with predominant environments, either aerobic or sulfate-reducing, that were recently reported (this conference). However, predominant methanogenic SBs showed a very low removal (ca. 43% average). Since both types of combined environments SB (PAM and M/SR) included the methanogenic pathway, one may speculate that this feature was associated with their poor performance. Non-bioaugmented control PAM and M/SR SBs that relied only on the native soil microflora showed lindane removals very close to bioaugmented units (15% and 23% for non-biaugmented PAM and M/SR, respectively). Methanogenic lindane-clastic bacterial counts were ca. 5 as log CFU for PAM and M/SR independently of sucrose or no sucrose addition. Sulfate reducing lindane-clastic bacterial counts were ca. 5 as log CFU for M/SR independently of sucrose or no sucrose addition. Aerobic lindane-clastic bacterial counts decreased from 5 to 4 (log CFU) at the end of 30-day operation in the PAM. It can be concluded that SBs with combined environments, where one of the anaerobic metabolism is methanogenic, were not as successful as SBs with one environment for removal of lindane from a heavy soil.

WEDNESDAY, 24 SEPTEMBER

ORAL COMMUNICATION

II.- Wastewater and solid waste management, treatment and reuse
III.-Strategies for the protection and remediation of natural environments
IV.-Microbial Ecology
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WEDNESDAY 24 SEPTEMBER 2008 ORAL COMMUNICATION

V ENVIRONMENTAL EDUCATION AND LEGISLATIVE ASPECT

VI NEW PERSPECTIVES

2528 EFFECT OF COMMUNITIES OF AMMONIA-OXIDIZING BACTERIA ON DEGRADATION OF 17-ALPHA-ETHYNYLESTRADIOL BY NITRIFYING ACTIVATED SLUDGE

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An endocrine disrupting compound, 17-alpha-ethynylestradiol (EE2), is a synthetic estrogen used as a key ingredient in oral contraceptives pill. This persistent organic pollutant, non biodegradable by most microorganisms, is discharged via municipal waste streams to natural receiving waters. Recently, it was found that ammonia-oxidizing bacteria (AOB) in nitrifying activated sludge (NAS) enriched with high ammonium loads can degrade EE2 via co-metabolism during ammonia oxidation. Nevertheless, the question arises about whether AOB in municipal wastewater treatment systems (WWTSs) that receive much lower ammonium loads than in the previous studies can co-metabolize EE2 or, if so, have the same co-metabolism patterns. Hence, this study aimed to investigate the degradation patterns of EE2 by NAS containing different AOB communities. To do so, the sludge taken from a municipal WWTS was enriched in three continuous-flow reactors receiving inorganic medium containing different ammonium concentrations of 2, 10 and 30 mM (NAS-2, NAS-10, and NAS-30, respectively). Communities of AOB in NASs were analyzed using specific PCR application followed by DGGE, and sequencing of 16S rRNA genes. AOB communities were found to differ among the NASs depending on the ammonium loads supplied. AOB with high affinity to ammonia dominated in the NAS-2 (*Nitrosomonas oligotropha* cluster and *N. cryotolerans* cluster) and NAS-10 (Unknown *Nitrosomonas* cluster). AOB with low affinity to ammonia were predominant in the NAS-30 (*Nitrosomonas europaea – Nitrosococcus mobilis* cluster). All NASs were tested to co-metabolize EE2 (10 mg/l) under varying initial ammonium concentrations of 2, 10 and 30 mM. EE2 can be degraded by all NASs under all initial ammonium concentrations via co-metabolism of AOB (confirmed by allythiourea addition). The ratios of EE2 degraded per ammonium oxidized at the initial ammonium concentrations of 2, 10, and 30 mM were 31.02, 14.75, and 8.06 mg EE2/g NH_4^+ for NAS-2, 29.80, 15.56, and 9.80 mg EE2/g NH_4^+ for NAS-10, and 106.64, 64.78, and 118.13 mg EE2/g NH_4^+ for NAS-30. These suggested that the degradation patterns of NASs which dominated by AOB with high affinity to ammonia (NAS-2 and NAS-10) were different significantly from that predominated by AOB with low affinity to ammonia (NAS-30). This may cause by the differences in enzyme induction, enzyme expression, or enzyme activity among AOB species which need further clarification. In addition, initial ammonium concentrations affected the degradation of EE2. The higher the initial ammonium concentration, the more EE2 can be degraded. However, the amounts of ammonia oxidized and EE2 degraded were not increased proportionally. In conclusion, NASs containing distinct AOB communities have different patterns for EE2 degradation.

2561 MOLECULAR IDENTIFICATION AND PHYSIOLOGICAL CHARACTERIZATION OF THE VINYL ACETATE ELIMINATION PROCESS IN AN AXENIC AEROBIC CULTURE ISOLATED FROM AN AEROBIC-METHANOGENIC SYSTEM.

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The vinyl acetate (VA) is a chemical compound, commonly used in the elaboration of a wide variety of commercial products as paints, adhesives, paper, etc. Because of that, it is founded as contaminant of residual waste water of industries that used it, representing a big risk to aquatic life and human health. The physical-chemical treatment of the waste waters that contain this compound, doesn´t eliminate the problem and only produce more gaseous emission into the environment. It has been achieved the biological elimination of VA in residual waste water of a polymeric resin industry, with an aerobic-methanogenic system. In this work, under aerobic conditions a VA consumer bacterium was isolated from an aerobic-methanogenic system, by consecutive enrichments with VA and sowing in Petri dishes. The obtained axenic culture was characterized by: a) Microbiological studies: nutritional requirements by growing measure with different growth factors, colonial and microscopic morphology, by Gram staining and electronic microscopy respectively. b) Physiological studies: evaluating VA consumption velocity (qAV), Vmax and Ks for the respiratory elimination process, inoculum and axenic culture. c) Identification of axenic culture by 16S rDNA analysis: with DNA extraction, amplification and sequencing of this gene. The axenic culture obtained, shows simples nutritional requirements, a ramificated, flat and white colonial morphology, bacillus with round ends, Gram negative, mobile by peripheral flagells, with a capsule and growth velocity of 0.045 d^{-1}. Kinetic parameters obtained were: a) Inoculum: qAV = 2.38 ± 0.3 mg/L.h, Vmax = 13.95 mg/L.h and Ks = 457.1 mg/L; b) Axenic culture: qAV = 421.9 mg/L, Vmax = 14.29 mg/L.h and Ks = 3.12 mg/L.h. Because of the 16S rDNA sequence, the axenic culture was identificated with 99.3% of similarity as *Brevibacillus agri*. The results of this investigation, shows that oxygen addition to a methanogenic reactor, promotes the participation of an aerobic bacterium identificated as *Brevibacillus agri* in the elimination respiratory process of VA.

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GUEST LECTURE

1879 NATURAL ATTENUATION OF HYDROCARBON POLLUTED SOILS IN MEXICO

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Crude oil and hydrocarbon by-products are the most common pollutants in Mexico. In the last years, the two terms, contamination and remediation have being re-defined; also, based on both the scientific advancement and the human risk, the sustentability of remediation technologies and the definition of cleaning levels has been taking place. In this context, the Natural Attenuation of soils is a viable and low cost remediation choice, defined as the degradation of organic compounds without artificial stimulation, through microbial activity including physical processes, such as volatilization, dilution, sorption, and hydraulic dispersion. This work intends to describe the results from the monitoring of natural attenuation in two sites contaminated with petroleum hydrocarbons. This study consider three years of monitoring. Paredon 31 and Campo 10 were the two sites studied. For each site, 6 monitoring wells were set and 15 sampling locations were chosen, each one having three samples from variable depth. Physicochemical and biological parameters measured to soil and water included pH, soil texture, TPH´s, PAH's, heavy metals, conductivity , aerobic and anaerobic, both heterotrophic and oil degrading bacteria, as well as toxicity and risk studies. Reduction of contaminated compounds (up to 2257 mg TPHs/kg ds/year), the risk assessment and microbial activity showed that the two studied sites could be treated by natural attenuation. Microbial activity in both sites was established by bacterial count, the soil holds natural anaerobic, aerobic, heterotrophic and oil degrading populations between 8.5×10^6 to 1×10^9 bacteria/gram of soil; microbial degradation was proved in situ by CO_2, H_2S and CH_4 determination and laboratory biodegradation tests. Concentrations of TPH found in Paredon 31 were from 300 to 50,000 ppm, and 150,000 ppm in Campo 10. In both cases, the surface soil (0-1.5 m) was the most contaminated layer; however the upper layers showed TPH´s decrease through the study time. A clay layer was found which may have impeded the hydrocarbon migration to the deepest soil layers, by forming a mechanical barrier. Only if land use turns out, then potential risk for metals will appear.

2441 KINETICS PARAMETERS OF A SLURRY REMEDIATION PROCESS IN ROTATING DRUM BIOREACTORS

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The knowledge of biotransformation pollution dynamics in any system is important for design and optimization purposes of biochemical processes involved. This is focus to the determination of kinetics parameters such as the maximum specific growth rate (μMAX), saturation constant (Ks), biomass yield (YX/S; X: biomass, S: substrate) and oxygen consumption (YO_2/S; O_2: oxygen). Several approximations, based on Monod equation, have been developed for estimating kinetics parameters in terms of concentration and type of substrate, bioprocess type and microflora available. The purpose of this work was the estimation of the kinetics parameters of the total petroleum hydrocarbon (TPH) removal process from a polluted soil biotreated in rotating drum bioreactors (RDB) aerated by natural convection. It was used a silt-loam soil polluted with an average of 54000 mg/kg of TPH, which was biotreated in two RDB of 2.3 and 7.7 long/diameter (L/D) ratio, at 5, 15 and 25 rpm during 30 days. Experimental data of TPH removal and biomass produced were adjusted to a hyperbolic equation of Monod type. The kinetics parameters were determined with an error approximation of 15%: *μ*MAX = 0.06-0.55 day^{-1}; Ks = 13000-58000 mg/kg; YX/S = 0.72-1.04 gX/gTPH. The correlation of these parameters versus rotating speed and L/D ratio agreed with the oxygen availability in each RDB considering that oxygen was supply by natural convection. The *μ*MAX and YX/S values determined agreed with those reported in literature. The very high values of Ks are suitable with the complexity of the TPHs studied. In conclusion, the values determined for the kinetics parameters were assumed to be feasible even the relatively low error approximation obtained, which is reasonable due to the kind of polluted soil studied: high pollution and very complex organic structures.

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2883 NITRIFICATION IN TRICKLING FILTERS APPLIED TO THE POST-TREATMENT OF EFFLUENTS FROM UASB REACTOR: CORRELATION BETWEEN AMMONIA REMOVAL AND THE RELATIVE ABUNDANCE OF NITRIFYING BACTERIA

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The number and physiological activity of nitrifying bacteria in wastewater treatment reactors are considered the rate-limiting parameters for the bioconversion of nitrogen in sewage. Since the presence of ammonia and nitrite oxidizers can be correlated with their activity, in situ probe counts can be correlated with the nitrification rates in order to compare the efficiency of different media types. The research aimed to study the process of nitrification in four tricking filters post-UASB reactor with different media types so as the relationship with the relative abundance of nitrifyers. The packing medias tested was: blast furnace slag, random plastic rings, corrugated plastic tubing and Downflow Hanging Sponge (DHS). The experimental set-up was constituted of a 22 m^3 UASB reactor treating domestic wastewater and four 2.16 m^3 TF reactors, each one packed with a different material, in two phases: in the first phase the TFs were operated with surface loading rate (SLR) of 20 m^3/m^2.d, and organic loading rate (OLR) of 0.43 kg BOD/m^3.d. In the second phase, the SLR and OLR were 10 m^3/m^2.d and 0.24 kg BOD/m^3.d, respectively. The relative abundance of ammonia and nitrite oxidizers in the biofilms developed along the filters packing height was determining by Fluorescent in situ hybridization (FISH) with the probes Nso1225 and Nspa662 respectively. Preliminary results showed that the OLR around 0.20 kg DBO/m^3.d should be used in order to increase the nitrification, obtaining an ammonia removal efficiency between 40 and 50%. Ammonia effluent concentrations presented statistical differences among all packing media tested. In general, the TF packed with DHS generated better quality ammonia effluents, in comparison with the other packing materials. The intersticial voids of sponges can contribute for retention of nitrifyers in DHS system. The plastic rings and corrugated plastic tubing showed lower efficiency for ammonia removal despite their higher surface area. The lower presence of biomass observed on corrugated plastic tubing could be one of the factors for the lower efficiency of this packing material, suggesting that this material is not adequate for retention of nitrifyers in this system. Therefore, the relative abundance of ammonia and nitrite oxidizers along the filters will bring great contributions about the presence of nitrifyers in differents packing media such as the filters packing height.

2446 ISOLATION AND CHARACTERIZATION OF AEROBIC CULTURABLE ARSENIC RESISTANT BACTERIA FROM DIFFERENT WATER SOURCES SUCH AS SURFACE WATER AND GROUND WATER IN RAUTAHAT, NEPAL

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Arsenicosis is a problem that is primarily caused by the consumption of arsenic-contaminated water. The different surveys have identified that Terai region in Nepal is at risk of ground water arsenic contamination. With the recent adoption of the 50 part per billion (ppb) of Nepal and 10 ppb by United States Environmental protection agency standard for arsenic in drinking water, it has been estimated that the Rautahat has its share of “hotspots” that exceeds the level in drinking water. A number of bacteria have been identified to resist arsenic exposure furthermore, it’s been proved that they possess chromosomal as well as plasmid genes for arsenic resistance. In this study, the numbers of culturable chemoheterotrophic arsenic resistant bacteria present in surface water as well as ground water, considering the anthropogenic influence from locations Toribari Jungle, Toribari non-Jungle, Bagahi village, Mardhar village respectively at Rautahat District of Nepal were determined by standard plate count method. The arsenic resistant organisms were screened on the basis of their viable growth in plate count agar amended with arsenate ranging from 0, 0.5, 10, 40, 80 to 160 part per million (ppm). Despite the ppb (42-6 ppb) level of arsenic in the water sources, the viable count of bacteria showed appreciable arsenic tolerance demonstrating the widespread nature of arsenic resistance. Nine morphologically distinct potent culturable arsenate resistant bacteria were selected and characterized. Some isolates were capable of tolerating > 1000 ppm of arsenic as arsenate and > 749 ppm of arsenic as arsenite. On the basis of morphological and extensive biochemical tests, the potent arsenic resistant bacteria showed relatedness with *Micrococcus varians* (ASW3), *Micrococcus roseus* (ASO3), *Micrococcus luteus* 1 (ASJ8), *Pseudomonas* / *Xanthomonas* / *Stenotrophomonas maltophilia* (ASJ6), *Pseudomonas* sp (ASR1), *Vibrio parahaemolyticus* (ASR3), *Bacillus cereus* (ASR5), *Bacillus smithii* 1 (ASR8), *Bacillus smithii* 2 (ASR4), respectively. Likewise, the bacteria showed appreciable bioaccumulation capability of about 140 ppm to 166 ppm in 48 hrs.

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2178 A STUDY OF NATURAL RECOVERY IN AN AQUATIC ECOSYSTEM AFFECTED BY MINING: THE RODRIGATOS STREAM (EL BIERZO, LEON, SPAIN).

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This work takes place into the Bierzo Region, located in northeast of the province of León (Spain). In this area numerous open-pit and underground coal mines exist. Some of them are still in activity but almost have been abandoned. In any case, mining implies the presence of coal adits, spoil dumps, tailing dams, and coal-washing plants at the river bank. Most of them persist when mining have finished. These result in wastewater emission and acid mine drainages generation, which eventually reach and impact fluvial systems. The aim of this work is to study the different stages of biological recovering of aquatic systems that are taking place in Rodrigatos stream. This perennial stream was heavily polluted in 2003. Since then up to now, several mines that drained out into Rodrigatos stream have been closed. Moreover, some access roads (civil constructions) have been fit out with calcareous materials which provide alkalinity into the stream by means of runoff. In order to realize this study, water physicochemical analysis and aquatic macroinvertebrates and benthic algae taxonomy identification have been performed since 2003. The results belonging 2003 showed, except for a stream section, neutral pH, a persistent sulphate contribution (7 - 219 mg/L) and an increase in carbonates concentration (1 - 91 mg/L). This carbonates concentration was reduced when a new discharge joined up the stream (alkalinity consumption). Ferric hydroxide precipitates were covering the river bed and algae and macroinvertebrates samplings were not successful. In fact, only two families of macroinvertebrates (Quironomidae and Sialiidae) were found down stream, 1 km from the last emission source. On the other hand, the last results (belonging 2007) show a small pH increase, reaching 8, and increases in sulphates (304 - 407 mg/L) and carbonates concentration (53 - 105 mg/L). Furthermore, ferric hydroxide precipitates have diminished. Due to closure of mining activity and temporal rises in the level of the stream, water quality has considerably improved, making possible the establishment of new macroinvertebrates species. In fact, 10 new families have been identified in addition to families found in 2003.

1876 BIODEGRADATION OF PHENOLIC COMPOUNDS WITH OXIDASES FROM SORGHUM AND NON-DEFINED MIXED BACTERIUM MEDIA

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The biodegradation of the phenolic compounds is performed using oxidative enzymes, e.g. polyphenol oxidases (PPOs) and peroxidases (POXs). These oxidases displaying a wide spectrum for the oxidation of phenolic compounds were isolated either from sorghum or mixed bacteria. Spectrophotometric methods were used to assess the monophenolase and diphenolase activities of PPOs as well as the hydrogen-dependant oxidation of POXs. This has allowed monitoring the biodegradation of phenolic compounds. Activities of oxidases from sorghum are higher than those of bacteria. The monophenolase-PPO, diphenolase-PPO, and POXs activities are 1.6, 59.4 and 4.31 times higher, respectively, in sorghum than in mixed bacteria. Independent of the biological sources of POXs, their activities is several orders of magnitude higher than those of PPOs. The optimisation of the biodegradation of phenolic compounds has shown by the improvement of the biodegradation capacity of the bacteria tested during the acclimation. The rates of observed degradation of substrates by the bacteria were dependant of the structure of phenolic compounds. A complete degradation of phenolic compounds could be achieved by the bacterial oxidases after pre-treatment with sorghum oxidases, showing their synergistic effects for the depollution of phenolic compounds.

2341 RESOLVING AND MODELLING TRACE METAL PARTITIONING IN A FRESHWATER SEDIMENT

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Elevated concentrations of trace metals in sediments pose toxicological risks to biota and may impair water quality. The sediment-water interface is the site where gradients in physical, chemical and biological properties are the greatest. Both chemical and microbiological transformation processes are responsible for cycling elements between water and sediments. Reactive transport models are often used to simulate the complex interplay of reaction and transport processes in sediments. However, only few studies have used this approach to quantitatively describe trace metal cycling in freshwater sediments. In this study, results of pore water analyses (TOC, pH, O_2, major anions, Eh, dissolved sulphide content) and sediment extractions for Fe, Mn, Ni, Zn, Co, Pb and Cu in sediment of two rivers in South-East of France (Rhône and Durance) have been used, in combination with thermodynamic calculations, to examine how trace metal speciation changes with depth in sediments. These profiles have been modelled by coupling early diagenesis, sorption/desorption and interstitial diffusion processes: (1) The early diagenetic model used considers the different pathways of organic matter oxidation, the precipitation of carbonate and sulphide phases and the re-oxidation by O_2. The results show that it describes correctly the behaviour of nutrients. (2) A sorption/desorption model has been applied to Ni, Zn, Co, Pb and Cu. It considers the dominant sorption process of each trace metals, which is a function of sedimentary conditions, diagenetic parameters and metals. (3) The diffusion of dissolved and colloidal phases has been considered as being a function of the local tortuosity of the sediment, deduced from porosimetric and granulometric analyses. The results obtained show that the developped model describes correctly the vertical behaviour of nutrients (NO_3^-, SO_4^{2-}), of physico-chemical parameters (pH, O_2) and of solid-liquid fractionation of trace metals (Co, Cu, Ni, Pb, Zn). This global approach is aimed to contribute to a better understanding of trace metal in sediments to guide management efforts to improve water quality and ecosystem health of current waters.

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2282 CONTROL OF BIOMASS ACTIVATION FOR VOC TREATMENT IN BIOFILTERS: INFLUENCE ON THE START-UP AND SUBSEQUENT OPERATION

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A prerequisite for the effective treatment of contaminated gas flow in a bioreactor is the proper acclimation of the biomass to the target contaminants. Regarding the aromatic compounds, naturally acclimated biomass may be collected from contaminated locations where these compounds have been discharged. After collection, the biomass can be grown up selectively in the laboratory by feeding the pollutants into a nutrient-biomass containing solution. Thus, an acclimated innoculum will be obtained in order to shorten the start-up period of biofilters. The objective of the present study is the development of an effective method to obtain acclimated biomass for both, reducing the start-up period of a biofilter as much as possible and guaranteeing a subsequent high removal efficiency in the biofilter. The pollutants to be degraded are three single-ring aromatic compounds (toluene, ethylbenzene and p-xylene). The initial biomass was collected in a wastewater treatment plant. Subsequently, each organic compound was separately fed into the biomass solution by bubbling. The evolution of the solid content was measured throughout time and correlated with absorbance values at 650 nm. Likewise, the amount of death/alive cells in the biomass solution was measured throughout time by using LIVE/DEAD BacLight Bacterial viability kits (Molecular Probes, Leiden, The Netherlands). Static/discontinuous tests were also carried out in order to determine the pollutant degradation rate in each case. Finally, three biofilters were filled up with a previously characterized support material and the acclimated biomass was inoculated. These results allow for achieving the moment when the biomass is fully acclimated to the target contaminant. Thus, an effective inoculation and subsequent start-up and operation in biofilters can be ensured.

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2311 SEARCH OF HEXACHLOROCYCHLOHEXANE (HCH) DEGRADERS FROM CONTAMINATED TROPICAL SOILS

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Banana is a main agricultural production in the French West Indies (Guadeloupe and Martinique) since the 1960's. However this crop requires an intensive use of pesticides. Since these years chlorinated pesticides such as hexachlorocyclohexane (HCH), chlordecone (kepone) and dieldrine has been used until the beginning of the 1990's, resulting in a generalized diffuse contamination of soil and surface waters in the banana producing areas. Natural decontamination involves microbial degraders. This study focuses on detecting catabolic genes for HCH degradation in three soil samples collected from agricultural fields in Guadeloupe banana producing areas, in order to gain further fundamental knowledge on a possible decontamination process using bioaugmentation.
Degradation experiments were performed in the laboratory using HCH as a sole carbon source but also using a co-metabolic medium. To assess whether the biodegradation catabolic genes for HCH metabolism is present in soil samples, investigation through PCR amplification of dehalogenase genes using soil genomic DNA and sequencing were done.

2892 BIOREMEDIATION OF SEVERELY WEATHERED HYDROCARBONS: IS IT POSSIBLE?

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Weathering processes of spilled hydrocarbons promote a reduced biodegradability of petroleum compounds mixtures, and consequently bioremediation techniques are often ruled out within the selection of suitable remediation approaches. This is truly relevant wherever old spills at abandoned industrial sites have to be remediated. However it is well-known most of the remaining fractions and individual compounds of weathered oil are still biodegradable, although at slow rates than linear alkanes or one- and two-ring aromatics. In this context, the study presented here has been designed in order to evaluate the bioremediation of one soil affected with a severely weathered spill of crude oil, and specifically to determine which hydrocarbon families are still susceptible of being biodegraded, and which are accumulated in the final residues. We dealt with a sandy soil, with low initial levels of nutrients, affected by a very old crude oil spill (initial averaged TPH value of 4.000 mg/kg). A pilot-scale landfarming of this soil was carried out in five different parcels of 60 m^3 each; specifically the conditions used were: (1) Natural attenuation, (2) Tilling (T) and watering (W), (3) T,W, and slow-release fertilizer addition (SRF), (4) T,W, SRF and surfactant addition, (5) oleophilic fertilizer addition. To follow up the hydrocarbon evolution, periodical sampling and analysis by GC-FID (quantification of TPH) and GC-MS (qualitative study of different families) were performed. After five months of treatment, results indicated TPH reduction higher than 55% in parcels 3, 4 and 5, affecting mainly to isoprenoids (m/z= 113); whereas steranes (m/z= 217, 218) and hopanes (m/z= 191) were basically unaltered. As conclusion, in this particular case it was possible to face a successful bioremediation, even more being absent easily-degradable compounds such as n-alkanes and light aromatics.

2381 CHARACTERIZATION OF BACTERIAL CONSORTIA FOR ITS USE IN BIOREMEDIATION OF GAS-OIL CONTAMINATED ANTARCTIC SOILS

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Success of bioaugmentation of chronically-contaminated soils is controversial, mainly because the inocula are frequently unable to establish in the matrix under bioremediation. In Antarctica, the environmental conditions and the restriction for the introduction of non-autochthonous organisms (imposed by the Antarctic Treaty) prevent inoculation with foreign bacteria. For these reasons, our aim was to isolate and characterize hydrocarbon-degrading Antarctic bacterial consortia, to test their capacity to enhance soil bioremediation and to evaluate the effect of two different inoculum sizes on remediation rate. Bacterial consortia M10 and J13 were isolated by enrichment cultures from contaminated Antarctic soils. Their degradative capacity on hydrocarbons was evaluated in-vitro and by detection (using PCR and blotting) of genes (alkB, nahAc, xylE) involved in hydrocarbons catabolic pathways. Consortia showed similar T-RFLP profiles, suggesting the presence of the same phylotypes. Although composition and degradative profiles were similar, field trials proved that consortia differed in their ability to establish in the soil, J13 being less efficient to remove hydrocarbons. Thus, M10 was selected for further characterization. Total 16S rDNA was amplified by PCR and cloned. Sixty clones were screened and grouped by similar AluI ARDRA profile, sequencing one representative of each phylotype. T-RFLP of clones showed that all the peaks from the entire consortia were recovered. To avoid the problems of reproducibility in preparation of the inoculum from M10 stock, a culture-dependent approach was also used to isolate its components for further preparation of a mixed-culture with similar degradative performance than M10. Analyzing phenotypic and molecular traits, *Pseudomonas, Stenotrophomonas* and *Sphingobacterium/Pedobacter* were detected. By cloning, also *Brevundimonas* was found as minority representative. Effect of bioaugmentation with M10 on remediation of sterile and non-sterile hydrocarbon-contaminated biostimulated soils was analyzed on site using microcosms inoculated at two different levels (10^6 and 10^9 UFC/g). An only-biostimulated and an untreated soil were also tested. Total and degrading counts and hydrocarbon concentration were measured. Results showed that, in the presence of the natural microbiota, only bioaugmentation with a bacterial load several orders of magnitude higher than the number of autochthonous bacteria will improve hydrocarbon removal, shortening the length of the bioremediation process.

2515 INFLUENCE OF ORGANIC POLLUTANTS ON THE PHYTOEXTRACTION POTENTIAL OF *Halimione portulacoides*

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The capability of salt marsh plants to accumulate trace metals is, in principle, advantageous for phytoremediation processes. Sites contaminated with metals are frequently also contaminated with organic pollutants of different families, like petroleum compounds and pesticides. The simultaneous presence of pollutants of different families might change plants capacities for extracting and accumulating metals, thus changing phytoremediation efficiency. Studies of metal accumulation in plants from estuarine sediments contaminated with a mixture of inorganic and organic pollutants are scarce, although deserve investigation because estuaries are often considered sinks for varied pollutants. This study aims to investigate the effect of some organic pollutants which are frequently present in contaminated environments, like PAHs and pesticides (DDE), on the accumulation of Cu by the salt marsh plant Halimiones portulacoides (a plant commonly found in Portuguese salt marshes, which is capable of accumulating several metals, including Cu [1]). Two sets of experiments were carried out in the laboratory with plant roots exposed to: (1) elutriate solution and (2) sediment soaked in elutriate solution. Media were prepared with sediment and water retrieved from a salt marsh from Cávado River estuary. Plants grown in a greenhouse were exposed to known concentrations (concentrations similar to those observed in Cavado sediments) of both Cu (10 mg/L) and of an organic pollutant (5 µg/L each PAHs or 0.5 µg/L DDE), during 6 days. Afterwards, Cu was determined in the media and in plant tissues (roots, stems and leaves) by atomic absorption spectrometry, after digestion assisted by high pressure microwave. Results showed that the plant could absorb and translocate Cu from all media. PAHs promoted Cu absorption /adsorption by the plant when no sediment was present, but DDE had no influence on the accumulation of Cu in any cases. Both organic pollutants influenced Cu solubility in elutriate, even in the presence of sediments. Therefore, the simultaneous presence of pollutants of different families in a colonized site may influence metal accumulation by plants and should be taken in consideration in future studies of suitability of plants for phytoremediation.

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2591 A FULL-SCALE REMEDIATION PROJECT USING A COMBINATION OF TREATMENT TECHNIQUES: SOIL FLUSHING, SOIL WASHING, THERMAL DESORPTION, LANDFARMING.

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The purpose of this paper is to describe the full-scale project of environmental recovery of the soil occupied by one old storage tank facility of petroleum hydrocarbons, following the directives of the Royal Decree No. 9 / 2005. It was marked the target levels of acceptable human risks for future use anticipated. For the recovery of the soil, a combination of ad situ treatment techniques was applied, which has allowed the full recovery of contaminated soil. The plot area of the performance had a surface of 100,000 m^2 where the subsoil was contaminated mainly by different types of hydrocarbons distributed heterogeneous by the subsoil. During the execution of the project, 800,762 tons of soil has been necessary to move, of which 373,000 tons were contaminated. For the recovery of 373,000 tons of contaminated soil, has been used a combination of ad situ techniques based on the specific characteristics of each type of soil as well as the contamination associated with them. These techniques have been: soil flushing, soil washing, thermal desorption and landfarming. During the implementation of the Soil Flushing, have been pumped/infiltrated 168,946 m^3 of water, injected 20,000 litres of surfactant, achieving to increase the ratio of extraction from 1.1 to 3.6% and recover 352,000 litres of hydrocarbons. Using the technique of Soil Washing, has been treated 302,000 tons of contaminated soil, being the average concentration of hydrocarbons at the entrance of the treatment 12,896 mg/kg and the output 416 mg/kg. It has been able to recover 90% of the treated soils. It was treated by Thermal Desorption 45,000 tons of contaminated soil, being the average concentration of hydrocarbons at the entrance of the treatment 16,876 mg/kg the output 148 mg/kg. It has been able to recover 100% of the treated soils. The Landfarming technique has been used to recover 50,000 tons of contaminated soil, being the average concentration of hydrocarbons before process 3,570 mg/kg and after the same 892 mg/kg. It has been able to recover 100% of the treated soils.

2238 A ROLE FOR PLANT-ASSOCIATED BACTERIA TO IMPROVE *IN SITU* PHYTOREMEDIATION OF BTEX AND TCE: EVIDENCE FROM 2 FIELD EXPERIMENTS

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Phytoremediation of highly water soluble and volatile organic contaminants is often inefficient because insufficient degradation of the pollutants by plants and their rhizospheres can result in phytotoxicity and/or volatization of the compounds through the leaves, potentially resulting in new environmental problems. Endophytic bacteria, if equipped with the appropriate degradation pathway, can assist their host plant to tolerate higher concentrations of contaminants and to reduce the evapotranspiration of water-soluble pollutants (Barac *et al.*, 2004; Taghavi *et al.*, 2005). In order to investigate the potential role of endophytic bacteria to enhance *in situ* phytoremediation of TCE and BTEX, two field experiments were performed. For the first field experiment, a site was chosen where TCE was present in the groundwater in concentrations up to 100 mg l^{-1}. TCE-concentrations were determined in two transects through a small wood (*Quercus robur* and *Fraxinus excelsior*) planted nearby about 25 years ago. Sharp decreases of TCE through these transects suggested that TCE degradation was taking place due to the presence of this wood. The microbial population associated with Quercus robur and *Fraxinus excelsior* was isolated and characterized. As 82% of the isolated bacteria could grow in the presence of TCE, it seems to be clear that these plant-associated bacteria contribute to the TCE-degradation. Additionally, in 2006, rows of hybrid poplar trees were planted perpendicularly on the contamination plume in order to accomplish the already existing bioscreen consisting of English Oak and Common Ash. These poplar trees will be (*in situ*) inoculated with bacteria that are able to degrade TCE and by consequence have the potential to improve phytoremediation of this TCE contaminated site. For the second, long term field experiment, 275 poplar trees were planted in 1999 on a BTEX contaminated experimental field in order to install a bioscreen. Measurements, conducted over a 6 years period after the planting of the trees suggested that poplar trees and their associated micro-organisms have, once the plant roots reach the contaminated groundwater zone, an active role in the remediation of the BTEX plume present. Analysis of the microbial communities associated with poplar showed that enrichment occurred of both rhizosphere and endophytic bacteria that were able to degrade BTEX contaminants. Interestingly, once the BTEX plume was remediated, the numbers of toluene degrading rhizosphere and endophytic bacteria decreased below detection limits. The results of both field experiments demonstrated that within the plant-associated bacterial communities, strains were identified that could potentially be used to improve phytoremediation strategies e.g. by in situ inoculation of these bacteria.

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2868 SOIL REMEDIATION: NEW STRATEGY BY THE COMBINE OF F.I.S.H. AND GEO-RADAR TELEDETECTION

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One of the most important targets in bioremediation to treat contaminated soils is the developing of new technologies. In this project we have developed a new strategy to eliminate in situ pollutants from hydrocarbons contaminated soils, supported with two specific techniques: FISH and Geo-Radar teledetection. With these techniques we try to delimit the pollutants distribution in soil, and to develop DNA and PNA biosensors with high specificity to evaluate the natural attenuation soil capacity using fluorescence in situ hybridization (FISH). This strategy gives us the following information: Pollutants presence analysis, deep teledetection without damage techniques (GPR-Geo Radar), determination of pollution compounds, in situ decontamination strategy and efficacy uptake pollutants from soil to environmental recovery. Different soil recovery solutions will be proposed after determining the contamination extension. The biosensors used in this work are based on the existence of soil bacteria with genetic skills for biotransformation of pollutant compounds, in order to avoid conventional chemist analysis. The biosensors are useful to analyze quickly the soil contamination from several environmental samples: soil, mud, sediments, sludge, waste water and underground water. The system is able to be adapted to detect specific pollutants, making and designing DNA and PNA probes highly specific. First of all, we have to elaborate the contamination map, using the GPR Geo-radar technique. Then, we make laboratory scale assays to decide the decontamination in situ strategy, and estimate the time and the cost of the process. The research in new molecular techniques applied to decontamination process, has allowed the use of FISH to detect the presence and remain of bacteria in soil, sludge and water. This technique is based on the use of oligonucleotide as specific hybridization probes to locate homolog sequences. These probes have been used to detect different bacteria as Pseudomonas, Burkholderia and Klebsiella, and to monitor changes in the bacteria from several environmental samples too. GPR Geo-radar is a technique based on the use of electromagnetic waves into the underground being able to detect dielectric differences between different materials, and the reflection generated is registered in a radagram or graphic of the underground. This is a non-damaging and non-destructing method, in order to perform big amounts of analysis without change in the prospected environment. The main advantage of this method, compared with other analysis systems is the possibility to have a complete sample of the contaminated ground, allowing and delimiting the extension of the contamination in ground with high precision, not only with random samples.

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2237 METAL ACCUMULATION IN PLANTS WITH ADDED ECONOMICAL VALUE GROWN ON METAL CONTAMINATED SOILS: SUSTAINABLE USE OF THESE SOILS FOR BIO-ENERGY PRODUCTION AND POSSIBILITIES FOR PHYTOEXTRACTION

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Phytoremediation has been proposed as an economic alternative for remediation of metal contaminated soils. It can be applied over extended surface areas and targets the "bioavailable" soil fraction of heavy metals, which is the most relevant fraction from an environmental risk assessment perspective. The most important drawback is the long remediation period required (years to decades). The use of willow, poplar and maize allows for gradual contaminant removal, while the produced biomass can be used for production of renewable energy. This in turn allows for economical valorisation of contaminated soils during the remediation process. An increasing number of reports support the opinion that phytoextraction will get only economically feasible if, in addition to metal removal, plants produce biomass with an added economical value. In a large scale field experiment performed on a moderately contaminated soil (4-5 mg Cd kg^{-1} soil), several willow and poplar clones proved to be capable of annually removing up to 121 g Cd per hectare in case only wood was harvested and up to 240 g Cd per hectare if also leaves should be harvested. This corresponds with a projected annual removal of respectively 0.043 $mg.kg^{-1}$ and 0.080 $mg.kg^{-1}$ from the topsoil layer (upper 25 cm). The feasibility of the proposed system was considered to be limited to the metals Zn and Cd. Biomass conversion by f.i. wood gasification allows for the production of 1.25 $MWh.t^{-1}$ electricity and 9 $GJ.t^{-1}$ thermal energy (Vervaeke et al., 2003). Energy production in this manner is considered CO2 neutral since the amount released during energy production is absorbed by the growing energy crop. Substitution of fossil resources by plant biomass would result in a reduction of greenhouse gas emission by 18 - 32 t CO_2 per hectare of energy crops. In addition to willow and poplar, also maize cultivars (for bioethanol) were tested. Also pyrolysis of biomass is considered as an alternative. Besides of energy, this process can deliver high valuable products for chemical industry. As an added incentive for regions with intensive animal production, such as Flanders and the Netherlands, biomass cropping is a nutrient requiring process and could therefore serve as an additional sink for excesses in animal manure overproduction. The different plant species and cultivars will be discussed for their biomass production, potential for economical valorisation and phytoextraction efficiency.

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2427 MERCURY UPTAKE BY METAL TOLERANT SILENE VULGARIS GROWN ON HG CONTAMINATED SOILS

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Mercury is a highly toxic element because of its accumulative and persistent character in the biota. It has been introduced to the environment from natural and anthropogenic activities. Silene vulgaris is a well-known example of facultative metallophyte or pseudometallophytes, which have populations on both normal and metalliferous soils. The facultative metallophytes represent probably the most frequent and arguably the most interesting category of metal-tolerant plants. The objective of this work was to evaluate the effectiveness of Silene vulgaris to uptake Hg from artificially polluted soils. To achieve this goal, a pot experiment was carried out in a rain shelter, with temperature and radiation closely resembled ambient conditions for a full growth period. Two different soils were used: A (pH=8.55, O.M. 0.63%), V (pH=7.07 O.M. 0.16%). Treatments included (1) Control, no metal addition; (2) low dosage: 0.6 $mg{\cdot}kg^{-1}$ Hg); (3) high dosage: 6 $mg{\cdot}kg^{-1}$ Hg. Mercury content in plant and soil samples was measured using an advanced Mercury Analyser (AMA-254, LECO company). Plants were grown healthy and showed good appearance throughout the study. No effect was found in biomass production. Mercury uptake in plant was and positively correlated with the mercury concentration found in both soils. Differences were statically significant (one-way ANOVA followed by a post-hoc multiple comparisons of means using the Duncan test ($P < 0.05$). Transfer factor (TF) or bioaccumulation factor which can be used to evaluate the plant capacity to translocate metals from soil to shoot was indeed affected by the Hg concentration in soils. The higher the concentration of mercury was, the greater the value of TF. Therefore, it is concluded that Silene vulgaris, grown in the conditions describe in this work, is capable to tolerate Hg concentrations in their shoots without biomass decreasing. In this context, It is suggested that it could be used in phytoremediation of Hg polluted soils.

2490 COMBINED USE OF BACTERIAL COMMUNITY ANALYSIS TECHNIQUES AND GEOGRAPHIC INFORMATION SYSTEMS (GIS) FOR THE EVALUATION OF ANTHROPOGENIC IMPACT IN COASTAL WATERS

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Marinas and recreational boats have an impact in coastal environments because they constitute point sources for low intensity but recurrent pollution by oil hydrocarbons. Since marinas tend to be located in areas of great natural beauty, and/or of high value economic activities, the pollution they contribute is assumed to be important and increasing, though currently-available information is very incomplete. It is therefore essential to assess the ecological impact of pollution derived from marinas on surrounding coastal ecosystems, and to develop effective detection tools to evaluate this impact. We performed a comparative study on the composition of bacterial communities in the coastal area surrounding a marina (Puerto Adriano), at the West of Mallorca Island. Interpolation of concentration of hydrophobic compounds, chlorophyll and bacterial counts (DAPI stain and FISH with EUB338 probe) in seawater over the geography of the sampling area using Geographic Information Systems (GIS) techniques allowed the delineation of two different habitats: bay and marina (with low and high levels of impact, respectively), and a transition zone between them. Interestingly, a link between the presence of high levels of hydrocarbons and high total- and active-cells was observed. Furthermore, gradual changes in certain bacterial populations (i. e. SAR11 and Roseobacter group) were diagnostic of impact. Two other analysis were performed: the first, a unique station in a crowded beach at Palma de Mallorca bay (S'Arenal), and the second in Santa Ponça bay, where there is a marina, two crowed beaches, and discharges of a wastewater treatment plant. Interestingly, a link between the geographical location of these point sources of anthropogenic impact and higher bacterial counts was observed. Our results thus suggest that simple (total- and active-bacterial cell counts) or more exhaustive (detection of specific bacterial groups) bacterial community analysis techniques combined with GIS are helpful to evaluate the geographical range of anthropogenic impact in coastal waters.

2239 MODIFIED ENDOPHYTES FOR IMPROVING PHYTOREMEDIATION OF MIXED CONTAMINATIONS OF HEAVY METALS (Ni) AND ORGANIC CONTAMINANTS (TOLUENE)

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Phytoremediation is a promising technology for the remediation of soils and groundwater contaminated with heavy metals and organic pollutants. However, large-scale application of phytoremediation faces a number of obstacles including the levels of pollutants tolerated by the plant, the bioavailability of the contaminants and, in some cases, the evapotranspiration of volatile organic pollutants. A possible solution for these problems can be the use of plant-associated bacteria. These bacteria can be isolated, eventually equipped with interesting characteristics and re-inoculated in the plant to enhance their beneficial effects. In order to improve phytoremediation of heavy metal contaminated soils, plant-associated bacteria can be equipped with a metal-sequestration system making the heavy metals less toxic to the plant (Lodewyckx *et al.*, 2001). Besides, rhizosphere bacteria producing siderophores and/or organic acids can increase the bioavailability of heavy metals. Phytoremediation of highly water soluble and volatile organic xenobiotics is often inefficient because plants do not completely degrade these compounds and the contact time between the microorganisms in the rhizosphere and the pollutants is not long enough. Since the pollutant is transported through the plant vascular system, endophytic bacteria, possessing the genetic information for efficient degradation of a xenobiotic, can promote degradation resulting in a decreased phytotoxicity and evapotranspiration (Barac *et al.*, 2004). In addition, the application of phytoremediation is often limited due to the fact that in many field cases plants have to deal with mixed contaminations of heavy metals and organic contaminants. The aim of this study was to investigate the potential of endophytic bacteria to overcome these limitations. In order to test this, yellow lupine plants (*Lupinus luteus*) were inoculated with the endophytic strain *B. cepacia* VM1468 possessing the pTOM-Bu61 plasmid and the ncc-nre Ni resistance. Inoculation of plants with the *B. cepacia* VM1468 strain resulted in a reduction of toluene and Ni phytotoxicity as a clear protective effect of *B. cepacia* VM1468 on the growth of yellow lupine was observed at all toluene and/or Ni concentrations tested. These results confirm our hypothesis that endophytic bacteria, when equipped with the appropriate capacities, help plants to survive under conditions of elevated levels of heavy metals and organic contaminants. Besides, the plants inoculated with B. cepacia VM1468 released less toluene through the leaves and accumulated more Ni, compared to the control plants. This shows that the toluene degrading, Ni resistant strain *B. cepacia* VM1468 not only protects his host plant against toluene and Ni phytotoxicity, but also contributes to a significant decrease in toluene-evapotranspiration and to an increase in Ni accumulation.

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1786 AIR POLLUTION TRANSPORT TO THE EL TAJIN ARCHAEOLOGICAL ZONE IN VERACRUZ, MEXICO

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Features of atmospheric transport to El Tajin, an important archaeological site in Veracruz, Mexico, are investigated. The study is motivated by the need for an increased understanding of the meteorological processes contributing to acidic precipitation at Mesoamerican heritage sites. Using a variety of meteorological resources and analysis techniques we find that both large-scale and local meteorological forcings favor transport from easterly and northerly sources. Significant acid precursor emissions exist in both of these directions, including a large area of active oil platforms in the southern Gulf of Mexico.

2454 SPECIFIC DEVELOPMENT FOR CATALYTIC EVALUATION OF CARBON MICRO-FIBERS COVERED WITH TIO_2–V_2O.

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The atmospheric contamination is one of the most important problems in the actuality, in Mexico the petroleum and chemical industries are the principal sources. On the present work we used carbon micro-fibers with a TiO_2 (anatase) or TiO_2-V_2O_5, TiO_2-CuO, and TiO_2-NiO covering as oxidation catalysts of n-C_4. The carbon micro-fibers were obtained from acrylic precursor fibers which are mainly conformed for acrylonitrile and two monomers in low concentration. The steps of the carbon micro-fibers production process are reaction, extrusion, oxidation and carbonization systems. The production process was optimized using experimental tools for to determinate the principal variables than impact the quality properties of the precursor and the final carbon fibers. With this methodology was defined a new technology than permits the control of important physical and chemical characteristics of the carbon micro-fibers. For catalysis applications the porosity, chemical structure and thermal response were controlled. In this work were analyzed two different carbon micro-fibers. The covering process of the micro-fibers with metallic oxides was a sol-gel technique. The micro-fibers were characterized using the following techniques: Raman spectroscopy, spectrophotometry, calorimetric scattering and optical microscopy. The evaluation of the catalytic micro-fibers was achieved in a pilot plant with a catalytic microreactor that was designed specifically taking account the experimental conditions for this process and using a gaseous flow for n-C_4, N_2 and O_2 of 2.85 lt/hr. The monitoring of the reaction products was achieved by gas chromatography technique. The results obtained showed that the catalytic fiber covered with TiO_2 (anatase) gave the best conversion, comparing with the rest of the catalytic fibers. In the initial experimental design, it was used the same catalyst weight; 0.2 gr, but different flows of nC_4-N_2 and O_2; after the weight was changed to 0.4 gr. The total oxidation system was modeling for to determinate different gas conversions.

2473 DEGRADATION OF BENZO[A]ANTHRACENE AND BENZO[K]FLUORANTHENE BY TRAMETES VERSICOLOR

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Polycyclic aromatic hydrocarbons (PAHs) are among the most frequently detected environmental pollutants and belong to EPA's priority pollutants. They are present in polluted soils from gas production, petroleum refining and wood preserving facilities. The remeditation of these sites represents an interesting challenge. High molecular PAHs (from 4 to 6 rings), like benzo[a]anthracene (BaA) and benzo[k]fluoranthene (BkF), are unlikely degraded by bacteria consortiums. The fungus *Trametes versicolor* has shown ability to degrade a wide range of environmental pollutants such as textile dyes, chlorinated aliphatic hydrocarbons and PAHs. Our study focuses on its capacity to degrade high molecular PAHs as a preliminary phase for the application of T. versicolor, alone or combined with bacteria, for soil remediation processes. Experiments were carried out in liquid medium in 500 mL bottles with Teflon® caps. Each bottle contained 50 mL of a defined medium with 10 g/L of glucose as carbon source and was inoculated with an amount of fungal pellets equivalent to 3 gDCW/L. The concentration was 20 ppm for both pollutants. In addition, medium containing non-ionic surfactants (Tween20 and Tween80) were used with the purpose of increasing the solubility and, therefore, the bioavailability of both pollutants for the fungus. Three types of measures were used: an abiotic control, a killed control and fungal inoculated, each one by triplicate. After 12 days, the initial content of glucose was completely consumed and lacasse activity was observed as well. Both pollutants were biodegraded in a 20% of the initial content in medium without surfactants. On the other hand, in medium containing non-ionic surfactants the biodegradation increased to 48% for BkF and 95% for BaA, respectively. In order to elucidate the biodegradation mechanisms and to identify the breakdown products, experiments with purified lacasse and Cyt-P450 inhibitors were undertaken. It has been demonstrated that using purified lacasse of T. versicolor in vitro, in presence of mediators, it is capable to degrade some PAHs. However, from our results it seems that degradation of BaA and BkF in vivo could be attributed to Cyt-P450 which is involved in degradation pathways of other pollutants by ligninolytic fungi.

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2298 CHLOROPHENOLS DEGRADATION IN SOIL COLUMNS INOCULATED WITH THE WITE-ROT FUNGUS ANTHRACOPHYLLUM DISCOLOR IMMOBILIZED ON WHEAT GRAINS.

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Anthracophyllum discolor, a white-rot fungus of southern Chile, produces ligninolytic enzymes specially manganese peroxidase (MnP), and has been studied for its potential use on bioremediation of contaminated soils with chlorophenols. The main purpose of this study was to evaluate the chlorophenols degradation in soils columns inoculated with *Anthracopyllum discolor* immobilized on wheat grains. Columns (35 x 5 cm) packed with a mixture of 50% quartz sand and 50% of allophanic soil (Andisol) activated by acidification, were tested for 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP) adsorption. Wheat grains colonized by *Anthracopyllum discolor* were put on the upper part of each column. The columns were operated in continuous and were fed with the respective chlorophenol (100 mg L^{-1}) under a flow rate of 1.5 mL min^{-1}, at room temperature during 29 days approximately. The enzymatic activity of Manganese peroxidase (MnP) was periodically evaluated. The chlorophenols concentration was analyzed by HPLC. PCP adsorption in the columns was high in the first 12 h and decreased suddenly up to 48 h. Between days 5 and 14, the PCP concentration was stabilized in the range of 30 mg L^{-1}. After that, PCP concentration increased and the column was saturated on day 16. The lowest values of PCP coincide with the production of MnP, showing that degradation favored by the A. discolor occur. Similar situation was observed when the columns were fed with 2,4-DCP and 2,4,6-TCP, except that no strong initial adsorption was observed. Between days 5 and 14, the 2,4-DCP concentration was stabilized in the range of 20 mg L^{-1}, and the column was saturated in day 24. When 2,4,6-TCP was treated in the column, the stabilized concentration (40 mg L^{-1}) was obtained between days 4 and 8, the column was saturated in day 11 and, a lesser quantity of the MnP enzyme was produced. 2,4-DCP was detected (aprox. 6 mg L^{-1}) as metabolic product of 2,4,6-TCP degradation. In conclusion, chlorophenols degradation increased in soil columns inoculated with the fungus A. discolor immobilized on wheat grains, increasing the capacity of the soil columns to adsorb and eliminate these contaminants.

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2552 CHASING HALORESPIRERS: HIGH THROUGHPUT MULTIPLEX DETECTION OF DECHLORINATING BACTERIA USING PRI-LOCK PROBES.

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Bioremediation management strategies for sites contaminated with chlorinated compounds require monitoring technologies that enable simultaneous detection and quantification of a wide range of microorganisms involved in reductive dechlorination. Many multiplex, quantitative detection methods available suffer from compromises between the level of multiplexing, throughput and accuracy of quantification. In this paper we report the development and application of a high-throughput, ligation based assay for simultaneous quantitative detection of multiple reductive dechlorinating populations and their key reductive dehalogenases. The ligation probes, designated Plant Research International-lock probes (PRI-lock probes), are long oligonucleotides with target complementary regions at their 5' and 3' ends. Upon perfect target hybridization, PRI-lock probes are circularized via enzymatic ligation, subsequently serving as template for individual, standardized amplification via unique probespecific primers. Adaptation to OpenArraysTM, which can accommodate up to 3072, 33 nl PCR amplifications, allowed high-throughput real-time quantification. This assay combines the multiplex capabilities and specificity of ligation reactions with highthroughput real-time PCR in the OpenArrayTM, resulting in a flexible, quantitative multiplex detection system (1). PRI-lock probes targeting 16SrRNA genes from 10 phylogenetic groups involved in reductive dechlorination were used to demonstrate the application of the detection system. All probes specifically detected their corresponding targets and provided discrimination against non-target organisms with very similar ligation target sites. The PRI-Lock detection system is currently being optimised for reductive dehalogenase genes and should be easily adaptable for versatile detection and monitoring purposes based on other functional genes involved in bioremediation.

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2452 OXIDATION OF ALKANES COUPLED TO CHLORATE REDUCTION BY *Pseudomonas chloritidismutans* AW-1T

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Microbial (per) chlorate reduction is a unique process that yields molecular oxygen during the dismutation of chlorite. The oxygen thus formed is used as terminal electron acceptor and may be used to destabilize persistent hydrocarbons through oxygenases. Bacteria are known which can couple aromatic hydrocarbons oxidation with chlorate reduction. But up till now no bacteria are known which can couple oxidation of aliphatic hydrocarbons to chlorate reduction. Here, we report that *Pseudomonas chloritidismutans* AW-1T previously isolated on acetate and chlorate is able to grow on alkanes with chlorate as electron acceptor. This strain degrades alkanes with oxygen and chlorate, but not with nitrate, thus suggesting the involvement of oxygenases in the breakdown of alkanes. The key enzymes chlorate reductase, chlorite dismutase and alkane hydroxylase were assayed and found to be present. The specific growth rate on decane and chlorate was 0.50+0.06 per day (doubling time was 2.13+0.73 day). Similar values were found with decane and oxygen.

2477 ANTHRACENE DECOMPOSITION IN SOLID PHASE BY SIMPLE OZONATION

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In this work the degradation of anthracene in solid phase by ozonation is studied. The ozone soil remediation is an attractive technology because it can be applied in three available modes: *in situ*, *on site* and *off site*. In most of real contaminated sites, anthracene is always combined with other polycyclic aromatic hydrocarbons (PAHs), for this reason it has been selected as a model compound for the preparation of the contaminated model soil, (baked and moisten sand) with the initial concentration 3 mg/g. However there are few studies where determination of ozonation by-products of PAHs in solid phase has been done. The model soil was treated to different ozonation time from 5 to 90 min at an initial concentration of ozone of 16 mg/L. The decomposition degree of anthracene was determined by UV/VIS spectrophotometry, HPLC-UV/Vis, and HPLC-MS. Based on the HPLC data, the obtained results confirm that in the case of baked sand, anthracene may be decomposed by simple ozonation during 15 min, at the same time from the HPLC it is possible to observe that there are at least 7 by-products during the first 5 minutes of the ozonation process, some of the main by-products have been identified as 9 - 10 anthraquinone, anthrone, and phthalic acid by HPLC-MS. Furthermore, analysis of the influence of the soil moisture content is presented proving its negative influence in the anthracene decomposition rate, taking around 30 minutes to be eliminated by simple ozonation.

1006 RESEARCH AND PERSPECTIVES IN SOIL ENZYMOLOGY

Spanish Group of Soil Enzymology.
www.ivia.es/soilenzymology.

During the last years, interest on soil enzymology has been increased due to the fact that measurements of extra-cellular enzymatic activity can be employed either to assess the soil biological activity, or as an indicator of the behaviour of nutrients cycles, and thus of the soil fertility. Furthermore, the study of enzymes and their activities may be a useful tool for the interpretation of changes occurring in ecosystems. This latter fact appears to be extremely interesting at the time being, when soil is subjected to diverse environmental impacts, such as aggressive anthropic actions, excessive use of agrochemical, residues management, etc.
Spanish Group of Soil Enzymology established in 1997 focus its interest on the unification of criteria on the behaviour, state and location of soil enzymes, as well as on the advances in analysis methodologies. Additionally, it is intended to create a forum, which serves as a basis for the contrast of different points of view and technologies, and for the enlargement of knowledge on soil enzymology.
The group organizes dissemination activities as training courses or International Meeting with the aim of spreading the use of the soil enzymology as a useful tool in environmental analysis.

1885 BACTERIAL COMMUNITY SHIFTS CORRELATED WITH CHANGES IN PERFORMANCE IN A MESOPHILIC ANAEROBIC BIOREACTOR TREATING DAIRY WASTEWATER

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Because anaerobic digestion is a series of metabolic reactions performed by a consortium of various symbiotic organisms, complete conversion of organics to methane is dependent on the synchronized activity of distinct microbial populations. Intensive efforts have been made to investigate and understand microbial behavior in anaerobic processes, but the majority of previous studies have focused on methanogens directly responsible for producing methane. However, the overall process should also be viewed in light of acidogens since they play a primary role in producing major substrates for methanogens. Therefore, this study aimed to investigate bacterial community shifts in relation with process data in a mesophilic anaerobic batch reactor treating whey permeate. Temporal variations in total bacterial and archaeal populations were quantitatively monitored using real-time PCR and the results were well correlated to process data. Significant increase in total bacterial population corresponding to the fermentation of substrate was observed during the initial period of operation. Biphasic growth of total archaeal population with the diauxic consumption of acetate and propionate, was also clearly exhibited during the methanogenic period. Changes in bacterial community structure were further analyzed by denaturing gel gradient electrophoresis (DGGE) of 16S rRNA genes. The results suggested that an *Aeromonas*-like microorganism was responsible for the initial fermentation of substrate carbohydrate in the reactor. Several sequences were closely related to *Clostridium* spp. capable of carbohydrate, lactate or ethanol fermentation or homoacetogenesis. Statistical analyses of the DGGE profiles showed that the structure of bacterial community varied with operating time, and such shifts reflected changes in process performance. The clustering analysis based on the DGGE profiles clearly demonstrated that a significant shift in bacterial community structure corresponded to a considerable event in process. Our experimental results suggested the possibility of diagnosis and prediction of an anaerobic digestion process by monitoring bacterial community shifts.

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2464 **AMMONIUM TOLERANCE OF *Methanoculleus* sp.**

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Ammonia, released during anaerobic degradation (AD) of protein rich materials, such as slaughter house waste, pig manure or waste water from fish industries, are inhibitory to methanogens causing instability of the AD-process. Previous studies showed that ammonia initiated an alternative methane producing pathway, likely caused by the inhibition of the acetate utilizing methanogens. In this pathway, syntrophic acetate oxidation (SAO), acetate is degraded by a two organism consortium consisting of a acetate oxidizing bacterium and a hydrogen utilizing methanogen. The hydrogen utilizing methanogens from five different biogas digesters, operating with SAO at high levels of ammonium (5-7 g NH_4^+-N/L), were isolated and all shown to be *Methanoculleus* sp. The aim of this study was to investigate the ammonia tolerance of these isolates. The isolates were cultivated with mineral medium on H_2/CO_2 (80:20), at ammonia concentrations ranging from 0.1 - 25 g NH_4^+-N/L at a constant pH of 7 (giving free ammonia concentrations between 1 - 1070 mg/L). The isolates were either directly transferred from a low ammonia level to different higher levels, or allowed to gradually adapt to increasing levels of ammonia. Methane production curves were then used for calculation of growth rates from the exponential growth phase. The production of osmolytes was analysed with high-resolution magic-angle spinning intracellular (HR-MAS) NMR. The results showed that both non adapted and adapted cells grow equally up to approx. 500 mg/L ammonia. At higher levels there was a quick decline in growth rate of the non-adapted cells. However, adapted cells could grow up to as high as 1070 mg ammonia/L, albeit at decreasing rates. This level of ammonia tolerance is much higher then previously reported for methanogens. HS-MAS analysis showed presence of high intracellular concentrations of the osmolyte beta-lysin during growth with ammonia. With methanogens, this osmolyte has previously only been detected during growth in presence of high levels of sodium chloride.
The project is a part of the thematic research program MicroDrivE (Microdrive.slu.se).

2388 **IN SEARCH OF FUNCTIONALITY-DIVERSITY RELATIONSHIPS IN ANAEROBIC MIXED CULTURE FERMENTATIONS**

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Based on the work described in this paper we will postulate that in environmental ecosystems with a weak selective pressure no clear relationship exists between the ecosystem functionality and the microbial diversity and microbial composition. In the past years we have been investigating the anaerobic fermentation of glucose, xylose, and glycerol, and mixtures of these substrates in continuously stirred tank reactors (CSTR) inoculated with an activated sludge characterized by a very rich microbial diversity. The overall objective of this project was to determine if a clear relationship could be found between the operational characteristics in terms of environmental conditions and the fermentations patterns observed. Experiments were conducted at different dilution rates, pH-values, substrate concentrations, and with mixtures of substrates. Most of the experimental results could be explained based on generalized biochemical pathways and thermodynamic considerations (Temudo et al. 2007; Temudo et al. 2008). To which extent the fermentation pattern in the system (functionality) could be related to the microbial diversity and composition in the bioreactor was the objective of this research. DNA was extracted from biomass samples from the reactors and the 16S rDNA fragment was analyzed using PCR-DGGE. Particular attention was paid to the response of the microbial community to changes in the operational characteristics, like the substrate concentration. The results of this work have demonstrated that the microbial response of the system upon a change in operation was largely unpredictable: In some cases a clear shift in the functional properties was not associated with a shift in the microbial population. This was the case when the substrate concentration was increased in the glucose and glycerol fed chemostat type reactors, In other cases a shift in functional properties was directly related to a shift in the microbial population composition. This was the case when glucose grown cultures were established at different pH-values or when the substrate concentration was increased in the xylose grown culture. To complicate things further, a predictable metabolic response in some cases can, but in other cases cannot be associated with a change in microbial population. This was what we observed when the influent composition of a bioreactor was changed from a single substrate to a mixture of substrates. The product spectra of the mixtures of substrates fermentation were found to be in line with the product spectra of the individual substrates. This anticipated functional response, was in some cases associated with a complete shift in population, but in other cases no change in the population composition was observed.

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2408 PHYLOGENETIC ANALYSIS OF *Bacteroidales* 16S rRNA GENES FROM HUMAN AND ANIMAL FECAL SAMPLES

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To effectively manage shellfish and water quality in coastal areas, it is necessary to identify the sources of fecal contamination. Microbial Source Tracking (MST) methods based on the identification of host-specific fecal targets serve this purpose. *Bacteroidales*, anaerobic bacteria, present in both human and animal gastrointestinal tract were retained in this study. To identify host-specific genetic markers, *Bacteroidales* 16S rRNA gene libraries were constructed using DNA extracted from feces and effluents (human, bovine, pig) and seabird feces (n= 10 samples for each host library). Chimeric sequences were eliminated (Mallard software) and resulted sequences were assigned to Operational Taxonomic Units (OTUs) when they shared 98% similarity. Phylogenetic trees were built by using neighbour-joining method (500 time resampling analysis). Of the 672 clones screened, a total of 508 clones yielded unambiguous and different sequence data. They resulted in 185 OTUs including 36, 73, 51 and 15 OTUs for human (feces and sewage treatment plant or STP, water samples), bovine (feces and slurry samples), pig (feces and slurry samples), and seabird (feces samples) origin, respectively. The main part of OTUs were *Bacteroides*-like in fecal samples from human (71 and 67% for feces and STP water samples, respectively), bovine (77 and 86% for feces and slurry samples, respectively) and seabird (64% for feces samples) origin whereas 92 and 67% of the OTUs were *Prevotella*-like in pig feces and slurry samples, respectively. The distribution of OTUs revealed at least one distinct host-specific cluster from human, bovine, pig and seabird fecal origin useful for designing host-specific real-time PCR primers to discriminate between human and animal sources of fecal contamination.

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2407 ESTIMATION OF PIG CONTAMINATIONS IN CATCHMENT BY REAL - TIME PCR USING TWO PIG-SPECIFIC *Bacteroidales* 16S rRNA GENETIC MARKERS

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The microbiological quality of coastal and river waters can be deteriorated by fecal contamination from human or animal activities. In Brittany, France, pig farming is one of the most important animal activities and can cause fecal contamination of surface water due to spreading of pig manure to agricultural soils and in a lesser extent with the use of lagoon surface water for irrigation. In order to discriminate these fecal pollutions from others in the environment, a library independent microbial source tracking method was selected: *Bacteroidales* host-specific 16S rRNA gene markers. Based on *Bacteroidales* 16S rRNA gene sequence analysis from pig feces and pig slurry, 27 and 24 Operational Taxonomic Units (OTUs) were obtained. These OTUs were used to build a phylogenetic tree and to design 2 pig-specific *Bacteroidales* markers (Pig-1-Bac and Pig-2-Bac) for real time PCR using TaqMan assay. The two markers showed 100% sensibility and specificity. Quantification was performed on 20 pig fecal samples and on 17 slurry and 9 lagoon water samples representing 2 steps of slurry treatment. A decrease in the contamination was observed throughout the fecal, slurry and lagoon water samples with an average of 8.5 $\log_{10}$ copies per g of wet feces, 6.7 and 4.6 $\log_{10}$ copies per 100 ml of slurry and lagoon water samples respectively. These results indicated that despite the action of slurry treatment, pig-specific *Bacteroidales* markers could be amplified. These two pig-specific *Bacteroidales* markers were applied to river water samples from the catchment of Daoulas estuary (Brittany, France) collected on 14 different sites with 6 sites being located near pig farms. Amplification with both markers was observed in the 6 sites near pig farms and on 2 sites downstream the estuary with a concentration ranging from 2.6 to 4.1 $\log_{10}$ copies per 100 ml of water. The two markers had the same efficiency for slurry and lagoon water samples. However, the Pig-2-Bac marker was better for the quantification of pig-specific *Bacteroidales* in natural water samples. Real-time PCR using this marker will be applied to environmental studies aiming at discriminating pig pollutions from other animal or human contaminations.

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2332 AN ASSESSMENT OF SYNTHETIC LANDFILL LEACHATE ATTENUATION IN SOIL BENEATH A LANDFILL AND ITS SPATIAL AND TEMPORAL IMPACT ON BACTERIAL COMMUNITY DIVERSITY

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Diverse redox environments may develop within a leachate plume as a consequence of strongly reduced leachate entering the soil beneath a landfill. The development of such environments is dependent on the biological and chemical characteristics of the leachate and the receiving subsurface soil. The aim of this study was to investigate the temporal and spatial succession of Bacteria involved in the potential attenuation of a synthetic leachate entering the subsurface below a landfill. The temporal fate of parameters such as redox potential; pH; phenol; copper and zinc, of a synthetic landfill leachate was assessed by irrigating a series of sequential soil microcosms (SSMs) at two hydraulic loading rates (HLRs). We chose HLRs that were representative of areas in South Africa receiving high (HLRh) and low (HLRl) rainfall. Polymerase Chain Reaction (PCR) and Denaturing Gradient Gel Electrophoresis (DGGE) were used to assess the temporal and spatial succession in Bacterial community diversity for each SSM. DGGE fingerprints generated data that was applied to the Shannon-Weaver Index *(H')* and Shannon-Weaver Evenness Index (EH). Moreover, the effects of phenol concentration, redox potential, and pH on Bacterial community diversity were tentatively assessed by three-dimensional graphical representation on PlotIT 3.2 software (Scientific Programming Enterprises). The results suggested that there was a significant shift in Species Richness (S) and *H'* for both HLRs but a significant change in EH was only observed for HLRh. Temporal comparisons of S and *H'* between both HLRs revealed significant differences throughout the investigation. Canonical Correspondence Analysis (CCA) revealed spatial distribution of bacterial community diversity with depth. Overall Bacterial community diversity showed a decrease with elevated pH and phenol concentrations along with decreasing redox potentials for both HLRs This study provides important evidence with respect to the effects of rainfall / leaching rates on spatial and temporal Bacterial community succession; and the ability of soil, previously unexposed to landfill leachate, to initiate natural attenuation of leachate constituents. Furthermore, it demonstrates the capacity of PCR-DGGE to fingerprint successional changes in Bacterial community diversity, and the potential to clone and sequence selected members of bacterial associations for future reference in environmental remediation strategies.

2393 EFFECTS OF ORGANOCLAYS ON SOIL MICROBIAL POPULATION ASSESSED BY MOLECULAR APPROACHES

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In recent years organoclays have attracted great interest because of their academic and industrial importance (Ray and Okamoto, 2003). Organoclays are an important type of modified clay material; they are synthesized by introducing cationic surfactants such as quaternary ammonium compounds into the interlayer space through ion exchange (Chaiko, 2002). Recent studies have shown the role of organoclays in the adsorption of pesticides and their applicability for bioremediation and development of herbicide formulations capable to retard the release and reduce the leaching of the active ingredient.
Studies concerning the toxicity of organoclays towards some microorganisms have been reported (He et al., 2005; Yang *et al.*, 2007), however nothing has been done on the interaction between these materials and soil microbial communities. On these bases, the objective of this study was to evaluate the effects of three different organoclays (Cloisite 30B, Nanofil 804 and Dellite 26C) on the microbial population isolated from compost by ARDRA and DGGE approaches. An enrichment test was carried out in Nutrient Broth containing the organoclay and the microbial population. Four transfers were made, each after 7 days incubation at 30 °C, in the dark and under continuous shaking. The molecular analyses on the microbial population were performed before treatment and 7 days after each addition of organoclay. DNA was extracted according to Martin-Laurent (2001), amplified with eubacterial primers, finally analysed by ARDRA and DGGE. ARDRA and DGGE gels showed similar profiles in terms of number and intensity of bands among the three different tested organoclays. However, the profiles of the samples treated with each organoclay showed the absence of a band, the appearance and an increase in the intensity of others. These bands were excised from the gels in order to identify, by DNA sequencing, microorganisms influenced by organoclays.

1829 THE EXOTIC INVASIVE PLANT, AMARANTHUS VIRIDIS, SUPPRESSES THE GROWTH OF NATIVE ACACIA BY ALTERING SOIL MICROBIAL COMMUNITIES STRUCTURE AND FUNCTIONALITIES IN A SAHELIAN ECOSYSTEM

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The functioning and stability of terrestrial ecosystems are mainly determined by plant specific richness and composition, which in turn, are closely interlinked with soil organisms development, in particular, soil microorganisms. One of the main success ways of invasive plants was these exogenous organisms-mediated modifications in soil microbial communities' composition and diversity as well as their functioning, thus compromising native plant survival. The aim of this experiment was to study the effect of the invasion of *Amaranthus viridis*, an exotic herbaceous plant, on soil microbial communities' structure and functioning in a Sahelian ecosystem, and on the survival of indigenous acacia species seedlings. At IRD field research station in Dakar (Senegal), soil samples have been collected on areas colonised by *A. viridis* plants and on areas colonised by others non invasive herbaceous species. Chemical characteristics, arbuscular mycorrhizal fungi spores and hyphal length, bacterial communities' structure (molecular fingerprints) and microbial enzymatic capabilities were determined on the soil samples. Moreover, we tested the effect of inoculation with mycorrhizal propagules on the growth of acacia seedlings. The results indicate significant higher soil content in C, N and P after the invasion by *A. viridis*. Moreover, these changes are accompanied by a higher overall microbial activity and a higher number of 16S gene copy in soil under the invasive plant. However, this exotic plant significantly decreases arbuscular mycorrhizal propagules present in soil and alters soil bacterial communities' structure and soil microbial functioning. Furthermore, an increase in soil mycorrhizal infectivity could make it possible to mitigate the depressive effect of this invasive plant on the development and nodulation of acacia species. These results clearly highlight profound shifts mediated by the exotic plant *A. viridis* during its invasive process, and, these modifications therefore alter the global functioning of plant communities. Moreover, mycorrhizal symbiosis can act as potential biological tool for invaded area restoration.

2396 MICROBIAL DIVERSITY OF A HIGH SALINITY OIL FIELD

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This work is a preliminary study to investigate the microbial diversity of an onshore oil field. It aims to compare results obtained from molecular methods, physicochemical analyses and cultivation. A core of 1150 m depth sediments (*in situ* T = 45 °C) was collected and immediately frozen with liquid nitrogen prior to further investigation. Macroscopic and Scanning Electron Microscopy analyses were performed. The sample corresponded to unconsolidated, porous sandstones with rich quartz/feldspar and lithic grains. Most of the grains were coated with clays. Salt crystals were abundant on the grain surfaces and within the clay coating. pH of the sediment was measured at 8.8, salinity corresponded to saturation. The sediment contained high amount of hydrocarbon. Major and minor metal concentrations were measured by ICP-AES. Carbon, nitrogen, sulfur and phosphorus concentrations were also measured. DNA extraction was performed with commercially available kits. To improve our DNA recovery, washing steps were performed prior to extraction according to Fortin *et al.*, 2004. PCR was effected with primers targeting 16S rRNA genes (archaeal and bacterial) and functional genes involved in sulfate reduction, nitrate and nitrite reduction, methanogenesis, alkane degradation and carbon assimilation. PCR products were cloned using the TOPO TA cloning kit according to the manufacturer's instructions. We didn't obtain amplification of *dsr*AB, *nar*G and *mcr*A genes whereas *nir*S and *nir*K genes were amplified. According to the in-situ physico-chemical conditions and to the cloning-sequencing results, culture conditions were defined to isolate representative microorganisms. No sulfate-reducing, nitrate-reducing or methanogen have been isolated. In conclusion, geological investigation showed that the sediment was representative of a inland body of salt water, contemporary to the Atlantic ocean opening. Present microbial flora could have been then fossilized.

2281 MICROBIAL ECOLOGY OF ARTISANAL ITALIAN CHEESE: MOLECULAR MICROBIAL CHARACTERIZATION BY CULTURE-INDEPENDENT METHOD

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Present study will treat the next topics: ecology of the natural and man made environments and functional diversity of bacteria. The microbial communities in artisanal goat cheeses produced in mountain pastures (typical farms) in Piemonte mountain (North of Italy) change a lot during processing and ripening time. Moreover cheese microbial ecosystems are different in each small dairy because adventitious microflora can come from the environment and contaminate the milk before the cheese making process and the product during manufacture and ripening. For this reason it becomes very important to study the microbial communities that are present on different products because the final cheese typicity could be species or even strain dependant. The aim of the present study was to obtain pictures of the dynamics of microbial ecosystems from different artisanal dairy products at different times by using culture-independent methods. This included DNA extraction from milk and cheese and environmental samples and LH-PCR analysis. The fingerprints of the bacterial communities was obtained after amplification of partial region of the 16s rRNA genes from different producers. The microbial identification of the LH-PCR patterns was obtained both by sequencing 16s rRNA of isolates from counting media and by direct identification from clone libraries after they were analyzed by LH-PCR. So the resulting peaks were compared to the bacterial pattern of the cheese. This study investigated different farms that produce goat cheese in spring in valley and in summer in mountain pasture in order to underline the differences in microbial ecosystem between the products coming from semi-industrial and from typical environment. The LH-PCR fingerprints of products were quite different in the small dairies and the LH-PCR profiles changed a lot during cheese making and ripening time. LH-PCR revealed a useful and reproducible tool to quickly control the cheese microbial population.

2345 BACTERIAL AND FUNGAL INDOOR AIR CHARACTERIZATION IN TWO NEW BIOCLIMATIC BUILDINGS

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Nowadays, people spend most of their time inside buildings where the air quality might not be good enough due to the high concentration of pollutants like Volatile Organic Compounds (VOCs), bacteria or fungi. These factors could be the cause of allergies, asthma or a group of symptoms called Sick Building Syndrome (SBS). Most of the new saving energy buildings have a poor natural ventilation of their rooms that provokes an increase in pollutants and causes a discomfort feeling to the people exposed. On this work it has been studied the evolution of fungal and bacterial populations in two new bioclimatic buildings located in Spain. Three sample collections have been done and several indoor and outdoor points were analysed. Bacterial samples were incubated using Nutrient Agar Petri Dishes. Every colony was isolated and the 16S rRNA gene was amplified by the Polymerase Chain Reaction (PCR), sequenced and the closest relative identified by sequence comparison. Fungal samples were collected using Malt Extract Agar growth media. Every fungal colony was isolated and identified by their morphological and microscopic features. Some of the retrieved bacterial sequences corresponded to *Bacillus subtilis, Bacillus cereus, Pseudomonas syringae* and *Arthrobacter aurescens*. Fungal identifications showed the presence of species mainly belonging to Dematiaceous group, Mucorales order and the genera *Aspergillus* and *Penicillum*. Most of the species identified have been isolated previously in soil, water and plants, and some are of human origin. Furthermore, some strains could be human pathogens and their level should be controlled to avoid the transmission of airborne diseases. Details about the sampling and results will be presented in this communication.

2383 MICROORGANISMS INTERACTING IN A BIOFILTER

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Biofilm microorganisms developed on a biofilter support media allow the metabolism of volatile organic compounds (VOCs) to carbon dioxide and water. VOCs are present in polluted gaseous streams from varied industrial activities. The main objective of this study was to identify the microorganisms present in the biofilm developed on a biofilter support media using molecular biology techniques. These microorganisms degraded methyl alcohol as the model pollutant. The identification of the biofilm native microorganisms was carried out when an elimination efficiency of methanol greater than 80% was achieved in the biofilter. Grape seeds were used as support media in the biofilter. The isolated microorganisms were characterized using the biochemical tests API-20NE galleries for bacteria, and API-AUX and API-32C for yeasts, and the genomic DNA extraction method. Biofilm microorganisms were also identified using the oxidase and catalase enzymes tests. The molecular analysis of the isolated bacteria by 16S and 26S rRNA-based assay was realized according to the modified protocol of Ballesteros-Rodea (2006). The growth of the filamentous fungi was made in subcultures in plate with malt extract agar and potato dextrose agar (Bioxon). These fungi were identified by their morphology and in according to the taxonomic keys of Raper and Fennell (1997), Booth (1997) and Ellis (1971). Moulds and yeasts were identified by molecular analysis according to the modified protocol of Tapia-Tussell et al. (2006). The isolated DNA of the moulds was quantified with spectrophotometry with a DNA concentration ranging from 0.08425 to 4.85 Ìg. The isolated bacteria were identified first with API galleries and then with readings of the sequence data at the National Center of Biotechnology Information (NCBI). *Stenotrophomonas maltophilia, Pseudomonas putida* and *Pseudomonas fluorescens, Micrococcus* sp, *Kocuria rosea* were identified with an homology greater than 98%. Other microorganisms that were also identified include the yeast *Rhodotorula mucilaginosa*, associated to the microflora of the grape, *Trichosporon asahii*, and the moulds *Cladosporium cladosporioides*, and *Fusarium oxysporum*. The results showed that the bacteria and moulds identified were native microorganisms of the grape seeds. It is hypothesized that moulds can also degrade methanol in the biofiltration system.

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2877 INTEGRATED ENVIRONMENTAL MANAGEMENT OF WASTEWATER DISCHARGED FROM A CLUSTER OF FIVE TEXTILE FACTORIES

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The study focuses on the cleaner production opportunities for a cluster of five factories discharging their wastewater into agricultural drain prior to wastewater treatment the financial and environmental benefits accrued upon the implementation of the proposed cleaner production schemes have been estimated. This was followed by a laboratory study to propose the appropriate treatment system. Composite wastewater samples representing the wastewater discharged from the five factories were mixed (according to their discharge flow) and subjected to treatment. The investigated treatment schemes included: chemical, biological (aerobic & anaerobic), anaerobic followed by chemical and anaerobic followed by aerobic. The results obtained indicated that the use of chemical treatment or anaerobic treatment is not sufficient to produce a wastewater complying with the National standards regulating discharge of industrial wastewater into receiving water bodies. However, activated sludge at a hydraulic retention time of four hours, or anaerobic treatment followed by either chemical or activated sludge treatment produced treated wastewater which can be safely discharged into the agricultural drain. Based on these results, an up-flow anaerobic sludge blanket reactor (UASB) has been operated on a continuous basis. The effluent of the reactor was subjected to either an activated sludge treatment or chemical treatment. The results obtained indicated that the use of an UASB reactor at a hydraulic retention time of 8hr, followed by activated sludge treatment (HRT-2hr) achieved very good results. Also, the combination of UASB reactor with chemical coagulation/flocculation /precipitation, using ferrous sulphate and lime was very promising. Both treatment schemes produced treated wastewater which can be safely discharged into the agricultural drain. Based on cost analysis, the treatment scheme consisting of UASB followed by chemical treatment was found to be the most economic.

2308 HYDROLYSIS OF RESIDUALS OF BARLEY STRAW USING WHITE-ROT BASIDIOMYCETES

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The imminent term of the fossil fuels has generated different initiatives focused to the development of alternative fuels in the entire world, one of the main alternatives for the biocombustibles production is the agricultural waste, all they have as main characteristic those of being compound for 3 biopolymers that represent, one of the biggest renewable sources of energy. However, one of them, the lignin, for its complex structure chemistry, avoids so much the hemicelluloses as the celluloses to be available to be metabolized by microorganisms. Diverse methods of hydrolysis of the lignocellulosic residuals have been developed, until the moment, the chemical and physical methods those that dominate this process, however, the hydrolysis yields don't reach beyond 20%. This low yield is attractiveness for biofuels production. In the nature exist microorganisms that are able to degrade mainly to the lignin leaving as residuals to the hemicelluloses and cellulose. These microorganisms are the calls white-rot basidiomycetes. These mushrooms generate a complex enzymatic battery compound mainly for the enzymes laccase, lignin peroxidase, manganese peroxidase and peroxidases. The objective of the present work was the one of increasing the degree of hydrolysis of agricultural residuals using mushrooms basidiomycetes, to reach this objective; two mushrooms basidiomycetes isolated of a subtropical area of Mexico were grown on barley straw. After 45 days of incubation in static reactors, it was analyzed the degradation of the ligning and the liberation of fermentable sugars using an enzymatic extract of *Tricoderma reseii*. The results showed that the strain 52.2 was the it showed the biggest index in degradation of the lignin, since to the hydrolizar the product of the fermentation with the extract of *T. reseii*, a yield of 25 % hydrolysis it was obtained.

2465 APPLYING INDUSTRIAL ECOLOGY IN AN ORGANIC FARM

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This project aims to show an organic farm as a model of industrial ecosystem and to improve some aspects in order to be able to apply in it some of the Industrial Ecology (IE) principles. Industrial Ecology can be defined as a multidisciplinary approach the ultimate goal of which is to have industrial systems operate like natural ecosystems by having industries, society and nature interact mutually in cycling matter and increasing process efficiency. One of the aims of the mutual cooperation of industries is to achieve zero emission/zero waste. This can be partly accomplished by having an industry use by-products and waste from another similar to the cycling of matter in natural ecosystems. The Rural Production System Xochimancas was chosen (Delegation Tlalpan, Mexico, D.F.) as a case of study that operates under the vision of Organic Agriculture and the exchange of wastes as raw materials. It may be seen as an industrial ecosystem model because of the great number of material and energy exchanges and sinergies existing in this farm. Xochimancas produces organic vegetables, and biofertilizers by means of vermicomposting, Bocashi type composting and by anaerobic decomposition. There are also some animals in the farm and a Temascal (a vapour bath). In order to apply the IE criteria on the farm, material, energy and information flow charts were developed. Physical and chemical analyses were also made to determine the quality of the product and wastes of each process; these analyses were: humidity, pH, nitrogen, phosphorus and organic matter. A sustainable set of indicators (environmental, social and economic) was defined to measure the sustainability of the system and some of the indicators value was calculated. The analysis of the charts allowed us to made new proposals in order to improve the exchange of wastes as raw materials. One of them is the use of the methane produced in composting as fuel. Physical and chemical analyses of the composting showed they achieved the nutrient requirements for their use as fertilizers. Based on the indicators value we say that the Xochimancas system tends to sustainability.

2455 EFFECT OF TYPE OF FUNGAL CULTURE, TYPE OF PELLETS AND pH ON THE SEMI-CONTINUOUS POST-TREATMENT OF AN ANAEROBICALLY-PRETREATED WEAK BLACK LIQUOR FROM KRAFT PULP INDUSTRY

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It is well known that fungi belonging to the Basidiomycete (such as *Trametes versicolor, Lentinus edodes, Phanerochaete chrysosporium*) are microorganisms with a demonstrated capability of degrading lignin and its derivatives using a powerful and diverse group of enzymes. Because of these features, ligninolytic fungi have been used for the treatment or post-treatment of a variety of recalcitrant and toxic effluents, those of the Kraft industry among them. Yet, most of reported fungal treatments so far required the supplementation with glucose or other soluble carbohydrates, pH 4 to 4.5, and their effective performance was demonstrated only for short periods of operation time. Information on extended treatment and performance at higher pH, although paramount for practical application and feasible scale-up of fungal treatment, is still scarce. For instance, it is useful to know whether a fungal treatment could still achieve a reasonable removal efficiency of pollutants at pH near neutrality, since this could mean significant savings in construction materials of the bioreactor. The objective of this work was to evaluate the semi-continuous post-treatment of anaerobically-pretreated weak black liquor wastewater (AP-WBL) from Kraft pulp and paper industry by aerobic post-treatment using hybrid pellets of *Trametes versicolor, Lentinus edodes* and defined mixed culture of the two fungi at pH 4.5 and 6.5. A statistical factorial experiment was carried out with three types of fungal culture (*Trametes versicolor*, Tv; *Lentinus edodes*, Le; and a defined mixed culture of Tv and Le named here DMC), pellets (double and triple pellets) and two pH levels (4.5 y 6.5). All fungal cultures were immobilized either on a mixture of oak-holm sawdust and powdered activated carbon (triple pellets) or only on sawdust (double pellets). In this way, biocatalysts that were true hybrid pellets were formed (mycelium surrounding support media). Tests were carried out in repeated batch loads of anaerobically-pretreated weak black liquor with incubation times of 7 days per cycle. A total of 10 cycles were run. At the end of each cycle, spent AP-WBL was removed and fresh AP-WBL was loaded, whereas the hybrid pellets remained in the flasks remained during the whole experiment. Control flasks with sterilized hybrid pellets were also run. The main response variables were the conventional removal efficiency and the unit, net removal efficiency of color, ligninoids, and COD. Triple pellets presented the highest values of unit, net removal efficiency of color, ligninoids and COD with 44, 28 y 40% respectively. For specific, unit, net removal a similar pattern was observed. Regarding the effect of culture type, it was observed that the treatment with defined mixed culture achieved the highest unit, net removal of color and lignoids, with averages of 31 y 28 % respectively. The highest color and lignoids unit net removals were registered at pH 4.5 with values 30 and 28%, respectively. In general manganese peroxidase of crude extracts showed higher activity than those of lignin peroxidase and laccase. Interestingly, maximum activities of Mn P were associated to high values of proteases. In conclusion, best conditions for semi-continuous postreatment of APWBL are pH 4.5, defined mixed culture, and triple pellets.

2558 NITROGEN RECOVERY FROM CONCENTRATED AMMONIUM WASTE STREAMS THROUGH PARTIAL NITRIFICATION

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With the current increasing energy prices, sustainable techniques for recovery and reuse of nitrogen from concentrated ammonium waste stream become more interesting and competitive. In this work, we propose an innovative N-recovery process scheme in which valuable ammonium nitrate (fertilizer) is produced from the high N-content liquid fraction of anaerobically digested manure. The process consists of biological partial nitrification and subsequent product concentration by vacuum evaporation. Efficient use of thermal energy generated from the combustion of biogas (produced in the anaerobic digester) enables this N-recovery within the plant by providing energy for concentration of valuable (by)-products. These high added value products will increase the overall economic viability of the manure digestion plant. This study particularly aimed at investigation of biological partial nitrification to produce ammonium nitrate from such highly concentrated ammonium streams i.e. 6000 mg NH_4 - N/L (+430 mM-NH_4^+). We succeeded to produce stable (> 6 months) NH_4NO_3 solution through aerobic partial nitrification in a 10 L continuously mixed reactor (CSTR) at 35 ºC and 5 days hydraulic retention time (HRT). A synthetic concentrated wastewater medium containing NH_4HCO_3 and trace elements was used in the experiment. Reactor was started-up with lower initial N-load and gradually increased in time. The process was self-regulated, no pH control was needed. The proton produced by microbial ammonium oxidation is buffered by bicarbonate through stripping of CO_2. Steady-state results showed that approximately 50% of the ammonium was biologically oxidized to (about 212 + 5 mM) nitrate, with virtually no residual nitrite (< 2 mM). To our knowledge, this was the first time an attempt has been successful to achieve NH_4NO_3 product at extreme N-concentration in CSTR. Batch experiments and modelling were being performed to determine the kinetic and growth characteristics of nitrifying bacteria (ammonium and nitrite oxidizers) at this extreme condition. These characteristics will be the basis for further process optimization. Several molecular techniques for identification of microbial community and diversity i.e. FISH (fluorescence in situ hybridization) and PCR-DGGE (denaturing gradient gel electrophoresis) is also being performed. These results are to be included in the final paper.

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2317 SURFACE RENEWAL ANALYSIS FOR ESTIMATING TURBULENT SURFACE FLUXES.

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A decade ago, the need for a long-term surface monitoring was recognized to better understand the soil-vegetation-atmosphere scalar exchange and interaction processes. The AmeriFlux concept emerged in the IGBP workshop (La Thuile, IT, 1995). Continuous acquisition of surface fluxes for different species such as temperature, water vapour, CO_x, halocarbon, ozone, etc., and momentum allows determination of the influence of local (canopy) exchanges, fossil fuel emissions, large-scale biotic exchange on ambient concentrations which are crucial to take decisions for protecting natural environments and water resources, to develop new perspectives for modern agriculture and forest management and to better understand the global climate change. However, projects such as AmeriFlux that involve the most advanced technology at α scale are prohibitive for most countries. Hence, development of affordable and reliable measurement methods is a challenge for new perspectives.
Here, it is shown the performance of Surface Renewal (SR) analysis for estimating sensible heat (H), latent heat (LE) and carbon dioxide (F) fluxes over rangeland grass as part of Ameriflux at Sierra Nevada biome (Ione, CA, USA). According to Drexler et al. (2004), SR analysis (Paw U et al., 1995 and 2005) is the lowest budget micrometeorological method for estimating turbulent fluxes and offers tremendous advantages over other existing methods. Climate patterns at Ione are those typical from cold and humid fronts passing through a mountain from late fall to late spring, and no rainfall, light winds, high temperatures and formation of regional advection for the rest of the year. Similarity theory is limited under such conditions. Net radiation (Rn), soil heat flux (G), the three-wind speed components, temperature, water vapour and carbon dioxide were continuously measured at 10 Hz during years 2001-2004. SR analysis was applied as described in Castellvi (2004) because enhance earlier SR analysis models as an independent method. Regardless of the weather conditions, the half-hourly SR flux estimates for all three scalars were close to those measured using the Eddy Covariance method and provided a good closure of the surface energy-balance equation (Rn - G = H + LE + F). It was concluded that SR analysis provided reliable flux estimates under non-ideal conditions. This study is described in Castellvi et al. (2008).

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1881 BIODEGRADATION OF ORGANIC CHEMICAL POLLUTANTS AND THEIR BIOCONVERSION INTO USEFUL COMPOUNDS WITH THE DISCOVERY OF NOVEL ENZYME AND GENE SYSTEMS

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Microorganisms maintain the biosphere by catalyzing biogeochemical processes, including the biodegradation of organic chemical pollutants. Although most organic chemicals are artificially synthesized, some microorganisms can degrade them into inorganic end products, carbon dioxide, ammonia, water, etc. These organisms are thought to be mutated in the environment and to obtain the enzyme and gene systems responsible for catalyzing the decomposition of new artificial chemicals. For these reasons, it is expected that novel enzymes and genes would be found through the studies on microbial degradation of organic chemical pollutants.

Aromatic amines are important industrial raw materials and intermediates used for the production of dyes, plastics, herbicides, etc. Studies of the biodegradation of aromatic amines as environmental pollutants have been made. *Pseudomonas* sp. AP-3, which was isolated from soil and decomposed 2-aminophenol, produced a unique dioxygenase, 2-aminophenol 1,6-dioxygenase. The sequence analysis of its gene revealed that the enzyme was quite novel. This enzyme catalyzed the ring cleavage of 2-aminophenol to form 2-aminomuconic 6-aldehyde, which was then converted nonenzymatically into picolinate. Thus the dioxygenase served as an excellent catalyst to produce various picolinate derivatives from 2-aminophenols, because the enzyme had extensive substrate specificity.

Bacillus cereus 10-L-2 isolated on a medium containing 4-phenylenediamine and peptone converted a wide variety of anilines including 4-phenylenediamine into their corresponding acetanilides. The enzyme catalyzing such acetylation was purified from cell extracts of the strain and identified as arylamine *N*-transferase. The enzyme, in particular, acetylated only one amino group of 2- and 4-phenylenediamine.

Aniline-degrading *Rhodococcus* sp. AN-22 was found to retain a constitutive expression system for the catabolic *catABC* genes encoding catabolic enzymes for aniline. Because the actinomycetes *Rhodococcus* strains have metabolic abilities of substituted hydrocarbons and other chemicals and solvent tolerance, an AN-22-based host-vector system will be useful for many applications to bioremediation, biodegradation, and bioconversion without an inducer such as IPTG.

We thus found a variety of novel enzymes and gene expression systems for catabolizing environmental pollutants, aromatic amines, in soil bacteria. These will provide us with new methods for the production of useful compounds and systems without adding an excessive load to the environment.

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THURSDAY 25 SEPTEMBER 2008 ORAL COMMUNICATION

IV MICROBIAL ECOLOGY

VI NEW PERSPECTIVES

GUEST LECTURE

2871 EFFECT OF SULFADIAZINE ON THE ABUNDANCE OF GENES INVOLVED IN THE N TURNOVER IN AGRICULTURAL SOILS

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Veterinary antibiotics are frequently used in animal production to treat diseases but also to protect health and to improve growth rate and feed efficiency. Sulfadiazine (SDZ) belongs to the group of sulfonamides which are administered to a great extent in pig production. They are poorly adsorbed in the animal gut so that most of them are excreted unchanged in urine and feces. Via manuring of animal waste, veterinary drugs may enter the environment and possibly compromise important soil functions, e.g. biochemical turnover processes in the N cycle.
In a pre-experiment, we investigated the impact of pig manure spiked with two concentrations of SDZ on the functional diversity of indigenous soil microbes involved in the N transformation processes in two different agricultural soils. The functional genes amoA, nirS, nirK, and npr coding for the ammonia monooxygenase, the cytochrome cd1- and copper-containing nitrite reductase, and the neutral metallopeptidase, respectively, were quantified by real-time PCR in the different treatments after up to 60 days. The results revealed in the SDZ treatments in both soils after 32 days clearly reduced numbers of amoA and nirK genes and simultaneously slightly elevated copy numbers of nirS genes. Interestingly, the number of archaeal amoA genes increased at the same time when bacterial amoA genes decreased, indicating an alteration of the functional microbial community due to SDZ application. In the main experiment, fresh and aged manure of pigs that have been fed with and without SDZ were applied to an arable soil. Data of the influence of SDZ on the abundance of the functional genes will be presented.

GUEST LECTURE

2911 ## MICROBIAL TREATMENT OF HEAVY METAL LEACHATES

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Ore-mining, metallurgy and other industrial activities represent the source of heavy metal and radionuclide contamination in terrestrial and aquatic environments. Physico-chemical processes are employed for heavy metal removal from industrial wastewaters. However, limitations due to the cost-effectiveness and use of contaminating reagents make these processes not environmentally friendly. Biotechnological approaches can overcome the limitations of physico-chemical processes. Biological alternatives for heavy metal removal are based on physical, chemical and metabolic properties present in microorganisms. Among the different methods, metal precipitation by biogenic sulfide and biosorption are the most relevant. Biogenic sulfide produced by bacteria in an anaerobic process dependent on the use of sulfur moieties as electron acceptors. Due to the combined removal of acidity, metals and sulfate, sulfate-reduction is the most promising process for the treatment of acid mine drainage. On the other hand, biosorption is based on the microbial uptake of organic and inorganic metal species by physico-chemical mechanisms, described as adsorption.

Due to the number and geographical distribution of mines, the Bolivian Andean region is seriously affected by mining contamination. Moreover, scarce technology resources make it difficult to manage the metal pollution. For these reasons, the development of a robust system to produce biogenic sulfide, which will be operated with a minimum of surveillance, is needed. In this context, two important issues were addressed: 1) dependency on the concentrations of electron donor and acceptor and/or 2) an increase in the available sulfate-reducing bacteria (SRB) biomass growth-surface. The use of agricultural and solid wastes, widely available, proved to be a good source of electron donors for sulfide production. Bacteria able to produce sulfide can either utilize macromolecules or use organic matter fermentation products as electron donors.

The use of cellulosic/hemicellulosic raw materials and volatile fatty acids resultant from degradation of solid organic matter were demonstrated to be suitable electron donors alternatively to those traditionally employed (e.g. lactic acid, ethanol). Sulfide productions of 5 mM and 16 mM were achieved when wheat straw and total volatile fatty acids were used, respectively. The augmentation of cell density through the establishment of microbial growth in biofilm packed bed reactor, allows the enhancement of sulfide production. Productions of 15 and 9 mM were achieved using Poraver® and pumice stones respectively using lactic acid as electron donor. While using total volatile fatty acids, a production of 16 mM was achieved.

The utilization of molecular tools such as 16S rRNA gene amplification, nested PCR and Fluorescence *in situ* hybridization (FISH) allowed the identification of sulfidogenic bacteria present in the different inocula employed in the present study. Additionally, two new bacterial species *Clostridium boliviense* and *Clostridium algarum*, which are able to produce sulfide using xylan as electron donor and sulfite and thiosulfate as electron acceptors, were described.

Heavy metal precipitation and biosorption by algal-bacterial biomass have the possibility of being combined with waste and/or wastewater treatment processes such as anaerobic digestion and, biological oxygen demand (BOD) and nutrient removal contributing further to achieve a sustainable process.

Since most of the mines are located in areas where accessibility is limited, combination of anaerobic digestion of biomasses available in the region and biological sulfide production may be advantageous. On the other hand, the advantage of using the algal-bacterial biomass is that selective removal of a particular heavy metal can be achieved. In addition, the algal bacterial biomass used as adsorbent agent can be regenerated and reused.

Metal sulfide precipitation over 90% was achieved with a mine leachate (from a mine located in the Altiplano) containing Pb(II), Zn(II) and Cu(II), while comparing with algal bacterial heavy metal biosorption, Cu(II) was selectively removed from a mixture of metals in a artificial leachate.

Key words: Bioremediation, heavy metals removal, sulfate-reducing bacteria, municipal and agricultural solid wastes, organic matter degradation, biogenic sulfide, metal precipitation, biosorption, algal-bacterial symbiosis.

2400 GROWTH RESPONSE AND TOLERANCE TO HEAVY METALS OF TWO SWAMP SPECIES INOCULATED WITH A PLANT GROWTH-PROMOTING RHIZOBACTERIA.

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Due to the sensitivity and the sequestration ability of the microbial communities to heavy metals, microbes have been used for bioremediation. Recently the application of plant growth-promoting rhizobacteria (PGPR) for the bioremediation of this kind of contaminants has been done. This study evaluated the growth response and the tolerance to heavy metals of two swamp species: *Cyperus elegans* and *Echinochloa* sp. inoculated with a rhizobacteria (strain XIII) and exposed to cadmium and zinc. Seeds of both species were surface-sterilized with 10% sodium hypochlorite and then thoroughly rinsed with sterile distilled water, these sterilized seeds were incubated for 30 minutes at room temperature either in sterile distilled water as a blank control or in a bacterial suspension in distilled water of the strain XIII, adjusted to an absorbance of 0.5 (5×10^7 CFU/mL) at 600 nm. Twenty seeds of each species treated and non treated with bacteria, were placed separately in baby food flasks with Magenta SIGMA caps, containing mineral medium and 6% bacteriological agar, supplemented with the appropriated Cd ($CdSO_4$): 0.01, 0.05, 0.1 mM and Zn ($ZnCl_2$): 3, 4, 5 mM concentrations and the control experiments were considerate without the addition of the metals. All the experiments were performed by triplicate and maintained at 30 °C in a growth chamber with photoperiod of 12:12, for 45 days. The growth of plants were evaluated by the measure of their root and shoot length and obtained the tolerance index (TI), expressed as the ratio of the shoot and root lengths of plants grown in the presence and absence of a specific added metal. The growth of both species were favoured by the presence of the strain XIII. The results of TI obtained from *Cyperus elegans* for both metals, showed that the development of plants inoculated with the rhizobacteria was constant and stable in the presence of metals even their concentrations, with a growth percentage between 50 and 70% compared with the growth plants without contaminants and bacteria. Thus, this kind of rhizobacteria thus promotes the plant growth and the tolerance to heavy metals in these species.

2261 ASSESSMENT OF THE EFFECT OF THE HERBICIDE TERBUTHYLAZINE ON A GROUNDWATER BACTERIAL COMMUNITY

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The ability of groundwater to recover from contamination of pesticides is primarily dependent on the presence of a microbial community able to degrade them. Nevertheless, the study of bacterial populations living in groundwater is quite scarce because it is strictly dependent on methods able to identify and characterize their community structure and functioning. In the present work we assessed the structure and functioning of the autochthonous bacterial community of a phreatic aquifer in the presence/absence of the herbicide terbuthylazine (100 μg/L). For this purpose a degradation study was performed for more than 170 days at 15 °C, using laboratory microcosms. The herbicide and the metabolite desethylterbuthylazine were analyzed both in microbiologically active and sterile groundwater samples by gas-chromatography. For all the experimental conditions, and at each sampling time microbiological analyses were performed: total cell number, using DAPI counts, cell viability, using a two-dye fluorescent bacterial viability kit (live/dead), bacterial carbon production, using [^{3}H]leucine incorporation, and bacterial community composition, applying Fluorescence *In situ* Hybridization, using Cy3-labelled oligonucleotide probes were assessed. The chemical results showed that the bacterial community had an active role in the herbicide degradation. In fact in microbiologically active microcosms terbuthylazine was halved in about 150 days, while in the sterile ones about 70% of the initial concentration still remained at 175 days. The overall microbiological analysis showed a change in bacterial community structure and functioning, which was particularly evident between days 80 and 124. In particular, a significant increase in the *Beta-proteobacteria* group was observed in the presence of the herbicide. The last result was confirmed by the isolation and characterization from the same microcosms of two bacterial strains (*Janthinobacterium lividum* and *Adevenella* sp.) belonging to this bacterial group.

2901 NEW BIOCHEMICAL PATHWAY FOR BIPHENYL DEGRADATION IN PLANTS: STRUCTURAL, MECHANISTIC AND BIOTECHNOLOGICAL ASPECTS

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Polychlorinated biphenyls (PCBs) and other structurally-related xenobiotics are amongst the most relevant organic pollutants known today. While some bacterial species can metabolize PCBs, with varying efficiency, no catabolic pathways have yet been described in plants. This is so despite the great potential of (at least some) plant species for soil and groundwater decontamination, a technology known as phytoremediation. In this talk we present a proteomic approach to identify key enzymes in the model organism Populus (poplar, aspen), the genome of which has been fully sequenced. Poplar plantlets were exposed to different concentrations of biphenyl or PCB congeners, and changes in the proteome at different times of exposure were monitored through high-throughput two-dimensional gel electrophoresis (IEF x SDS-PAGE) and mass spectrometry. To avoid microbial interference, all experiments were carried out under axenic conditions, both in hydroponic cultures and on solid media. This strategy has allowed us to identify several poplar proteins induced by biphenyl / PCB treatments. Here we present the characterization of an oxidorreductase, termed Po BIP3 (Poplar Biphenyl - Induced Protein 3) presumably involved in the specific breakdown of biphenyl rings. So far, this activity has been described exclusively in a few prokaryotic enzymes. After isolating the complete gene for Po BIP3, we have followed a multidisciplinary approach that includes (1) the three-dimensional modelling of the mature protein and a detailed study of the enzyme-substrate interaction; (2) an analysis of the possible catalytic mechanism, including thermodynamical aspects (Pacios et al, submitted); (3) the over-expression of Po BIP3 in *Escherichia coli* to analyze its biochemical activity in vitro and undertake structure-relationships analyses; (4) the complementation of *Pseudomonas* mutants deficitary in bph enzymes involved in PCB degradation; and (5) the ectopic over-expression of Po BIP3 in transgenic *Arabidopsis* and *Populus* plants to evaluate its biotechnological potential.

2301 ARBUSCULAR MYCORRHIZAL SYMBIOSIS ALLEVIATES DIESEL TOXICITY ON *Melilotus albus*

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Petroleum hydrocarbons (PH) affect plant growth and development by limiting water absorption and nutrient availability. Arbuscular mycorrhizal fungi (AMF) have been demonstrated to increase plant tolerance of grass species to PH, but the performance of AMF on legume species during phytorremediation of PH-contaminated soils has been scarcely understood. Thus, this research evaluated the effects of AMF on tolerance and growth of *Melilotus albus* in a diesel-contaminated soil. Seedlings of *M. albus* Medik, were inoculated with the arbuscular mycorrhizal consortium *Glomus* Zac-19 (AMF), and established in diesel contaminated sand (7,500 mg kg^{-1}). Non inoculated plants (Non-AMF) under contaminated sand were used as controls. After 60 days, all the plants were harvested to measure total dry mass (DM), leaf area, specific leaf area (SLA), root to shoot ratio (RSR), number and DM of nodules, and the AMF-colonization via the fungal alkaline phosphatase (ALP) vital stain. Diesel exerted significant negative effects on plant growth. Although no significant differences were observed among treatments, AMF plants had greater total leaf area and total DM, and number and DM of nodules, but reduced SLA and RSR than Non-AMF plants. Nevertheless, AMF-plants had significantly increased ($P<0.05$) shoot and leaf DM than Non-AMF. The extent of enhanced shoot DM and leaf DM due to AMF inoculation was 64% and 89% greater than Non-AMF plants. Root AMF-colonization measured by the positive reaction of the fungal ALP in the cortical cells, was significantly reduced by the presence of diesel along the experimentation. The inoculation of the AMF-consortium *Glomus* Zac-19 alleviated diesel toxicity on *Melilotus albus*, by significantly increasing the plant biomass production.

2886 BACTERIAL COMMUNITY IN NATURAL GRASSLAND OF URUGUAY: ASSESSMENT OF EFFECTS CAUSED BY CATTLE GRAZING

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The Campos in Uruguay, the Pampa in Argentina and southern Brazil comprise one of the largest areas of natural temperate sub-humid grasslands. In Uruguay, 87% of the country is occupied by natural grasslands grazed by domestic herbivores, mainly cattle and sheep. Grazing is a key disturbance that shapes grassland communities, drastically altering plant species composition. However, less is known about its impact on soil microbial communities. The objective of this work was to assess the effects of grazing on soil bacterial communities of grassland. Composite soil samples were taken 2 years in autumn and spring, from 3 contiguous areas of natural grassland: (1) a grazed area (G), (2) an ungrazed area which had been excluded to cattle for 10 years (Ex10), and (3) an ungrazed area which had been excluded to cattle for 20 years (Ex20). Abundance of fluorescent *Pseudomonas, Bacillus* spp. and actinobacteria was determined by plate-counting on selective media. The diversity of these bacterial groups and *Bacteria* domain was assessed by DGGE (Denaturing Gradient Gel Electrophoresis) of 16S rDNA fragments amplified by PCR from total soil DNA. In both ungrazed plots, actinobacteria abundance was significantly lower than in the grazed plot. *Bacillus* spp. did not show a consistent response to treatments: in spring 2004 numbers were higher in Ex10 and Ex20 than in G plot, but the opposite was observed in autumn 2006. In this last season, fluorescent *Pseudomonas* population was diminished in G plot. By DGGE analysis, a high genotypic diversity of *Bacteria* was observed. Cluster analysis showed clear grouping of samples by treatment in autumn, with linkage distances of 0.5 between Ex10 and Ex20, and 0,9 between G and ungrazed plots. However, spring was not an appropriate season for detecting treatment-dependent community structures. Impact on microbial diversity (IDM) index was calculated based on DGGE results, showing significant differences between grazed and ungrazed areas. This study has contributed to characterise the impact of grazing on grassland microbial communities. Its results will help in the designing of soil management strategies towards the conservation of agroecosystems and its sustainable functioning.

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2495 ASSESSMENT OF EFFECT OF PHOSPHATE FERTILIZATION INPUTS ON PLANT GROWTH PROMOTING MICROBIAL COMMUNITIES IN SOIL

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Phosphorous (P) is among the most essential and limiting macronutrients which is required to increase crop yield, agronomically the amount of P loss in "runoff" per annum through supplement addition is generally inconsequential. However, from the perspective of water quality small concentrations of P are sufficient for water bodies to become eutrophic, which is a major environmental protection issue throughout the globe. Some plant growth promoting bacteria and fungi have the ability to solubilise adsorbed phosphate in soil and provide this directly to plants, this biofertilisation can be used in reducing "runoff" caused through excess application of fertilizer. The phosphate solubilising ability and diversity of these microbes is hypothesised to vary in soil receiving different inputs of P as it is a limiting nutrient in soil. Hence it would be important to analyse the diversity of the associated microflora. In this study we are using molecular tools such as microbial fingerprint techniques e.g. PCR-TGGE and metagenomics to investigate field sites that have been running a long term experiment using reduced chemical inputs. Preliminary results based on 16S rRNA gene library analysis from different samples receiving high and reduced P inputs suggest higher microbial diversity in plots with reduced P inputs compared with the field site receiving high P input, However, the exact functional diversity of these microrganism present in soil will be clearer on further analysis of the library and the soil metagenome. This work will enhance our knowledge of the microflora involved in phosphate solubilisation which will provide benefits in reducing water pollution, increasing agricultural yield and contributing in implementation of soil and water policies for sustainable agricultural systems.

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2162 EFFECT OF DISTANCE AND DEPTH ON SOIL MICROBIAL BIOMASS, N MINERALIZATION AND GENETIC DIVERSITY OF RHIZOBIA UNDER *Acacia senegal* TREE

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The relations between plants and soil biota involve positive and negative feedbacks between soil organisms, their chemical environment, and plants. Then, the characterization of microbial community functioning and their diversity are important to understand these linkages. An experiment was conducted in a field system for two years (2005 and 2006) to investigate the effect of distance from tree stem on soil microbial biomass, N mineral content and the diversity of rhizobia associated to *Acacia senegal*. Soil samples were taken along transects (R_0, foot tree; $R_{/2}$, approximately 0.50 m distance from the stem; and R, approximately 1 m distance from the stem) and at different horizons: 0-25 cm, 25-50 cm and 50-75 cm of the rhizosphere of trees. Soils sampled during dry season and rainy season were incubated under laboratory conditions and *in situ*. For each sampling, nitrogen mineralization and total microbial biomass were analysed. *Acacia senegal* root nodulating bacteria diversity was assessed by PCR-RFLP of the intergenic spacer 16S-23S DNAr of strains trapped on roots of *A. senegal* seedlings from soils samples. Results showed a decrease of mineral nitrogen content and total microbial biomass according to the distance from tree trunk and the depth. The maximum of mineral nitrogen and total microbial biomass was found at the foot tree (R_0) and at 0-25 cm. Mineral nitrogen decreased during the wet season whereas total microbial biomass increased. The mineral nitrogen was in nitrate form (NO_3^-) during the dry season whereas at raining season it was in ammoniacal form (NH_4^+). Interestingly, results showed a great genetic diversity of rhizobia nodulating *A. senegal* with a high diversity at the foot of tree (R_0) and at 0-25 cm depth. Therefore, *A. senegal* trees seemed to have a positive influence on microbial density and diversity.

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2323 SOIL MICROBES AND SOIL MICROBIAL PROTEINS: INTERACTIONS WITH CLAY MINERALS

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Bacterial enumeration in soil environments estimates that the population may reach approximately 10^{10} g^{-1} of soil and comprise up to 90 % of the total soil microbial biomass. Bacteria are present in soils as single cells or multicell colonies and often strongly adsorb onto mineral surfaces such as sand and clay. The interactions of microbes and microbial biomolecules with these minerals have profound impacts on the physical, chemical and biological properties of soils. The objective of this work is to characterize the interaction of soil microbes and soil microbial proteins with montmorillonite and kaolinite clay minerals. Microbes were propagated from a light clay-loam soil collected from Carlow, Ireland and the microbial titre determined. Microbial proteins were isolated and the concentration evaluated using the Bradford assay. Quantified microbes and proteins were sorbed to montmorillonite and kaolinite and their equilibrium adsorption determined (initial concentration – equilibrium supernatant) as the number of cells and/or milligram of protein per gram of mineral. Clay mineral-complexes were characterized by X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Fourier-transform infrared (FT-IR) spectroscopy. Montmorillonite and kaolinite adsorbed 3.71×10^{10} and 3.33×10^{10} cells/g, respectively. Equilibrium adsorption of proteins onto montmorillonite and kaolinite was determined at 920 mg/g and 728 mg/g, respectively. XRD showed that microbes and proteins intercalated the interlamellar region of montmorillonite resulting in montmorillonite-microbial and montmorillonite-protein complexes with 001 d-spacing of 16.1 and 15.3 Å, respectively compared with pure montmorillonite 001 d-spacing of 13.2 Å. SEM indicated that microbes and proteins also adsorb on montmorillonite surfaces. XRD spectra confirmed that microbes and proteins did not penetrate kaolinite, indicating that adsorption occurred primarily on external surfaces, as also suggested by SEM. FT-IR spectra were indicative of montmorillonite-microbial and –protein, and kaolinite-microbial and -protein complexes. Montmorillonite indicated a greater affinity for soil microbes and soil microbial proteins when compared with kaolinite. The implications of these interactions on the total contribution of proteins and other microbial constituents to soils are currently being considered using novel NMR approaches.

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2287 ELECTROCHEMICAL CHARACTERIZATION OF A MICROBIAL FUEL CELL (MFC) THAT UTILIZES COW MANURE AS ENERGY SOURCE

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Microbial fuel cells are new types of bioreactors that convert the chemical energy encountered in organic matter directly to electricity. The efficiency of this energy conversion is potentially higher than described in other environmental technologies focused on energy production from organic wastes, such as biogas utilization. A MFC reactor utilizes microorganisms as catalysts to transfer electrons from the biological oxidation of the organic matter to an electrode (anode) in anaerobic conditions. The electrons flow through a conductive wire from the anode to a cathode, which is placed in a second compartment with oxidizing conditions. Here the electrons complete the circuit through transfer to an oxidant (such as O_2). The two compartments are physically divided by means of a non-conductive separator, enabling diffusion of protons and ions between the anode and cathode compartments to complete the electronic circuit. In the cathode cell, water is produced from the electrons and protons that reduce O_2. Therefore a continuous and profitable electric current between the electrodes is formed, without biogas accumulation (except CO_2). MFC technology is still in its infancy and most of surveys have been carried out using biodegradable synthetic media (glucose and volatile fatty acids) in reactors constructed from too expensive materials for their scale up and commercial application. The results presented show that cheap materials such as cow manure and polyethylene membrane are feasible for MFC operation, achieving higher electricity production than described for other MFC reactors that oxidize complex organic wastes. The voltage and the maximum power output density obtained in the presented MFC reactor were 580 mV and 277 mW / m^2, respectively. Polarization curves showed that acetate amendment (50 mM) increased significantly (20%) the power output to 360 mW/m^2 (3.5Ω of external resistance), and the maximum current density to 1883 mA/m^2, whith a maximum cell voltage of 711 mV. Further research will focus on the improvement of energy efficiency and production to reach economical competitiveness, and to understand the ecophysiology of microbial populations that inhabit MFC reactors constructed with low cost materials.

2895 MICROBIAL FUEL CELL DESIGN AND CHARACTERIZATION FOR ELECTRICITY PRODUCTION FROM WASTEWATERS

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The purpose of this research was to design and characterize a lab scale, one chamber microbial fuel cell (MFC) that will be used for the treatment of a variety of wastewaters, acidogenic extracts, and leachates. The MFC consisted of a horizontal cylinder built in plexiglass 78 mm long (between electrodes) and 48 mm internal diameter. The cylinder was fitted with a circular anode made of stainless steel plate 1 mm thickness and a cathode made of a sandwich of 3 circular layers (from inside to outside): proton exchange membrane, flexible carbon-cloth containing 0.5 mg/cm^2 platinum catalyst, and a perforated plate of stainless steel 1 mm thickness. The cathode was in direct contact with atmospheric air on the metallic plate side. The surface area of anode per unit volume was 12.82 m^2/m^3. The MFC was inoculated with 143 mL inoculum from a methanogenic, complete mix reactor. Biomass concentration in the inoculum was ca. 200 mg VSS/L. The MFC was loaded with 7 mL of a model leachate concocted with a mixture of organic acids and mineral salts with ca. 16 g COD/L. The MFC was batch-operated for 50 h at 37 ºC. Initial and final pH were 7.66 and 8.32, respectively. The circuit of the MFC was fitted with an external, variable resistance device in order to estimate the value of the internal resistance of the MFC. This value is one of the main characteristics of a MFC, because according with the Theorem of Jacobi of maximum power delivered by an emf, a MFC fitted with an external resistance equal to its internal resistance will give a maximum power output. In this regard, we carried out the polarization curve of the MFC, that is, cell voltage (VMFC) and current intensity (IMFC) against external resistance value. First, the open circuit voltage was determined to be ~0.36 V. After 1 hour, the polarization curve was determined by monitoring the VMFC and IMFC at increasing values of the external resistance and viceversa. The average polarization curve was non linear. An average slope to such curve was calculated and gave a value of ~32 kΩ for the internal resistance of MFC. Afterwards, the open circuit voltaje of the cell was monitored again and a value of 0.35 V was found. The latter agreed very well with the first value of the open circuit voltage, further validating the whole polarization curve procedure. The MFC developed a power output near 5x10^{-5} mW when tested with an external resistance of 32 kΩ. This value is considered to be low, and it was determined by the high value of the internal resistance. Hence, further research is needed regarding ways to minimize the internal resistance and increasing power output of our MFC model.

2487 COMBINED BIOGAS AND BIOETHANOL PRODUCTION IN ORGANIC FARMING

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Bioenergy production from local bioresources has a great potential. It is important to reduce dependency on fossil fuels and decrease green house gas emission in organic agriculture (OA). Both biogas and bioethanol can be produced in OA and significantly contribute to the sustainability of organic farms. The presented study is part of the BioConcens project (http://www.bioconcens.elr.dk/uk/). This study is focused on characterization of relevant feedstock for co-production of biogas and bioethanol within organic farming. Clover grass silage, dry clover grass and dried grass from meadows were selected. Biogas potential batch tests were performed for each substrate individually. The strong acid hydrolysis was performed to determine the total content of sugars in lignocellulosic biomass. The three main sugars (glucan, xylan and arabinan) were measured by HPLC (high pressure liquid chromatography) and finally theoretical ethanol yield was calculated based on the total glucose content. The most important was the polymer of glucose – glucan. In typical yeast based ethanol fermentation only glucose is converted into ethanol. The theoretical ethanol yield was calculated and it was found to be 14.9 and 13.4 [g/100gTS] for dry and silage clover grass, respectively. The lower ethanol yield is due to that the sugars are converted to lactic acid during ensilaging. The rest of the sugars remaining from the yeast based ethanol production will be present in the process effluent and can be either used as an animal feed or further fermented to methane through anaerobic digestion process. The biogas potential for the dry grass, dry clover grass and clover grass silage was 304 ± 21, 269 ± 14 and 352 ± 5 $mLCH_4$/gVS, respectively. Clover grass silage seems to be the most promising feedstock from the presented ones. Energy crop in form of silage might be more effective for the biogas production than dry lignocellulosic material, due to easier access for microorganisms to the valuable biodegradable organic compounds. It is expected that the on-farm production of the bioenergy would improve not only sustainability of such a farm but as well economics. Further investigations will be carried out.

2254 BIOHYDROGEN FROM WASTES AND COMBINATION OF BIOLOGICAL PROCESSES FOR ENERGY YIELD IMPROVEMENT: A REVIEW

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Hydrogen is one of the best contemporary energy alternative because it exhibits the highest energy density (its calorific value is 3 times higher than that of gasoline); it is versatile and can be used both as a primary or secondary energy source; it is compatible with electrochemical and combustion-based energy conversion processes; and it is a clean energy source since water is its main combustion product and no aggressive pollutants such as SO_x, CO, CO_2, SO_x, hydrocarbon vapors are generated. Although there has been several recent reviews on one-stage biohydrogen processes, there is no a critical overview of series and series-parallel biological processes that using at least one biohydrogen stage, lead to maximize the yield of bioenergies and secondary resources from wastes. Then, this work is a review on the combination of biological processes for obtaining bioenergy and other resources from organic susbtrates and centered in biological hydrogen processes. There is potential for increasing the energy yield from organic substrates and wastes by the combination of biological: *(i)* hydrogenogenic fermentative processes in series with methanogenic digestion, *(ii)* the dark hydrogenogenic fermentation followed by hydrogenogenic photoheterotrophic fermentation of the spent liquors or extracts; *(iii)* the so-called indirect biophotolysis process that is 3-stage process, i.e., algae or cyanobacteria biomass synthesis by biophotolysis, followed by dark fermentation of such biomass, with a last stage of photoheterotrophic fermentation of organic acids from the second step; *(iv)* the dark hydrogenogenic fermentation of organic substrates followed by electricity generation from spent acidogenic liquors or leachates in microbial fuel cells; *(v)* integration of hydrogenogenic processes in a novel concept coined as Biorefinery of Wastes that allows for the recovery of energy (hydrogen, methane, electricity) and by-products (organic chemicals, recyclables, soil amenders, and protein-enriched solids for animal feed). Several areas of future research have been identified: *(i)* more work is needed on the hydrogenogenic fermentation step of organic wastes using microbial consortia because it has distinct advantages compared to fermentation of expensive substrates using pure cultures (neither sterilization nor light required, possibility of reclaiming several industrial and municipal wastes, etc.); *(ii)* further research on obtaining added-value products such as enzymes from spent solids of solid substrate fermentation hydrogenogenic processes; *(iii)* it is required more fundamental research on the structure and dynamics of microbial communities of the several biological processes involved in the combined approach, its evolution as related to the operating conditions of bioprocesses, identification of key H_2-producing microorganisms and their metabolism, and eventually genetic manipulation of key species for improving their H_2-producing capabilities. Molecular biology tools are of outmost importance and application in dealing with these issues; *(iv)* Experiments and projects dealing with the scale-up of combined processes is a must; *(v)* Studies from a systemic point of view and life cycle analysis of integration of hydrogenogenic and other biological processes within the framework of waste management, recycling and by-products recovery will help in demonstrating the value and feasibility of biohydrogen as a key issue on sustainable development of modern societies.

2343 EFFECT OF TEMPERATURE AND HYDRAULIC RETENTION TIME ON HYDROGEN PRODUCING GRANULES: HOMOACETOGENESIS AND MORPHOLOGICAL CHARACTERISTICS

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The effect of temperature and hydraulic retention time (HRT) on the homoacetogenesis and on the morphological characteristics of hydrogen producing granules was investigated. Hydrogen was produced using an expanded granular sludge blanket (EGSB) reactor, fed with glucose and L-arabinose, under mesophilic (37 ºC), thermophilic (55 ºC), and hyper-thermophilic (70 ºC) conditions. Apparent homoacetogenesis was observed only at mesophilic conditions. Glucose was utilized preferentially over L-arabinose at thermophilic and hyper-thermophilic conditions. Higher yields of hydrogen production were linked to the decrease of lactic acid and the increase of n-butyrate in all temperatures tested. The total filaments length per VSS (TL/VSS) increased and the apparent granules density (VSS/TA) decreased with an organic loading rate (OLR) of 5 kgCOD/m^3/d at 37 ºC. When the OLR was increased to 10 kgCOD/m^3/d and 16 kgCOD/m^3/d, TL/VSS decreased and the VSS/TA increased suggesting an increase of granules density. An increase of TL/VSS and decrease of VSS/TA only occurred at the highest OLR (16 kgCOD/m^3/d) at 55 ºC. However, at 70 ºC, an increase of TL/VSS and a decrease of VSS/TA was already observed at an OLR of only 10 kgCOD/m^3/d. Granules size was not affected by temperature but an increase in higher OLR led to a decrease in the percentage of projected area of aggregates with an equivalent diameter (Deq) larger than 1 mm.

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2579 FERMENTATIVE HYDROGEN PRODUCTION FROM COMBINATION OF TOFU PROCESSING AND ANAEROBIC DIGESTER SLUDGE WASTES USING A MICROBIAL CONSORTIUM

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The combination of Tofu manufacturing waste and anaerobic digester sludge was studied for fermentative H_2 production in batch and continuous modes using a mixed culture originated from sewage sludge. In order to increase the solubilization of organic substrates from Tofu waste, various pretreatments including heat-treatment, acid/alkali treatment, and sonication were examined alone or in combination with others. Heat-treatment at 110 ºC for 2 hrs in presence of 0.1 N HCl increased the soluble carbohydrate content of the Tofu processing waste from 2.2 to 15 g/L. Anaerobic digester sludge was added to the Tofu processing waste as a supplementary nitrogen-source and minerals, and incubated with a mixed H_2-producing culture. Optimal mixing ratio of anaerobic digester sludge was 20% (v/v) for H_2 production under batch conditions at 60 ºC and pH 5.5. In continuous experiments using a CSTR, the reactor was initially fed with synthetic media containing 1% (w/v) glucose at a HRT of 12 hrs and the ratio of the pretreated Tofu processing waste and anaerobic digester sludge was gradually increased from 20% to 100% (v/v). After the stabilization of the reactor for H_2 production, the effects of HRT and pH were investigated. With decreasing HRT, volumetric H_2 production rate increased. The H_2 production performance was not affected by pH 5.5 – 6.0, but, at pH 5.0, the reactor became unstable. The composition of headspace gas in the reactor was constantly kept at 50-60% (v/v) of H_2 gas and no CH_4 was detected during the fermentation. The maximal volumetric H_2 production rate was 7.8 L H_2/L/day. This study indicates that the combination of Tofu processing waste and anaerobic digester sludge could be considered as suitable wastes for H_2 fermentation.

2536 EFFECT OF SUBSTRATE CONCENTRATION ON HYDROGEN PRODUCTION

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In Mexico City 44,000 tons/day of waste are produced. The anaerobic digestion (AD) process has been used over a century for the stabilization of organic waste. The end-product methane is a useful energy source; however, it is a low value end product with relatively less energy content. Its combustion by-products are powerful greenhouse gases. There is a stress to explore alternative environmental technologies that not only stabilize the organic solid waste (OSW), but also generate benign high value end products. From this perspective, anaerobic bioconversion of organic wastes to hydrogen gas is an attractive option that achieves both goals. Previous studies developed in an anaerobic digester (AD) used for treating sorted OSW supplied by Mexico City food central market showed that under the conditions of the OSW, low pH and easily degraded organic matter, H_2 production was favored instead of methane production. Batch tests showed that heat treatment (80 ºC, 30 min) of the mesophilic sludge obtained from the AD enhanced the hydrogen production two times with respect to control (without heat treatment). The initial substrate concentration plays an important role in the volume and the rate of hydrogen production. Batch tests were conducted at increasing glucose concentrations (3, 5, 7, 10, 15, 20, 30 g/L), maintaining the pH around 5.5, the temperature of incubation was 35 ºC. At a relatively low initial glucose concentration, the rate of fermentation was also low. The maximum cumulative hydrogen productions (365, 461 mL) were accomplished with 10 and 15 g/L of initial glucose concentration, respectively. This corresponds to 3.6 and 3.05 mol H_2/mol glucose. The carbohydrate removal efficiency was 99% at initial glucose concentration up to 15 g/L and then began to decrease. The glucose was consumed in around 20-24 hr for the lower concentrations. It has already been reported that substrate inhibition gets predominant at higher glucose concentration because this modifies the metabolic pathways. The lower H_2 production rate indicates that the carbon flux at high glucose concentrations is directed to the production of reduced by-products such as ethanol and organic acids. More experiments are necessary to establish the conditions to enhance the continuous H_2 production.

2578 MICROBIOLOGICAL HYDROGEN PRODUCTION BY ANAEROBIC FERMENTATION AND PHOTOSYNTHETIC PROCESS

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Hydrogen gas is a clean and renewable energy carrier. Microbiological hydrogen production from glucose or starch by combination use of an anaerobic fermenter and a photosynthetic bacterium, *Rhodobacter sphaeroides* RV was studied. In 1984, the co-culture of *Clostridium butyricum* and RV strain to convert glucose to hydrogen was demonstrated by Miyake et al.[1]. Recently, we studied anaerobic fermentation of starch by a thermophilic archaea, *Pyrococcus furiosus* followed by photosynthetic process with RV strain. Due to the difference of the optimal growth temperature of each bacterium, the hydrogen production was done in a two-stage process. *P. furiosus* fermented starch to hydrogen and acetate with a high rate and yield. RV strain was able to convert acetate to hydrogen under illuminated conditions. However, some adjustment of composition of culture medium will be required; sodium chloride was inevitable for growth of *P. furiosus*, but inhibitory for hydrogen production from acetate by RV. As another microbiological combination for hydrogen production, an immobilized co-culture of a lactic fermenter, *Lactobacillus delbrueckii* [2] or *Rhizopus oryzae* and RV in agar gel was studied, which converted glucose to hydrogen with high molar yield of 7 to 8 per glucose. The hydrogen production rate of the co-cultures rate might be lower than that by *P. furiosus. L. delbrueckii* and *R. oryzae* do not produce hydrogen but do lactate, which is an appropriate substrate for hydrogen production by RV. We will evaluate and compare the two systems for microbiological hydrogen production described above.

2395 EFFICIENT METHANE PRODUCTION FROM LIPID-RICH WASTEWATER IN HIGH-RATE ANAEROBIC TREATMENT

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Anaerobic digestion allows, simultaneously, wastewater treatment and production of biogas, a renewable energy source. The energy yield of this process is higher for more reduced compounds, such as long chain fatty acids (LCFA), the main products of lipids hydrolysis. However, LCFA conversion to biogas is not always complete, and tends to decrease with the increase of the organic loading rate applied (OLR). Recent results obtained in our research group showed that efficient methane production from lipid-rich wastewater can be accomplished if acclimation of the inoculum is performed through discontinuous or pulsed feeding of fat.
In this work, continuous treatment of a synthetic dairy wastewater was studied in an upflow anaerobic reactor inoculated with sludge previously acclimated to oleate. Acclimation was performed in a lab scale reactor operated in cycles. During the experiment, OLR from 5 to 31 kg COD m^{-3} day^{-1} were applied and the performance of the reactor was monitored. LCFA were quantified in samples collected during the reactor operation and the specific methanogenic activity (SMA) in the presence of acetate and H_2/CO_2 was determined at the beginning and at end of the operation.
From 5 to 21 kg COD m^{-3} day^{-1}, COD removal averaged 98% and remained higher than 90% until the end of the experiment. Maximum conversion to methane was obtained for the OLR of 12 kg COD m^{-3} day^{-1} (100%). Nevertheless, continuous treatment of an OLR as high as 21 kg COD m^{-3} day^{-1} was possible with a methane yield of 72%. LCFA accumulation was observed mainly during the periods of higher OLR, reaching a maximum value of 3.5 g COD gVS^{-1} at the end of the operation. Palmitic acid was the main LCFA quantified during the experiment, representing 40–100% of the total LCFA detected. Oleic and stearic acids were also present in quantities ranging 10–30% and 0–15%, respectively. Despite the high OLR applied at the end of the experiment, SMA in the presence of acetate and H_2/CO_2 increased 18 and 15 times, respectively, from the beginning until the end of the operation.

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2607 IMPROVEMENTS IN FERMENTATIVE BIOLOGICAL HYDROGEN PRODUCTION THROUGH METABOLIC ENGINEERING

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Dramatically rising oil prices and increasing awareness of the dire environmental consequences of fossil fuel use, including startling effects of climate change, are refocusing attention world-wide on the search for alternative fuels. Hydrogen is poised to become an important future energy carrier. Renewable hydrogen production is pivotal in making it a truly sustainable replacement for fossil fuels, and for realizing its full potential in reducing greenhouse gas emissions. To this end, we have been investigating biological hydrogen production by microbial fermentation, a process that could use readily available wastes, thus solving another environmental problem, as well as presently unutilized bioresources, including enormous supplies of agricultural and forestry residues. Although it is likely that only relatively simple bioreactor and bioproccess technology would be required, currently obtainable yields must be increased in order to make this process practical. Our recent results show that substrate to hydrogen conversion efficiencies can be improved through nutrient limitation, and genetic modification to increase hydrogenase expression and eliminate competing pathways. Both batch and continuous culture studies have shown that yields are highest with limitation for nitrogen or glucose, approaching the theoretical maximum of 2 moles H_2/ mole of glucose for enteric bacteria. Likewise, compared to the isogenic parent, yields are much higher with a strain lacking uptake hydrogenases, mutated in lactate dehydrogenase, and containing constitutive FhlA activity, allowing increased expression of hydrogenase 3. We have shown that dilution rate (hydraulic retention time) is an important factor controlling the overall efficiency of a hydrogen producing fermentation, with a trade-off during hydrogen fermentation between increased production and increased substrate conversion. Pathways exist to further increase yields, and efforts underway to build even more efficient strains through metabolic engineering and the heterologous expression of hydrogenases will be discussed. One resource that is currently produced in large amounts, and whose production will dramatically increase in the future, over one billion kilograms by 2010, is biodiesel-derived glycerol. We are presently developing a photofermentation process to stoichiometrically convert this substrate to hydrogen through, and are currently producing six moles of hydrogen per mole of glycerol, with improvements possible up to eight.

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2580 HYDROGENASES IN *Frankia*

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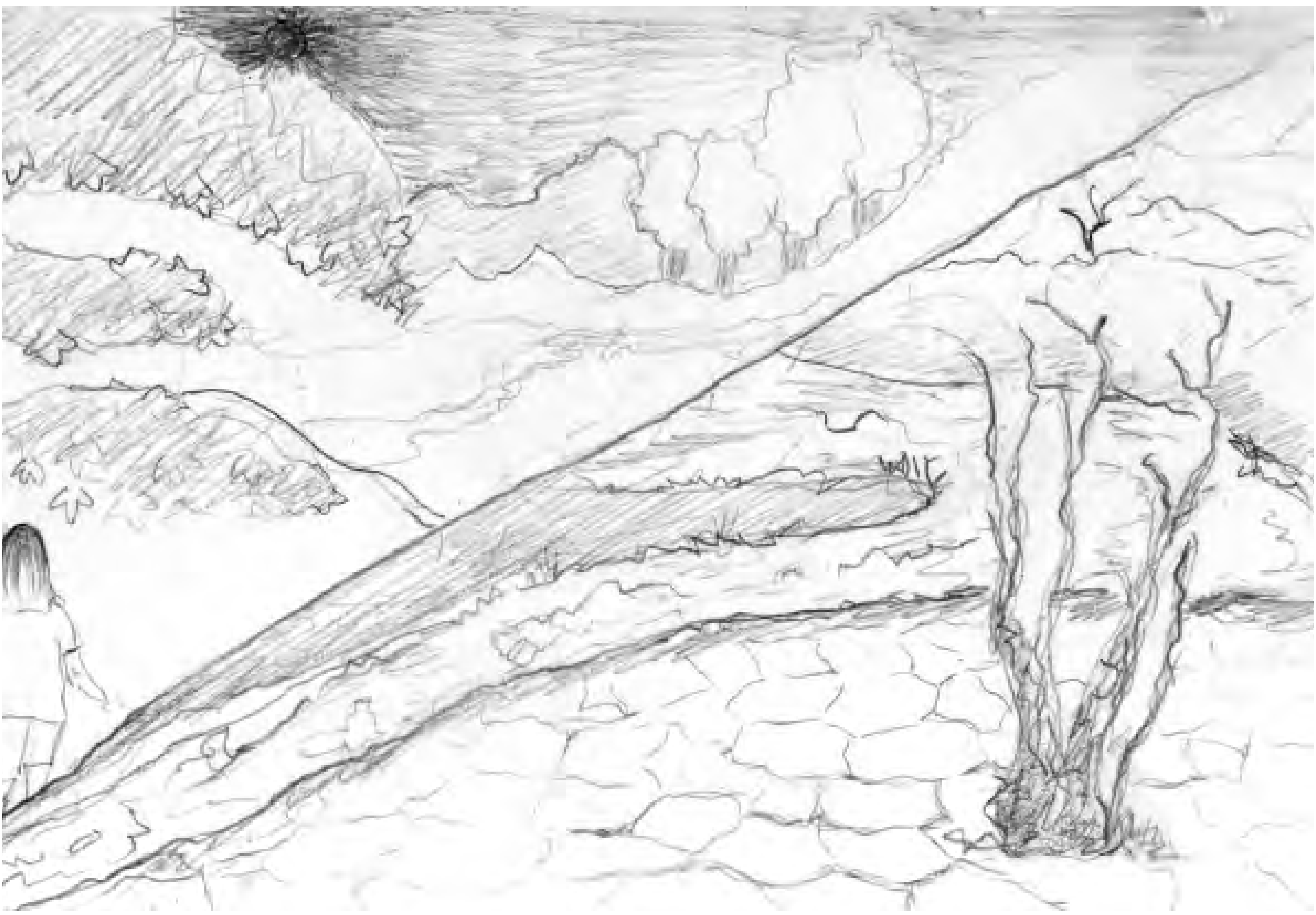
Frankia sp. are actinomycetous bacteria, shown to have hydrogenases. Strains isolated from ten different actinorhizal host plants showed uptake hydrogenase activity, which was further increased by addition of nickel. The uptake hydrogenase of *Frankia* is most probably a Ni-Fe hydrogenase.

Genome characterization of three *Frankia* genomes, revealed the presence of two hydrogenase genes (syntons) in *Frankia*, which are distinctively separated in all three. The structural, regulatory and accessory genes of both hydrogenase synton#1 and #2 are arranged closely together, but in a clearly contrasting organization, content and orientation. These hydrogenase syntons have sequences that are divergent from each other. Hydrogenase synton#1 and #2 of *Frankia* sp. CcI3 and *F. alni* ACN14a are similar in gene organization, content and orientation, while the syntons are both reduced and rearranged in *Frankia* sp. EANpec. The uptake hydrogenase synton#1 of *Frankia* are more expressed under free-living conditions whereas hydrogenases synton#2 are mainly involved in symbiotic interactions.

The hydrogen-evolving hydrogenase activity was recorded in eight *Frankia* strains: Nickel also increased the activity of hydrogen-evolving hydrogenases in *Frankia*. The fact that *Frankia* can produce hydrogen is reported only recently. The knowledge of the molecular biology of *Frankia* hydrogenase is, therefore, of a paramount importance to optimize the system in favor of hydrogen production. *Frankia* is an attractive candidate in search for an organism efficient in biological hydrogen production since it can produce a considerable amount of hydrogen.



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MONDAY 22 SEPTEMBER 2008 POSTER COMMUNICATION

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1833 ANALYSIS OF CAPTOPRIL IN SURFACE WATERS BY DIFFERENTIAL PULSE VOLTAMMETRY METHOD

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One of the important problems concerning waters ecosystems is the presence of pharmaceuticals remains in different kinds of surface waters. These compounds cause huge changes in waters environment. They cause genetic changes in water organisms, are not also neutral for people in case of penetrating into drinking water. The object of analysis was captopril, which it is a synthetic dipeptide serving as an orally active inhibitor of the angiontensin-converting enzyme (ACE) and has been widely used as antihypertensive drug and to moderate heart failure. Researches ranged preparation of captopril extraction procedure from surface water samples by solid–phase extraction (SPE) and elaboration of differential pulse voltammetry (DPV) method for its determination. All voltammetric measurements were carried out using a PC compatible ECO-TRIBO polarograph operating under ETP 2.0 software for storing and processing data (PolaroSensor) in combination with a mini-microelectrode system UMμE (Prague, Czech Republic). A three-electrode cell system was completed by means of a hanging mercury drop electrode (HMDE) as working electrode, Ag|AgCl|KCl$_{(sat.)}$ as reference electrode and platinum as auxiliary electrode. Different supporting electrolytes for determination of captopril were used. Researches concerning extraction of captopril from surface water samples were carried out with using different stationary phases in columns from set for SPE extraction J.T. Baker Company. Conditioning methods of stationary phases were researches and eluents for elution captopril after enrich on columns for SPE were chosen. Captopril stable in waters was also researched. Captopril recoveries from water samples were appointed and determinations by DPV method were effected in 0.2 –1.0 μg mL^{-1} range. This method can be successfully applied to routine determination of captopril in surface waters samples.

1852 ENZYMATIC AND BACTERIAL INDICATORS OF WATER AND SEDIMENT POLLUTION

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The work aimed to evaluate the bacterial and enzymatic potential in water and sediments of the Ariefl River (Romania), in order to detect the effect of some putative pollutants on this potential. Eleven sampling sites were established along a distance of about 100 km of the river. The analyses were carried out in the autumn of 2006 and 2007. The physico-chemical parameters of water analyzed were: pH, Eh, conductivity and O_2 concentration. The following four ecophysiological bacterial groups have been studied, both in water and in sediment: aerobic mesophilic heterotrophs, ammonifiers, denitrifiers and iron-reducers. The following four enzymatic activities have been measured in sediment: phosphatase, catalase, actual and potential dehydrogenase. Excepting the P11 site, the water pH was alkaline and the redox potential (Eh) was negative. The presence of all the four ecophysiological bacterial groups was registered, both in water and in sediment. The descending ranking of their abundance was: aerobic mesophilic heterotrophs > ammonifiers > denitrifiers > iron-reducers. The number of bacteria in sediments exceeds by approximately one order of magnitude the number of the corresponding group in water. Bacterial indicators of sediment (BISQ) and water (BIWQ) quality were calculated, taking into account the logarithm of the number of bacteria which belong to each group. The four enzymatic activities were noticed in all the studied samples. Enzymatic indicators of sediment quality (EISQ) were calculated, based on the values of each enzymatic activity. The lowest values of bacterial and enzymatic indicators registered in the P11 site indicate a strong local pollution. The pollution source might be the mining enterprise in Roşia Montană, which empties the waste water into the Abrud River, 10 km upstream from the site P11, where it flows into the Arieş. The values of the quality indicators were slightly higher in 2007, but the differences were not significant. Positive correlations were established between the bacterial and enzymatic indicators. The results obtained certify the validity of the synthetic enzymatic and bacterial indicators as efficient tools for estimating the microbial activity in water and sediments so as to monitor the effect of the polluting factors on these natural habitats.

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1853 DETERMINATION OF VULNERABILITY'S AREAS TO POLLUTION: CASE OF ALLUVIAL WATER TABLE OF TEBESSA (EAST ALGERIA)

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This work relates to the alluvial water table of Tebessa, which is characterised by a semi-dry climate and a very heterogeneous geology. To examine the pollution problem who seems exists, we have used two methods: the DRASTIC method who combines the information given by the seven parameters leading to the map of vulnerability to pollution and a second method that is based on hydrochemistry and take into account the results of the ratio Sr^{2+}/Ca^{2+}. The superposition of the two maps has shown that the most vulnerable areas are the most polluted: This is the case of the Chabro and Kebir Oueds (Sr^{2+}/Ca^{2+}> 3‰). On the borders, the ratio Sr^{2+}/Ca^{2+} is less than 1‰. This area is the least vulnerable. This work has allowed understanding the process of salinity acquisition.

1889 CHEMICAL QUALITY OF WATER IN *Anopheles stephensi* HABITATS AND ITS SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN SOUTH EASTERN OF IRAN

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Using of insecticides depends on the knowledge of the susceptibility levels of malaria vectors to these chemical. In this study, the chemical quality of water in the larval breeding habitats and the susceptibility levels of *Anopheles stephensi* to DDT 4%, dieldrin 0.4%, permethrin 0.75%, cyfluthrin 0.15% deltamethrin 0.05% and lambdacyhalothrin 0.05% were investigated according to WHO method in south eastern of Iran. Water quality analyses were done according to standard methods for the examination of water and wastewater. The LT50 values of different insecticides were calculated using the probit regression line. The results of water chemical analyses of larval habitats showed that water pH to range from 6.5 to 8.2, chloride from 2 to 120 mg/l, sulfate from 0.2–12 mg/l, DO from 4.5 to 7.5 mg/l, nitrate from 0.02 to 4 mg/l, phosphate from 0.08–1.2 mg/l and salinity from 85 to 130 mg/l. Results of the susceptibility tests showed that *An. stephensi* is resistant to DDT and dieldrin in this region. The LT50 value of the tested pyrethroids including deltamethrin, permethrin, lambdacyhalothrin and cyfluthrin was 3.50, 5.10, 3.95 and 5.38 minute respectively. This strain had high resistance to DDT, dieldrin with mortality rate of 63 and 70 min respectively. It is concluded salinity is one of the important factors for breeding site. Furthermore, other chemical parameters of water also exerted some impact on mosquito larval population. Performing complementary tests with other pyrethroids on this strain seems to be necessary in Sistan and Baluchestan region.

2154 THE ECOLOGICAL EVALUATION OF DEBED RIVER WATERSHED BASIN IN ARMENIA BY HYDROPHYSICAL, HYDROCHEMICAL AND HYDROBIOLOGICAL PARAMETERS

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The environmental researches are foremost in today's Armenia. During the last years seemed economic growth in country means additional pressure to environment, especially without introducing resource economizing new technologies.The water resources play important role for development of economy in Armenia. The pollution of surface waters, not complete management of water resources are really problems in the field of water resources management. The Debed river watershed basin is located in the North of Armenia. The river Debed is a trans boundary river and has an also regional importance. Debed river and its tributaries are used for household, irrigation and industrial purposes. The area of watershed basin is characterized with rich natural recourses, those stands out with developed industry and agriculture, causing huge ecological and environmental problems. The condition of surface waters gradually becomes shaper with restoration of economy after the years of economic crisis, because of industrial, household wastewaters and agriculture caused pollution. There were not operating wastewater treatment plants in Debed river watershed basin those the industrial and household non-treated wastewaters are dumped directly to rivers. Also the non-organized management of solid wastes cause pollution. Coming from these the ecological condition of Debed river watershed basin really needs attention. The objectives of the researches to study the ecological situation of Debed river and its watershed basin for giving the ecological evaluation of the area which will support to development an action plan for complete management and sustainable development policy for the area. In basis of research works is putted the Driving Forces-Pressures-State-Impact-Responses (DPSIR) framework, developed and accepted by EAOC for trans-boundary river basins, by which is possible to understand confluences between the deferent problems of water management. During field and laboratory researches have been studded the hydrophysical, hydrochemical and hydrobiological parameters of waters of Debed river and its main tributaries. As for researches evaluation, Debed river watershed basin is polluted with heavy metals, pesticides, organic matter and with other toxic pollutants; the water ecosystems cannot conquer the level of present anthropogenic pressure sufficiently which causes degradation of water ecosystems and disbalance of environment, health and socio-economical problems.

2157 DISTRIBUTION OF POLYCYCLIC AROMATIC HYDROCARBONS BETWEEN WATER, SEDIMENT AND COMMON EELS (*Anguilla anguillae*) IN THE URBAN STRETCH OF TEVERE RIVER

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The research has focused the attention on the occurrence of polycyclic aromatic hydrocarbons (PAHs) in water, sediment and common eels (*Anguilla anguillae*) in the urban area of Tevere River. These compounds can derived from point (e.g. oil spill) or non-point (e.g. atmospheric deposition) sources and are one of the most widespread organic pollutants. The selected PAHs are belong to the EC priority list of dangerous substances (fluoranthene, benzo *(b)* fluoranthene, benzo (k) fluoranthene, benzo *(a)* pyrene, benzo *(g,h,i)* perylene, indeno *(1,2,3-cd)* pyrene). Some of them are known or suspected carcinogens, and are linked to other health problems. They are primarily formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacco. The Tevere River is the second largest river in Italy, it crosses industrial districts, agricultural and urbanised areas including the city of Rome, before flowing into the Tyrrhenian Sea. Water and bed sediment were collected from five sampling sites along the most polluted stretch of the river in the neighbourhood of Rome; samples of fish were collected only from one site that was located in the southern part of Rome, 382 km from the source and downstream a municipal WWTP effluent. Solid matrices (sediment and fish's muscle) were extracted by sonication while water samples were extracted and preconcentrated by C_{18} SPE cartridges. Final extracts were analysed by HPLC and fluorescence detection. The results have shown the presence of these compounds in all the samples analyzed. The most polluted compartment was sediment with values that ranging between 21 to 41 ng/g (sum of the six PAHs) followed by water samples with a content between 5 to 28 ng/L; values of concentration of total PAHs in muscles of common eels varied from a minimum of 1 to a maximum of 7 ng/g f. w. These results are in agreement with other values reported in literature for European rivers and can represent a possible factor of risk for environmental health.

2253 OCCURRENCE OF ENVIRONMENTAL MYCOBACTERIA IN WATER FROM THE CENTRAL ZONE OF THE CHIHUAHUA, MEXICO

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Environmental mycobacteria are inhabitants of many reservoirs, including water, soil, plants, animals and humans. The aquatic environment is an important reservoir of environmental mycobacteria; where their presence is influenced by biotic and abiotic factors. The presence of environmental mycobacteria in water has been related with health problems in inmunosupressed patients. In Mexico, there are not reports of the ecology of mycobacteria; therefore, the aim of this work was to characterize reservoir sites, like creeks and drinking troughs, and to determine some biotic and abiotic environmental factors to understand the conditions leading to their environmental persistence. The isolation technique was standardized using a *Mycobacterium smegmatis* and a *Mycobaterium kansasii* reference strains, and show a sensitivity of 3.75 bacterial cells/ml. After standardization of the isolation technique, thirty samples were obtained from different areas of Chihuahua State, Mexico. Environmental factors analyzed included total coliforms, mesophilic aerobic count, temperature, pH, DO, COD, Zinc, Lead, Iron and Arsenic. Multiple correlation analysis was done among all environmental factors studied. Average values of conditions were: 18.1°C temperature, pH of 7.72, DO of 2.33 ppm, COD in the range of 20-65 mg/l and one sample with a value of more than 3000 mg/l, Iron of 0.3827 ppm and Zinc of 0.0374 ppm. Samples had a low value of Coliforms, except for one sample, and the average total aerobic count was of 3.0 log CFU/ml. Thirty three strains of environmental mycobacteria were obtained in 36.7% of the samples, and were identified by biochemical tests as *M. fortuitum, M. phlei, M. simiae, M. chelonae, M. flavescens, M. vaccae, M. agri, M. scrofulaceum, M. avium complex, M. terrae complex, M. ulcerans, M. chitae, M. smegmatis* species, and one unidentified strain. Most of them were of rapid growth, not pigmented, and has been isolated previously from water sources. There was no identified influence of biotic and abiotic factors on their persistence; however there were positive and negative correlations among some of the factors. This investigation contributes to the knowledge of geographical distribution of environmental mycobacteria in Mexico.

2266 EVALUATION OF THE POLLUTION OF SURFACE WATERS IN THE BASIN OF WEST ALGERIA BY ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDES

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The pollutants are pesticides which cover a whole range of chemicals designed to protect plants from pests and destroy unwanted plants. There are a very large number of pesticides and we do here that some of the major families (organochlorine insecticides, organophosphates, carbamates, pyrethroides, phenoxyalcanoic, herbicides). Their transfer from the treatment area will be mainly caused by rain (runoff, infiltration) but also by the rise in temperature or the wind that promotes volatilization substances or dispersal in the heart of the treatment. The detection of pesticides in water is complicated because she asked for technical analyses and costly (HPLC, GC, GC / MS…), on the other hand there is a wide variety of active substances requiring analytical capabilities very different. Some active ingredients are very difficult to analyze because they have the physico-chemical characteristics that make their extraction or detection difficult. In this study we will attempt to provide a procedure for the evaluation of pollution of surface waters in pesticides drainage area of the rivers Tafna in the west of Algeria. For this sampling campaign was launched on several points of the study area for a full two years 2002 – 2007, given the large number of pesticides and after the inquiries that have been made in the study area we limit our research on the Families of organochlorine and organophosphorus pesticides such as methyl parathion, methomyl and deltamethrin. Used a liquid-liquid technique extraction and after preconcentration of simples we used a gas chromatography analysis coupled with mass spectroscopy to make a qualitative and quantitative detection of micro organics toxic pollutants in surface waters near agricultural basin.

2344 PHYSICO-CHEMICAL AND BIOLOGICAL RESEARCH ON WATER FROM ARIES RIVER

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Water flow in most river systems has two principal components: surface run-of of precipitation and groundwater. Their contributions differ in each system and depend on the physical setting of the drainage basin as well as on climatic parameters. Most groundwater bodies are isotopically constant and closely reflect the average annual isotopic composition of local precipitation. We studied the Aries River which is one of the biggest rivers from Romania, and unfortunately it is much polluted. Water samples were prelevated from 11 sites along Aries course. The study was carried out in the year 2007, and the stations were sampled seasonally. We have measured de ^{18}O and D isotopic composition in Aries River to know the evolution of the two components of this river, and to connect this data with the isotopic composition of the aquatic plants and with the pollution of the river. Some ions from Aries water were also analyzed: NO_3^-, NO_2^-, PO_4^{3-} Cu^{2+}, Fe^{3+}. Analysis of diatom communities has been studied in order to establish the water pollution of Aries. All physico-chemical analyses revealed that the most polluted site is Abrud; the source of pollution is most probably the mining enterprise from Rosia Montana. Isotopic contents are increasing from upstream to downstream. As organic pollutants we identified halogenated hydrocarbons and some organochloride pesticides. Aromatic hydrocarbons have been identified only accidentally. Heavy metals have also been identified in all sites, Cu, Mn, Zn and Fe having the highest values. The structure of diatom communities is strongly influenced by the different pollution sources from this area: mine waters, industrial waters, waste products, land cleaning, tourism, etc. Mine waters sloping in mining area from Bucium – Rosia Montana – Baia de Aries induces the total desintegration of diatom communities in Abrud brook, upstream of Campeni and downstream of Baia de Aries. The level of water eutrophication has been observed from upstream of Campeni to downstream of Campia Turzii.

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2372 EFFECT OF ANTHROPOGENIC IMPACT ON “CALORE” RIVER IN SOUTH ITALY

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The “Calore” is the main affluent of the Volturno that is one of the most important river of Campania region (South Italy). Many years ago was a navigable river but in the 1958 its flow has been reduced as a consequence of water deviation to Puglia region. Reduced flow caused an increase of pollutants coming from urban and industrial waste, no controlled and unlawful dumping as well as numerous quarries. The aim of this study was to evaluate the pollution level of Calore River and to identify the main source of pollution, such as Tammaro and Sabato affluents, industrial complex and Benevento city. In this view the content of some elements (Cr, Cu, Cd, Fe, Mn, Ni, Pb, Zn), and organic carbon content were measured in the sediments. These were collected in ten sites before and after the industrial area, the city and the confluence of Sabato and Tammaro affuents as well as the confluence of Calore into Volturno. To statistically analyze the data, 5 cores (∅ 5 cm) were collected in the 0-5 cm layer and separately analyzed. The highest contents of metals were found in the sites forward Benevento, industrial area and near an urban solid waste dumping. The affluents also contributed to river pollution because their high contents of Ni, Cr, Pb, Cu, Fe and Zn. The confluence of Calore into Volturno river significantly contributed to pollution of this last. In fact, all studied elements, with only exception of Cd, had higher contents in the site of the Volturno after the confluence with Calore than in that before. By comparing the metal contents of sediments with the limits proposed by Preter and Anderson (1990), it evidenced that the most of sediments were polluted by all tested elements, with the exception of Cd, Fe and Mn. In particular, the sites after the city appeared very polluted by Cr, Cu and Ni. As concern organic carbon content, the values increased three times in the sites after the city whereas the contribution of Sabato and Tammaro affluents appeared no significant.

2433 IMPACT OF HUMAN ACTIVITIES ON THE WATER QUALITY OF THE RED RIVER (VIETNAM): PRESENT SITUATION AND VARIATION TRENDS IN THE NEXT 50 YEARS.

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The Red River which covers a watershed area of 156,450 km^2 with a total population near 30 million inhabitants is one of the biggest rivers in Vietnam. The main branch of the Red River receives two major tributaries, the Da and Lo Rivers, and then forms a large delta before discharging into the Tonkin Bay (South China Sea). The 3 upstream sub-basins and the Delta area differ widely in population density (from 101 inhab km^{-2} in the upstream basins to more than 1000 inhab km^{-2} in the delta), land use and agricultural practices. The objective of this work is to estimate the influence of human activities in its watershed to the water quality of a tropical river system, the Red River. Few data are available on water quality in the Red River system, both in China and in Vietnam, excepted those collected at the outlet of the rivers in the delta area. In this paper, we introduced the results of water quality of the Red River system in the period 2003 – 2007, basing on monthly sampling surveys. Results showed that nutrients in the upstream sector of the Red River with less human impact was classified in the oligotrophic level while in the delta area where population, agricultural and industrial releases strongly affected, water quality was in the eutrophication statement. Using the Seneque/Riverstrahler software, we explore the effect in terms of water quality and biogeochemical functioning of a variety of scenarios describing possible future changes in the Red River basin concerning hydrological management, land use and agricultural practices and population increase.

2458 ENVIRONMENTAL IMPACT ASSESSMENT IN AN ABANDONED METAL MINE IN SPAIN

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There is an increasing concern regarding the environmental effects of mine tailing sites. Tailing are produced during ore processing and are characterized by high levels of heavy metals. Toxic metals in the tailing can be released in the environment by erosion and leaching processes and they contaminate water, soil and plant ecosystems resulting in human health and ecological risk. In this work a model using risk assessment tools is proposed to evaluate ecological impacts in mine areas. Evaluation model was applied to an abandoned mine in Bustarviejo (Madrid, Spain). Fifteen soil sampling sites were selected in the mining area and surrounding, distributed between 3 and 1300 m. In these samples, total soil and $(NH_4)_2SO_4$-extractable metal concentrations were determined. Environmental impact assessment comprised four steps: hazard identification, exposure assessment, dose-response assessment and risk characterization. In exposure assessment, potentially affected receptors and exposure pathway were identified. Exposure through indirect routes was determined using models and by means of direct measures of contaminants in surface waters and plants. Toxicity data were selected from literature. For risk characterization total and weakly adsorbed and easy extractable concentration of metals and metalloids in soil were compared with L(E)C50 and No Observed Effect Concentration (NOEC) for the different environmental receptors to determine impact to short and long term, and with the Predicted No Effect Concentration (PNEC), which allowed established different impact levels. Hazard identification showed mainly concern to arsenic, cadmium, cupper and zinc. Total soil concentration of these metals ranged between 2000 and 3 mg kg-1, depending of metal and distance to the mine. Extractable fraction varied from 1.97 to 0.12 mg kg^{-1}. Receptors affected were soil organisms, aquatic organisms and terrestrial vertebrates exposed through the consumption of contaminated food and soil ingestion. As expected, environmental impact assessment showed differences between metals, sampling sites and organisms. Notable differences were also observed when the assessment was based in extractable fraction rather than total fraction, especially for arsenic according with the least availability observed for this metalloid.

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2469 ENVIRONMENTAL RISK ASSESSMENT BY MULTI-BIOMARKER RESPONSES IN AQUATIC BIOTA OF THREE TROPHIC LEVELS IN A LAKE OF THE MEXICAN CENTRAL PLATEAU

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Aquatic ecosystems continually are impacted by wastewater and lixiviation of the adjacent areas. The inputs of xenobiotics generate complex mixtures that provoke adverse effects in the aquatic biota. The use of a battery of biomarkers for the toxic effect assessment of xenobiotics in the aquatic biota is usefully for the environmental risk assessment (ERA). The aim of this contribution is to assess comparatively the biomarker response of the aquatic biota of three trophic levels in Yuriria Lake (YL) to approach an ERA by the input of several xenobiotics. YL is located in the Lerma river (LR) basin, one of the most important in México, by the population density, industrial and agriculture activities. The main tributary is LR, one of the most polluted in Mexico, which incorporates a great diversity of pollutants to YL. Sentinel species and biomarkers chosen were: in the first trophic level the green algae *Ankistrodesmos falcatus*, lipid peroxidation level (LPO) and algal growth potential (AGP), in the second trophic level the amphipod *Hyalella azteca*, LPO and acetylcholinesterase (AChE) and in the third trophic level the fish *Goodea atripinnis* (hepatic glycogen, and in gill, muscle and liver: alkaline phosphatase, gamma-glutamyl transpeptidase, glutamic-pyruvic transaminase and AChE activities, and LPO). Static assays were used to assess the effect of the water from three sites of YL: the inputs of LR, the channel "la Cinta" (CC) and the limnetic zone (LZ). In *A. falcatus*, the LPO was higher in the assays from both tributaries, while the higher AGP was in the LZ. In *H. azteca* the AChE showed higher inhibition during spring and fall. In the case of *G. atripinnis* differences were detected by tissue and site studied. LR exerted most important damages in gills biomarkers while CC provoked major damages in liver biomarkers. The presence of diverse xenobiotics in YL provoked oxidative stress and growth inhibition in the first trophic level. The second trophic level was affected by oxidative stress and neurotoxic damages and in the third trophic level the liver and gill tissues were affected by oxidative stress, neurotoxic and hepatotoxic damages.

2619 EFFECT OF CADMIUM ON THE GROWTH OF *Spirulina maxima (Arthrospira)*

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The removal of heavy metal by microorganisms can occur throug several mechanisms, such as simple adsorption, absortion, enzymatic synthesis or through the production of extracellular polymers. Cadmium (Cd), a metal released in most mining and metallurgical processes, is one of the main agents responsible for water pollution. In spite of the increasing amount of research published on the interaction of microorganisms with heavy metals, few work describe this interaction with cyanobacteria. Because *Spirulina maxima* is widely cultivated for use as a nutrional supplement, its suceptibility to incorporating heavy metals needs careful investigation in order not to compromise its use in the human diet. Cadmium do not enter the metabolism of living beings, so they are considered non-essential and act serious cell toxicants even at low concentration (Moreira, 2001). *Spirulina maxima (Arthrospira)* strain was obtained from the microalgae collection of the Technological of Superior Studies of Ecatepec. Experiments were carried out in 500 mL Erlenmeyer flask within 400 mL of modified Zarrouk medium and 10% of inoculums at exponential growth phase, then the seed was added respectively with 5, 10, 15 and 20 ppm of Cadmium from a solution of Cadmium nitrate. The cultures were grown at environment laboratory conditions of temperature (28 ± 20C), continuously aerated at 0.5 vvm, photoperiod 12h /12h (dark-light) at 170 μmol/m^{2}s of photonic flux density. The cellular growth was determined by chlorophyll quantification with an optical density of 700 nm by a hot methanol technique according to the methodology of APHA (1992). Finally, all the experiments were carried out by triplicate during 168 h. The results we have obtained shown that *Spirulina maxima* is able to grow in the presence of Cadmium at 5, 10 and 15 ppm. However, the concentration of 20 ppm turned to be highly toxic for the seed since it started to grow modestly and suddenly died at 60 h. It is also worth noticing that at 5, 10 and 15 ppm the growth stops and decays after 60 h.

Keywords: Spirulina maxima (Arthrospira), heavy metals removal, lead, nickel, cadmium, biochemical reactors.

2620 EFFECT OF NICKEL ON THE GROWTH OF *Spirulina maxima (Arthrospira)*

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Nickel is not produced in Mexico, but is a component widely used in the paper industry, fertilizers, iron foundry, and jewels fabrication among others. Therefore it is founded in residual waters coming form domestic and industrial. It is known that such a heavy metal is essential to the life of some microorganisms including microalgae, however at relatively high concentrations it turns to be toxic inside some physiological processes (Jin *et al.*, 1996). Singh et al., had shown that the presence of Ni^{2+} may inactivate the photo-system II in *Nostoc muscorum* as far as it alters and destroys the photosynthetic membranes. From a biochemical view point the metal is located in the active cite of enzymes like hydrogenise, ureases, dehydrogenize carbon monoxide, mainly linked to a cystine or histadine, whose reaction produces the presence of guanidine-triphosphates (GTP asses) (Maier *et al.* 1993). The goal, motivated by the previous, is to observe the effect of nickel on the growth of *Arthrospira* at different concentrations of the metal. *Spirulina maxima (Arthrospira)* strain was obtained from the micro-algae collection of the Technological of Superior Studies of Ecatepec. Experiments were carried out in 500 mL Erlenmeyer flask within 400 mL of modified Zarrouk medium and 10% of inoculums at exponential growth phase, then the seed was added respectively with 5, 10, 15 and 20 ppm of Nickel from a solution of Nickel Chloride. The cultures were grown at environment laboratory conditions of temperature (28 ± 20 ºC), continuously aerated at 0.5 vvm, photoperiod 12h /12h (dark-light) at 170 μmol/m^2s of photonic flux density. Cellular growth was determined by chlorophyll quantification with an optical density of 700 nm by a hot methanol technique according to the methodology of APHA (1992). Quantification of the cellular metal content was registered at regular time intervals by atomic adsorption spectral-photometry. Finally, the experiments were carried out by triplicate during 168 h. The experimental work revealed the capacity of *Arthrospira* to grow in presence of relatively high concentrations, 5, 10 or 15 ppm, of Nickel. In turn, when dealing with a concentration of 20 ppm of the metal such nice growth exhibited a diminution starting from 48 h.

2621 EFFECT OF LEAD ON THE GROWTH OF *Spirulina maxima (Arthrospira)*

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The pollution of aquatic ecosystems caused by heavy metals from industrial and domestic sources leads to the bioaccumulation of these toxicants in cyanobacteria and microalgae with damage or inhibition of specific enzymes and tranfer of metals through the food web (He *et al.*, 1998). Moreover, even though ions of some metals, such as zinc, are involved in the metabolism of algae. *Spirulina maxima (Arthrospira)* strain was obtained from the micro-algae collection of the Technological of Superior Studies of Ecatepec. Experiments were carried out in 500 mL Erlenmeyer flask within 400 mL of modified Zarrouk medium and 10% of inoculums at exponential growth phase, then the seed was added respectively with 5, 10, 15 and 20 ppm of Lead from a solution of bicarbonate of Lead $PbCO_3$. The cultures were grown at environment laboratory conditions of temperature (28 ± 20°C), continuously aerated at 0.5 vvm, photoperiod 12h /12h (dark-light) at 170 μmol/m^2s of photonic flux density. Cellular growth was determined by chlorophyll quantification with an optical density of 700 nm by a hot methanol technique according to the methodology of APHA (1992). Quantification of the cellular metal content was registered at regular time intervals by atomic adsorption spectral-photometry. Finally, the experiments were carried out by triplicate during 168 h. According to the results, *Spirulina maxima* is able to grow in presence of Lead in all the range of polluting metal we have handled. In the case of 5 ppm or 10 ppm, the growth in medium Zarrouk was 50% larger than in the reference medium. In turn, with 15 ppm the growth in Zarrouk medium was 20% larger than in the reference medium and with 20 ppm it turned to be, in Zarrouk medium, 10% lower with respect to the reference medium. When adding 20 ppm of Lead to the seed, the undesirable effect of inhibition of the cellular growth appeared.

1814 SOIL ORGANIC MATTER AS FACTOR OF CARBON CYCLE IN NATURE

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Soil organic matter represents most important pool of carbon in nature. Have been estimated content about 2000 Pg of carbon in soil cover of Earth and about 500 Pg C in plant biomass. In the global C cycle we can identify about 60 Pg C yearly removed from soil into the air and same quantities of C removed from air back to the soil. Carbon (as CO_2 mainly) is directly emitted into the air from soil organic matter (after mineralization) and back carbon removing from air into the soil is realized through soil and plant living biomass. Equilibrium between both ways of carbon cycle is critical principle of ecosystems stability and sustainability of nature. During of several years we determined and data collected concerning of CO_2 emissions from different soils of Slovakia. From results we can conclude that from every 1 hectare of soil can be emitted yearly from about 3 to 5 tons of carbon (in average it is about 4.2 t C-CO_2). It is depending on soil quality mainly. From less productive soils is this emission more intensive in comparison to most productive soils. From total area of agricultural soils (2.4 mil. ha) about 10 061 thousands tons of C-CO_2 is emitted yearly what is very near to CO_2 emissions from industry. Of course relevant amount of C-CO_2 is removed back from air to soils. Detail maps and information systems about CO_2 emission from soils of Slovakia are available.

1839 HEAVY METAL CHARACTERIZATION IN SOILS UNDER DIFFERENT WASTEWATER IRRIGATION PATTERNS

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An area closed to the city of Chihuahua has been traditionally irrigated with wastewater to produce agriculture products including animal foodstuffs. It has been hypothesized that metal levels could be found in these soils that were high enough to cause potential health and population problems. The objective was to determine heavy metal concentrations in different soils due to irrigation practices in an area close to the metropolitan area of Chihuahua, Mexico. Four soil types were evaluated; a soil with a history of irrigation with wastewater and still irrigated (S1), a soil with a history of irrigation with wastewater until 2003 (S2), a soil with no irrigation history (S3), and a soil close to S1 and close to the river where the wastewater is transported (S11). Three soil depths were evaluated; 0-15, 15-30 and 30-50 cm. A total of 150 soil samples were analyzed evaluating pH, EC, OM and the following elements; Na, K, Cd, Pb, Ni, Cr, Cu and Fe. The pH and EC were different for soil type but no differences were noted for soil depth and the interaction. Maximum pH levels were noted in S3 with a value of 8.74 while maximum EC were observed in S1 with about 0.850 dSm^{-1}. OM was different for soil types and soil depths but the interaction was not significant. S1 and S11 obtained maximum levels of OM while minimum levels were noted in S3. Maximum OM levels were observed at the 0-15 cm depth followed at the 15-30 cm depth and finally at the 30-50 cm depth. The metals K, Pb, Cu and Fe were observed in maximum levels in S1 or S11 while the metals Na, Cd, Ni and Cr were noted in maximum levels in S3. The conclusion is that some metals are present in the soils due to anthropogenic activities but some others are present in natural forms.

1883 HEAVY METALS IN SELECTED EDIBLE VEGETABLES AND THEIR DAILY INTAKE IN SANANDAJ, IRAN

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The levels of four different heavy metals [cadmium (Cd), lead (Pb), chromium (Cr) and copper (Cu)] were determined in various vegetables [leek (*Allium ampeloprasum*), sweet basil (*Ocimum basilicum*), parsley *(Petroselinum crispum*), garden cress (*Lepidium sativum*) and tarragon (*Artemisia dracunculus*)] cultivated around the Sanandaj city. The contributions of the vegetable to the daily intake of the heavy metals from the vegetables were investigated. One hundred composite samples (monthly 20 samples) were collected during five months. Atomic absorption spectrometry was used to determine the concentrations of these metals in the vegetables. The average concentrations of each heavy metal regardless of the kind of vegetable for Pb, Cu, Cr and Cd were 13.60 ± 2.27, 11.50 ± 2.16 , 7.90 ± 1.05 and 0.31 ± 0.17 mg/kg, respectively. Based on the above concentrations and the information of National Nutrition and Food Research Institute of Iran, the dietary intake of Pb, Cu, Cr and Cd through vegetable consumption was estimated at 2.96, 2.50, 1.72 and 0.07 mg per day, respectively. It is concluded that the vegetables grown in this region have a health hazard for human consumption.

2153 TOXIC EFFECT OF FOUR FABRIC SOFTENERS ON THE GROWTH AND CARBON DIOXIDE PRODUCTION OF SOIL MICROORGANISMS

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The fabric softeners contain potentially toxic substances, such as cationic surfactants, dyes and others additives. The fabric softeners are discharged in the wastewater produced in the laundry activities, and these wastes have potential pollution capability on the ecosystems that received wastewaters without treatment. Our principal aim was to determine the toxic effect on the microorganisms of soil that received fabric softeners without treatment. Two kg of soil sample was collected in the municipality of Cuautitlan, State of Mexico, Mexico. The soil was collocated in a polyethylene black bag and was maintained to 4°C. Texture, pH, organic material percentage and soil humidity were determined. The four fabric softeners with the biggest sales in Mexico were evaluated for the toxic effect on the growth of mesophilic aerobic bacteria (MAB) and fungi of agriculture soil. Five 0.5 L polyethylene bottles were employed. Each bottle contained 30 g of soil with humidity of 30%. The concentration of fabric softener evaluated was of 0.1%. One bottle was maintained with 30 g of soil without fabric softener as a control system. The bottles were maintained to environmental temperature. The microbial growth was evaluated for viable count in Bengala Rose Agar and Standard Count Agar, every day for 10 days. The plates were maintained to 37°C/48h and 28°C/5 days to determine the MAB and fungi respectively. The effect on the carbon dioxide microbial soil production was evaluated, using the fabric softener with the most toxic effect on the microbial soil growth. 0.5 L polyethylene bottles were employed with 30 g of soil (30% humidity) and 0.1% of more toxic fabric softener. One bottle with 30 g of fabric softener free soil was employed as a control system. The bottles were maintained to environmental temperature and the carbon dioxide production was evaluated using the Winkler method. The four fabric softener evaluated inhibits the MAB growth. The fabric softener identified as FS-2, produce a drastic bacterial growth reduction of $7x10^6$ to $1x10^6$ CFU/g. The inhibition of the microbial soil carbon dioxide was observed, when the FS-2 was applied. The reduction observed was to 506.67 μMolCO_2 compared with the control system.

2220 EQUIVALENT DOSE AND ACTIVITY CONCENTRATION OF RADIONUCLIDES IN SOIL AND PLANTS

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The main objective is to know radionuclide dispersion (238-U, 226-Ra, 210-Pb, 232-Th, 224-Ra) and biotransfer to tree species. The study area is northeast of Salamanca province (Spain), where there was mining uranium. Initially, were measured "in situ" equivalent dose to a Geiger counter. According to the data, were collected samples of soil and samples of plant. In these samples were determined activity concentrations of radionuclides by gamma spectrometry. The average values of the equivalents dose were 1.33 μ Sv/h in the most altered zone and 0.16 μ Sv/h to 500 metres away. Compared to the literature, mining waste showed low values for 238-U (3388 Bq/kg), whereas most disturbed soils have very high values of 238-U (18200 to 1038 Bq/kg) y 226-Ra (7700 to 4155 Bq/kg). The branches and leaves of trees rapid growth (*Populus alba*) presents transfer rates lower ($4.4E^{-3}$ and $2.60E^{-2}$ for 226-Ra) than those found in oak (*Quercus ilex*) ($1.17E^{-01}$ and $1.98E^{-01}$).

2233 SOIL EROSION AND RUNOFF RESPONSE IN ALMOND ORCHARDS UNDER TWO SHRUB COVER-CROP STRIPS IN A HIGH SLOPE IN SEMI-ARID ENVIRONMENT

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Soil erosion is one of the main physical processes of land degradation in Spain. Several studies in the Mediterranean environment have demonstrated the positive effect of vegetation covers on the reduction of water erosion and their indirect improvement of the soil physical and chemical properties, essentially by the incorporation of organic matter. The main aim of this study was to analyse two cover-crop strips: Andalusian thymus and rosemary effects on soil loss and runoff from hillslope with almond orchard, which is representative of those commonly found throughout the study zone. Soil loss and surface runoff patterns over a five-year period were monitored in erosion plots from hillslope with two different cover-crop strips: (1) non-tillage with thymus (*Thymbra capitata* (L.) Cav.) and (2) non-tillage with rosemary (*Rosmarinus officinalis* L. (var. postrata) of 3 m width, in Lanjaron (Granada) on the south flank of the Sierra Nevada of southeast Spain. The erosion plots were located on the hillslope at 35% incline, at 580 m in altitude and with 144 m^2 (24 m x 6 m) in area. The area selected for the experiment is the part of the rainfed orchard given entirely with almond *(Prunus amygdalus* Basch cv. Desmayo Largueta) trees, the planting grid were 6 x 7 m. The cover-crop strips were tested in order to provide information on the erosional response. In the *Thymbra capitata* strips, runoff ranged from 30.4 to 8.8 mm yr^{-1} and erosion from 0.79 to 0.15 Mg ha^{-1} yr^{-1}, while under *Rosmarinus officinalis* strips, runoff ranged from 49.8 to 5.8 mm yr^{-1} and erosion from 6.1 to 0.2 Mg ha^{-1} yr^{-1}. According to the results the strips of *Thymbra capitata* reduced the runoff and soil losses with respect to the *Rosmarinus officinalis*. In the last two years of the study a similar behavior is appreciated in the soil protection for the both strips. The goal of this study is to provide a decision support tool for an appropriate land management in the study area.

2234 COMPARISON OF THE PROTECTIVE EFFECT OF TWO HARVEST INTENSITIES IN THE SHRUB *Santolina rosmarinifolia* L. IN AN AREA OF THE SPAIN SOUTHEAST

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The objective of this work is to study the behavior of the *Santolina rosmarinifolia* L. as for its protective effect of the soil. Two harvest intensities are compared (25 and 50%), considered as the most suitable to guarantee a sustainable use of this species, as shown on previous study (Martínez Raya, A. *et al*. 2002). The methodology used was closed plots to allow us to have a continuous evaluation of the sediment emission and runoff. Four plots were monitored, two for each harvest percentage, with a surface for each one of 96 m^2, in a slope of 17.5% a planting grid of 1 x 1 m has also been used. The obtained results refer to a four years study, being controlled a total of 27 erosive events. The data refers to the mean value calculated for each harvest treatment. The annual values of precipitation, erosion and runoff are shown, as well as the average of the runoff and soil losses for the four years study. Being this value very inferior average to the estimated one by this team in bare soil (6.14 Mg ha^{-1}; 182 mm) (Durán, V.H. *et al.*, 2006), which demonstrates the effectiveness of this specie in the protection of the soil versus the erosion. The differences in the soil erosion and runoff values for the two harvest intensities are small in the four years study and in both cases the values annual means are considered acceptable (0.11 Mg ha^{-1} and 4.5 mm for 25% and 0.47 Mg ha^{-1} and 6.7 mm for 50%). With the obtained results, as per the production of annual biomass, essential oil and soil protection, it can be concluded that the appropriate harvest intensity, for this specie and under these soils and weather conditions to guarantee a sustainable use, it is that of 25%.

2259 ADVANCES IN CALIBRATING SALTIRSOIL AT PLOT SCALE: FIRST RESULTS

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SALTIRSOIL (SALTs in IRrigated SOILs) is a capacity-type, steady-state model developed to predict average major ion content (sodium, calcium, magnesium, chloride, sulfate) in the medium to long term in productive well drained soils. Once the algorithm verification was done the objective of our study was to calibrate SALTIRSOIL under one of several water quality and management scenarios in Mediterranean agriculture. Given that drip and basin are the irrigation methods most representative of irrigated agriculture in Valencia (Spain), this calibration is being performed with climate, soil, irrigation water (composition and management) and crop (species and management) information from an experimental plot drip irrigated with well water and cropped to water melon that has been monitored since late spring 2007. To carry out the calibration, firstly we have performed a sensitivity analysis. That is to change an input variable at a time within a range while maintaining the other variables unchanged and measuring the variability in the model output. Secondly, we have compared simulated saturation soil extract composition against measured data. According to the sensitivity analysis SALTIRSOIL calculations seem to be most affected by climate (rainfall) and water quality and less by soil physical characteristics. According to the first predicted-measured comparisons SALTIRSOIL does not seem to be affected by any systematic error and as a consequence, parameterization would not be needed at least under drip irrigation. On the other hand we have observed that i) the longer the climate data record used for the simulations the closer calculated and measured data are and ii) calculated results are closer to space-averaged measured data than to whichever single point measured data. More comparisons under different water quality and irrigation management practices are needed in order to calibrate SALTIRSOIL in the study area.

2285 ENVIRONMENTAL IMPACT OF INTRODUCING AROMATIC-SHRUB STRIPS IN ALMOND ORCHARDS UNDER SEMIARID CLIMATE (SE SPAIN): IMPLICATIONS FOR EROSION AND AGRICULTURAL RUNOFF CONTROL

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Erosion degrades soil quality in natural, agricultural, and forest ecosystems, thereby reducing the productivity of the land. Semi-natural vegetation and diverse cropping systems have been converted into monocultures with low tree densities, leaving the soil unprotected. Soil loss, runoff, and nutrient loss over a four-year period were monitored in hillside erosion plots with almond trees under different soil-management systems: (1) non-tillage with sage (*Salvia lavandulifolia* L. sub species *Oxyodon*) strips 3 m wide (SS); (2) non-tillage with rosemary (*Rosmarinus officinalis* L.) strips (RS), (3) non-tillage with thyme (*Thymus baeticus* L. Boiss. exlacaita) strips (TS), and (4) conventional tillage (CT) in Lanjaron (Granada, SE Spain). The erosion plots, located on a 35% slope, were 144 m^2 in area. The plant-cover strips, 3 m wide, ran across the slope. The most effective treatment proved to be TS, reducing soil loss by 93% and runoff by 80%, with respect to CT. The RS reduced soil loss by 91% and runoff by 82%, with respect to CT, while these percentages were 69% and 51%, respectively, for SS. In addition, all the treatments as a whole, in comparison with CT, revealed that the plant strips were the decisive factor in the reduction of NPK losses by surface runoff. The TS treatment decreased nutrient loading in surface waters and thus had a positive impact on the environment. Thus, the combination of orchard trees with shrubs provided a viable option to conserve soil and water in hilly areas and highlights the beneficial impact of plant strips in mountainous agriculture.

2286 SOIL EROSION AND NUTRIENT LOSSES CONTROL BY PLANT COVERS: ENVIRONMENTAL IMPLICATIONS FOR A SUBTROPICAL AGROECOSYSTEM (SE SPAIN)

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Soil erosion, in addition to causing on-site loss of topsoil and reducing the productivity of the land, brings about major off-site environmental effects such as water body pollution and eutrophication. In the Mediterranean area, this fact is especially relevant where precipitation is characterized by scarcity, torrential storms and extreme variability in space and time. To study the effects of soil erosion in runoff potential pollution we installed six erosion plots on the taluses of orchard terraces where an intensive irrigated agriculture based on subtropical crops has been established. The experimental erosion plots (4 x 4 m in area) were located on a talus of 214% slope at 180 m in altitude. Soil losses, runoff, nutrient losses (NO_3-N, NO_2-N, PO_4-P, and K) and carbon losses were monitored during a two-year period (2005-2006). The plant covers used were: *Thymus mastichina* (Th), *Lavandula dentata* (La) and native vegetation (Nv). The highest erosion and runoff rates was recorded in Th with 15.1 mm yr^{-1} and 1.96 t ha^{-1} yr^{-1}, and the lowest in Nv with 13.9 mm yr^{-1} and 1.3 t ha^{-1} yr^{-1}, respectively. It is concluded that Nv was the most efficient plant cover. The NO_3-N did not exceed the maximum admissible concentration in drinking water (50 mg L^{-1}) in any treatment, however, NO_2-N exceeded the maximum concentration permited for drinking waters (1 mg L^{-1}), and the level above 0.75 mg L^{-1}, which is considered to cause stress in fish. In relation to the NH_4-N concentrations from all treatments, especially Th exceeded the 0.5 mg L^{-1} standard for public water supplies. The 2 mg L^{-1} limit (level considered toxic to fish) was only exceeded in Th. The highest values for PO_4-P in runoff were reached in Th, where concentrations surpassed the limit for protection of fresh water (0.01-0.025 mg L^{-1}). The highest concentration of soluble K was reached in Th, but they were within the 12 mg L^{-1} upper limit recommended for drinking water. These results recommend the use of plant aromatic as barriers to control erosion and thus pollution of water bodies in the taluses of orchard terraces in a subtropical agroecosystem.

2431 IMPACT OF HEAVY METALS IN ENZYMATIC ACTIVITY OF SOILS FROM HIDALGO, MEXICO

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The soils from Valle of Mezquital, Hidalgo, Mexico have been irrigated with waste waters from Mexico City for more than 88 years. The present investigation was made in order to know the relationship between heavy metal contents and time of irrigation with waste waters and production of CO_2 and enzymatic activity in soils from Valle of Mezquital for knowing the disponibility of nutrients and degradation of soils. The soil samples (Ten in each plot). Three plots with different time of irrigation: 5 years (C1), 50 years (C2) and 100 years (C3). The soils associated with the plots were Leptosol Rendzic (C1), Calcaric Phaeozem (C2) and Leptic Calcaric Phaeozem (C3). The enzymatic activity and respiration were analized with three replicates, 30 superficial samples (0-20 cm) at differents times of incubation 0, 4, 7, 14, 21, 28 and 60 days. All samples were analyzed by alkaline phosphomonoesterase, dehydrogenase, urease and basal respiration (García et al., 2003). The heavy metals contents was evaluated by PIXE particle accelerator method, at 0 to 20 cm and 20 to 40 cm. SPSS package were used for the analyses and the ANOVA were determined to assess the statistical differences to level of $P<0.05$. The activity of dehydrogenase, phosphatase and urease show statistical differences at different times of irrigation and incubation. The higher values were observed at 50 and 5 years and the smallest values at 100 irrigation years. The respiration was low at 100 years of irrigation with waste waters. To 0 to 20 and 20 to 40 cm the contents of Cr, Co, Ni, Cu, Mn and Zn were similar. The lower concentration in all metals was observed between 5 and 50 years while the concentration was higher in samples with 100 years of irrigation with wastewaters. In the soil with 100 years of irrigation the Cr was the more phytotoxic with ratios of more than 100 mg kg^{-1}, the content of Zn was of more than 300 mg kg^{-1}, and the Co was similar to 50 mg kg^{-1}, the content of Cu was 80 mg kg^{-1}. The K, Fe and Ti were of similar values and at 5 and 100 years the contents were higher, the Ca was of low concentration at 100 years. The conclusion is that the irrigation with wastewaters at 100 years lower notably the enzymatic activity and respiration, this is associated with the high concentration of heavy metals and low content of Ca.

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2460 EFFECT OF RHIZOSPHERIC BACTERIA ON SUNFLOWER GROWTH UNDER ABIOTIC STRESS CONDITIONS

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The contamination is widespread all over the world affecting living beings, particularly the plants, reducing their biomass and harvestable parts. In this sense, the phytoremediation is a very promising technology for decontamination. Previously, *Pseudomonas fluorescens* biotype F was isolated from the rhizosphere of sunflower plants in heavy metal contaminated soil, identified and characterized as tolerant to arsenic and its protein profile, under arsenic stress conditions was determined [1]. It is known that some rhizospheric bacteria promote plant growth and tolerance to contaminants. Two cases were investigated – arsenic contamination and salty-stress. We studied the influence of the isolated strain and the strain *P. fluorescens* CECT 378 on the growth of sunflower under arsenic or salt stress conditions in growth chamber. The experiments were carried out during 35 days and different growth parameters and arsenic accumulation were measured. We observed that the inoculation is quite beneficial for the plant growth and accumulation of the metalloid in the tissues. The plant dry biomass in the treatment with *P. fluorescens* biotype F was increased with more than 20 % compared with the control treatment. On other hand, the bacteria were characterized for their ability to survive in salt or arsenic stress conditions in solid and liquid media. Furthermore, we assessed their abilities to utilize aminocyclopropane carboxylic acid (ACC) [2] as sole source of nitrogen, as well as production of indoles [3] and siderophores [4]. These parameters are of primary importance when estimating if some strain could be determined as plant growth-promoter or not. Finally, it was determined that the inoculation of these rhizobacteria is beneficial for the plant in order to overcome the abiotic stress conditions produced by arsenic or salt.

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2476 ASSESSMENT OF THE GENOTOXICITY OF OLIVE MILL WASTE WATER (OMWW) WITH THE *Vicia faba* MICRONUCLEUS TEST

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Olive mill waste water (OMW) can cause serious environmental hazards in olive producing countries, especially around the Mediterranean basin. In Morocco, olive mills are one of the foremost polluters: the volume of OMW produced annually is estimated at 250 000 m^3 during the season of production. The present study concerns the genotoxicity of OMW generated in mills producing olive oil in Morocco. The *Vicia faba* micronucleus test which detects chromosomal breakage and aneuploidy was employed in order to evaluate the genotoxic potential of OMW and the six major phenolic compounds identified by HPLC in this effluent. Five concentrations of OMW were tested: 0.1; 1; 5; 10 and 20%. Maleic hydrazide was used as positive control and aerated Hoagland's solution was used as negative control. The results showed that OMW was genotoxic at a 10% OMW concentration. For low concentrations, 0.1 to 5%, no significant difference in micronucleus frequencies was recorded in *Vicia faba* cell roots. In order to investigate the molecules involved in this genotoxicity, the six major phenols (oleuropein; gallic acid; 4-hydroxyphenylacetic acid; caffeic acid; paracoumaric acid and veratric acid) present in this effluent were studied at the concentrations corresponding to the genotoxic concentration of the OMW. Data showed that two phenols, gallic acid and oleuropein induced a significant increase in *Vicia faba* micronucleus frequency; the four other phenols had no significant genotoxic effect. These results suggest that in our experimental conditions, OMW genotoxicity was associated to gallic acid and oleuropein. This genotoxicity depend on the phenolic compounds concentrations, the pH of the culture medium and the presence of metal ions (particularly Fe and Cu).

2489 INFLUENCE OF A PHOSPHO-POTASSIC FERTILIZER SOLUTION ON YIELD AND QUALITY OF WHEAT CROPS

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There is currently interest in the use of industrial by-products to reduce the use of synthetic fertilizers. For this reason, in this paper the influence of a phospho-potassic fertilizer solution obtained from a aminoacid production process on wheat crops is studied. The positive influence on leaf potassium contents was most significant when the dosage of phospho-potassic fertilizer solution was applied to bread wheat. On the wheat grain, the levels of potassium were significantly highest with the highest doses of the phospho-potassic solution. Likewise, for phosphorus contents the differences were significantly more manifest in bread wheat. Finally, the additions of the experimental solution (but only in the highest doses) produce the highest yield of both varieties of wheat, and on the carotene content (wheat for semolina) and the Zeleny index for bread wheat.

2491 INFLUENCE OF FERTILIZER MATERIALS ON THE PHYSICAL AND CHEMICAL PROPERTIES OF SOILS: CASES OF WINE STEFANESTI – ARGES

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The overall objective is the preservation or improvement Heritage ground, avoiding the loss of organic matter. This objective is consistent with the general approach of sustainable agriculture is an investment in the long term. Organic materials play an important role in the global functionnement soil, through its physical, chemical and biological properties, which define the concept of fertility. The objective of this work is to illustrate the influence of organic matter (humus in the form of stable) from soil fertilized on the physical and chemical properties of soil in a vineyard-Stefanesti Arges. For each soil type, have been planned: V1 - a witness not fertilized; V2 - soil fertilized with grape marc; V3 - a soil fertilized with green manure; V4 - soil fertilized with manure. The study covers 3 years of experimentation in *Vitis vinifera* vines var Cabernet Sauvignon and Zweigelt. The physical characteristics of the soil (bulk density, degree of compaction and the degree of compaction, porosity and porosity drainage, distribution of porosity units per floor, soil moisture, its field capacity) and chemical (humus rate, pH , active carbonate, nitrate nitrogen, exchangeable K, phosphorus accessible to plants) for each variant experimental. Thus the parameters of soil in the plots are usually fertilized by improved qualitatively significant change in the density, degree of compaction, porosity and drainage (V2). Also, fertilizing with organic fertilizers induces a reduction of carbonate assets and an increase humus stable, especially in plots fertilized with manure and grape marc. Phosphorus accessible to vineyards in the fertilized plots with grape marc is also significant increases. The results show the positive effect of the incorporation of organic matter on the physical and chemical properties of soils.

2533 LONG-TERM EFFECTS OF OLIVE OIL MILL WASTEWATER SPREADING ON SOIL AND OLIVE TREES

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The olive oil extraction process produces huge amounts of liquid waste called olive mill waste water (OMWW). Large amounts of OMWW (30 million m^3) are produced in the Mediterranean regions that accounts for 95% of the total olive oil production worldwide. In Tunisia, OMWW constitutes a serious environmental problem due to the features associated with this type of agro-waste and to its diverse organic load which may reach values as high as 100 g/L and is mainly due to sugars, lipids, phenols, and tannins. The main purpose of this study is to investigate the long term effects of OMWW spreading on the soil characteristics, on olive tree growth and yield. The experiment was carried out between January 1995 and December 2007 in olive orchards located in the Châal (34° 3' N, 10° 20' E; Sfax, Tunisia). The experimental design consisted of 4 treatments (with three replicates of 6 trees each per treatment). The untreated OMWW was sprayed at 0, 50, 100 and 200 m^3 ha^{-1}. The effluent used in this study was taken from a three phase olive mill extraction. Physico-chemical characteristics of soils (pH, electrical conductivity, organic matter, soil macro- and micronutrients including N, P, K) were determined before and after each OMWW application. Samples were collected yearly at 3 and 12 months after OMWW application at 40 cm soil depth. Olive tree growth, yield and oil quality were evaluated every year. Comparing the different treatments with the control, the spray of OMWW at 50 m^3 ha^{-1} has slightly improved the soil fertility and the plant growth. However, the 200 m^3 ha^{-1} treatments has induced the soil salinization, reduced the soil permeability and thus the plant growth and olive yield were decreased. The supply of OMWW at 100 m^3 ha^{-1} has permitted a marked improvement of the soil fertility. Its organic matter, nitrogen and potassium contents have increased noticeably. The increase of organic matter content from 0.3% to 1.3% has allowed an improvement of the retention capacity and water permeability of the sandy soil. The spray of the OMWW in the sandy soil of the south of Tunisia, at acceptable level (100 m^3 ha^{-1}), could be an interesting alternative for the rational evacuation of this sewage. Furthermore, the OMWW spray has improved the soil stability and created mulch preventing water evaporation due to its binding and hydrophobic effects.

2550 ENVIRONMENTAL EFFECTS ON PROLINE ACCUMULATION AND WATER POTENTIAL IN OLIVE LEAVES (*Olea europaea* L. CV "CHEMLALI") UNDER SALINE WATER IRRIGATED FIELD CONDITIONS

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In arid regions in Tunisia suffering from limited water resources, the olive extension to irrigated lands has led to the urgent use of saline water, the most readily available water in these areas. Nevertheless, the effects of salt stress on olive tree seem to be reinforced by environmental conditions. The issue of this paper is to determine how does the olive tree respond to environmental stress in the Mediterranean climate under saline water irrigated field conditions with respect to leaf proline concentrations and water status? To answer this question we determined simultaneously proline concentration and water potential (ψLw) in olive leaves (*Olea europaea* L. cv "Chemlali") during two successive crop seasons and related them to environmental parameters. The experiment was carried out in the orchard of the Olive Tree Institute in Sfax, Tunisia. The environmental parameters we followed were: air temperature, rainfall, solar radiations. Similar patterns of change of proline levels and ψLw were observed during both crop seasons. Low leaf proline content, accompanied with low ψLw, was detected during both autumn and winter seasons. The increase of air temperature and solar radiations has led to the accumulation of proline and the decrease of ψLw. The olive trees tend to acclimatize to prolonged hot-dry periods by the accumulation of proline in order to activate water uptake to the growing tissues. Old leaves were found to sustain lower ψLw values and higher proline content than the young ones. The old leaves seem to play a protective role for the young ones. Water status and ψLw were affected more strongly by most environmental parameters during the vegetative growth phase and to a lesser extent during the rest phase. The olive tree develops several mechanisms to cope with such stress conditions.

2869 RESIDUAL SORPTION AND LEACHING OF THE HERBICIDE DIURON FOLLOWING DE-OILED TWO-PHASE OLIVE MILL WASTE ADDITION TO SOIL

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The residual sorption, desorption, degradation, and leaching of the herbicide diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a herbicide widely used in olive groves, was studied following the addition to soils of de-oiled two-phase olive mill waste (DTPOMW). Field experiments were conducted on an olive grove soil amended over seven years with DTPOMW. Treatments included an unamended control, and DTPOMW1 and DTPOMW2 (27 and 54 Mg ha^{-1} DW equivalent, respectively). Soil samples were collected two years after the last DTPOMW application to measure the residual effects. Diuron sorption and desorption isotherms for unamended and amended soils were obtained by short-term (<24 h) equilibration tests, and were fitted by the Freundlich equation. Leaching experiments were studied in hand-packed soil columns, collecting the leachates over 55 days. Half-lives (t1/2) were calculated with incubation studies in darkness, after adjusting the soil moisture content to -0.33 kPa. Due to the natural composting of DTPOMW in the soil, the diuron sorption isotherms showed a major residual increase two years after the last waste addition. Sorption increased with the amount of DTPOMW added, reflecting the affinity of diuron for the organic amendment, by factors of 11 and 15 over the unamended soil for DTPOMW1 and DTPOMW2, respectively. DTPOMW also influenced diuron desorption from the soil, and increased diuron sorption irreversibility. The incubation studies showed that DTPOMW addition to soil increased the herbicide's half-life. Diuron only leached from unamended soil, and the amount of applied herbicide recovered was 9%, 7.2 %, and 4.1 % for control, DTPOMW1, and DTPOMW2, respectively. DTPOMW may be useful as a means of reducing the risk of groundwater contamination as a result of diuron leaching.

2887 STUDY OF TOXICITY AND DISTRIBUTION OF MINE TAILING IN ARID/SEMIARID CLIME

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A total of 69 soil samples around a sulphur mine of Trovador (Almería, Spain) were studied. The samples were divided into three sectors. Sector 1 represents the mine waste, Sector 2 the drainage zones (ramblas), and Sector 3 the limestone high relief. The mining waste registered a very acidic pH, without carbonates, with low cation-exchange capacity, high electrical conductivity of the saturation extract, and a high concentration of S, Zn, As, Cd, and Pb. Meanwhile, the soils of Sector 3 presented a basic pH, carbonates, and low electrical conductivity. The S in the mining waste, on oxidizing, acidified and solubilized to trace elements that, in this form, were spread throughout the drainage waters, accumulating in the soils of Sector 2. The quantities of Cu, Cr, and Ni that accumulated were relatively low and do not represent a serious problem of toxicity. The Zn and Pb, although accumulating in greater concentrations, tended to precipitate rapidly and were transported only short distances, at least in toxic quantities. On the contrary, As and Cd tended to concentrate in the ramblas, especially in the lowest relief, near population centres, where they reached potentially toxic concentrations.

2888 EFFECT OF HEAVY METALS FROM SOILS AMENDED WITH BIOSOLIDS AND SOWED WITH FORAGES ON THE ABUNDANCE AND BIODIVERSITY OF EDAPHIC ARTHROPODS

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There are many studies about positive effects of biosolids application to ameliorate grain and forage production. However it is necessary to know more about the effects of this by-product on edaphic biota. Therefore the goal of this study was to know the effects of heavy metals from biosolids of the wastewater treatment plant of Aguascalientes city (Mexico) on edaphic arthropods in soils sowed with lucerne and corn amended with biosolids at 200 (low), 400 (middle) and 800 (high) ton/ha wet weight. Although Cd, Cr, Cu, Ni, Pb and Zn levels were below Mexican guidelines because most waste water is from domestic sewages, the effects observed with 200 ton/ha are alike with highest dose in several aspects. Regression analysis among heavy metals and the main arthropod groups showed positive correlation among Cd, Cr, Ni and Zn microelements and species richness, so mites, collembolan dipterous, himenopterous and spider abundance. Otherwise negative effects were observed between Cd and coleopterous abundance and between Pb and himenopterous abundance. Species diversity was not correlated with any heavy metal. Edaphic arthropod abundance increased with increase biosolids dose. Species richness was high lightly with 200 ton/ha than without biosolids application, with 400 and 800 ton/ha species number decreased in two forage species. Arthropod biodiversity estimated with Shannon index was higher with low dose than without biosolids in lucerne forage. Arthropod biodiversity in corn cultivation was similar without biosolids and with low dose. Middle and high doses caused a significative decrease in biodiversity in both forage species.

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2581 ECOTOXICOLOGIC DIAGNOSIS OF A SEALED MUNICIPAL DISTRICT LANDFILL

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Assessing the environmental impact of a soil-topped landfill requires an ecotoxicologic diagnosis. Here we describe a set of protocols for such a diagnosis as well as their application to a real case (the urban solid waste, USW, landfill of Getafe, Madrid). Since their initial sealing some 20 years ago with soils taken from the surroundings, waste deposition has continued in most USW landfills of the Comunidad de Madrid. This has hindered a precise evaluation of their impact on the environment and its affected ecosystems. To overcome these difficulties, the procedure proposed assesses three areas: edaphic, aquatic and ecologic. In this study, we consider: the soil variables that have proved most relevant (salinity due essentially to anions, and heavy metal and organic compound concentrations); these same variables determined in surface waters of the wetland most affected by leachates running down its slopes; and also characterize plant communities and microbial biodiversity. Finally, we present the results of a bioassay conducted in controlled conditions in microcosms prepared using soil polluted with $ZnCl_2$ (different Zn concentrations), in which we evaluate the ecochemical actions of a plant population (*Bromus rubens*) growing in abundance at the landfill site.

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2582 HEAVY METALS, SALTS AND ORGANIC RESIDUES IN SOLID URBAN WASTE LANDFILLS AND SURFACE WATERS IN THEIR DISCHARGE AREAS: DETERMINANTS FOR RESTORING THEIR IMPACT

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This report describes a continuous assessment of the impact of solid urban waste (SUW) landfills in the central Iberian Peninsula that were sealed with a layer of soil 20 years ago. Cover soils and soils from discharge areas have been periodically analysed. Soil concentrations of salts and heavy metals affect the biotic components of these ecosystems. Our analysis of this problem is highly conditioned by the constant reuse of many of the SUW landfills, since they have never been the target of any specific restoration plan. This has meant that subsequent to their initial sealing, the scenarios have received the input of new waste, which in large measure has not been domestic waste, although the landfills already contained mixed urban/industrial residues. In many of these regions, the problem of soil pollution has worsened over the years. Thus, current analyses continue to reveal the presence of heavy metals (Zn, Cu, Cr, Ni, Pb) in soils, salts (sulphates, chlorides and nitrates) in soils and surface waters, along with non-agricultural organic compounds, mainly aromatic and aliphatic hydrocarbons often appearing in very high concentrations, insecticides such as gamma-HCH, with high levels detected in soils. Around 50% of the water samples collected show COD levels in excess of 150 mg/l. Traces of phenolic compounds were detected in 15 samples, some of which exhibited high levels of 2-chlorophenol and pentachlorophenol. All these factors are conditioning both the revegetation of the "landfill systems" and the phytoremediation of their slopes and terrestrial ecosystems arising in their discharge areas.

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2225 VOLATILE ORGANIC POLLUTANTS IN IRON AND STEEL INDUSTRY

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It is a well known fact that iron and steel units generate about 25% from total gaseous emissions, and a significant part of these are diffuse emissions, which appear during technological stages. So that, apart from other types of pollutants, appear volatile organic compounds (VOC's) that contain a considerable number of diverse and complex substances that, even in small amounts, affect all environmental factors: air, water, soil. Part of these compounds are very toxic, carcinogenic, and odorous, others can cause the greenhouse effect by absorbing infrared radiations, thus leading to climate change, other compounds are involved in the destruction of the ozone layer or in the formation of the photochemical smog. VOCs are carbon-based compounds, who remain in the environment for a long time and become widely distributed throughout the environment, and in the end they accumulate in fatty tissue of living organisms. Considering all major implications that volatile organic compounds have on people's health (birth defects, cancers, disfunctioning of immune, development and reproductive systems, diminished intelligence) and on the quality of the environment in the long or in the short run, both from a regional and from a global perspective, a correct identification and quantification of such compounds becomes stringent, with a view to protecting the environment factors (air, water, soil) and reducing the environment risk as a result of exposure to organic compounds emissions. This action is determined by the fact that Romania has this obligation by signing international accords. During the technological processes specific for iron and steel units, the volatile organic pollutants reach the atmosphere from primary emission sources (controlled – chimneys) and mainly from secondary emission sources (uncontrolled, diffuse – permeable equipment and machinery, inadequate functioning parameters during technological operations). Moreover, industrial wastewater's containing such pollutants can affect natural receptors, and solid waste containing volatile organic compounds can, in time, lead to soil and water pollution. Environmental protection regulations denote narrow range in concentration of this pollutants (part per million - ppm or even part per billion - ppb) in water and atmosphere. As a result of the current situation in our country regarding the control methodologies for iron and steel industry volatile organic compounds and the limited possibilities to minimize/reduce the environment risk created by these pollutants once they are emitted in the environment (air, water, soil), the main objective of this paper is to characterize, and determine such emissions, by identifying the organic pollutants emitted in the environmental factors (air and water) as a result of iron and steel making processes, quantitative determination of organic pollutants emitted in industrial wastewater's and identifying the EU - Directives, regarding VOC's, and their transpose in national legislation. In iron and steel industry in the sintering process polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), are formed within the sinter bed itself, probably just ahead of the flame front as hot gases are drawn through the bed, or because of disruptions to flame front propagation, result in higher PCDD/F emissions. PCB can also be present in raw materials. Therefore, in wastewater from coke oven plants, before treatment, are comprised a big variety of different organic chemical compounds such as polycyclic aliphatic hydrocarbons (PAHs), up to 30 mg/l. The analysis of wastewater from iron and steel industry, for volatile trace level pollutants, can be made by physical - chemical methods, known for theirs accuracy: gas-chromatography method (GC) and gas-chromatography coupled with mass-spectrometry (GC-MS). Polycyclic aromatic hydrocarbons (PAHs) have received increased attention in recent years in water pollution studies because some of these compounds are highly carcinogenic or mutagenic. In particular, benzo[a]pyrene (B[a]P) has been identified as being highly carcinogenic, so for this pollutants was described in this paper analysis methodology by GC-MS. Because a big part of these VOC's came from diffuse sources, air pollution near source is imminent and exist a high risk for working personnel to get acute or chronicle intoxication, especially in coke area. So, knowledge of volatile organic pollutants properties generated in iron and steel industry processes and their variation limits is particular useful for taking measure that ensure framing of these pollutants in emissions regulations and, in this way, the risk of humans and environmental exposure at toxic action of such as compounds can be diminuated.

2280 MICROBIAL ECOLOGY OF ARTISANAL ITALIAN CHEESE: ENVIRONMENT AND WORKING CONDITIONS

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In agro-food sector the structural features of working environments and consequently their hygienic conditions are of primary importance for a safe and quality food production and to ensure comfortable and ergonomic working conditions. In particular, as regards high-mountain dairy production, the environment is important because it can affect the development of typical microbial ecosystem. In this type of production raw milk is still being used. Milk quality, depending on the state of hygiene in the environment, may enhance or reduce the quality of the final product. During the ripening the indigenous and environmental micro-organisms and different microclimate conditions (temperature and humidity) will come into play. This aim of the paper is to examine the health and safety conditions of the working environments of a sample of high-mountain dairies with the objective to identify structural elements (layout, coatings, spots in the facility where dirt can accumulate or facilitate weed-growing etc...), and management practice that can affect the safety and the final quality of cheese. The choice of the sample fell upon family-run farms still characterized by craft work and traditional methods. According to the methodology adopted in every dairy and in all processing premises, surveys were carried out to assess microbial contamination in air and on work surface (health, quality and typicity indicators). The level of microbiological contamination was measured either by means of laboratory analysis and instrumental field methods. The surveys were repeatedly carried out to reduce the daily and seasonal variability both during working hours and during resting hours to quantify the contamination due to processing activities. Microbial contamination was linked to the building features of processing premises and their layout. The comparison of these data has made it possible to devise some design and management guidelines to improve the hygiene and safety of processing premises, and consequently to produce good quality cheese.

2321 EVALUATION OF THE STRATEGY DESIGNED TO REDUCE THE INCIDENCE OF CASES PRODUCED BY THE BIOCONTAMINANT (*Legionella* SEROGROUP I) IN THE CITY OF CÓRDOBA - SPAIN

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Environmental protection is essential to the quality of life for present and future generations. The EU lines of priorities for reducing the adverse health effects associated with certain environmental factors, include “To prevent the risks associated with the facilities at risk in the transmission of certain diseases such as *Legionella*”. The objective of this work was to evaluate the actions undertaken to prevent and control biocontaminant *Legionella pneumophila* serogroup I, in cooling towers and evaporative condensers (TyCC) in the city of Cordoba. From 01/01/2005 to 31/12/2007, the number of cooling towers and evaporative condensers increased from 158 to 230. Maintenance plans were designed for 87% of these facilities according to local rules in force. The number of evaluation visits undertaken were 170, 313 and 238 in 2005, 2006 and 2007 respectively. The number of incident cases of *Legionella* increased substantially in the period of our study raising from 3 to 15 cases for a 300.000 inhabitants population. Although efforts have proved to be useful to unify criteria and to facilitate communication among stake holders (evaluators, facilities owners, institutions and maintenance companies) the ultimate goal of the health strategy, to reduce the incidence of the disease, has not been reached. TyCC are low energy consumption facilities that do not need chemical products as refrigerants and exhibit a great cooling power. Exhaustive evaluation visits, maybe under too strict criteria, have lead to the replacement of these facilities with other technologies less clean and efficient from an environmental point of view. There is a need to design different strategies to include controls for other facilities that, although not related to disease outbreaks, are associated to sporadic cases that is the type of cases that are detected in our municipality.

2445 HEAVY METALS, PAHS CONTENTS AND ECOPHYSIOLOGICAL CHANGES IN LEAVES OF HOLM-OAK IN URBAN AREA OF CASERTA (ITALY)

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Human health as well as that of animals and plants is severely affected by air pollution. In urban areas the vehicular traffic, in particular, contributes to the release of contaminants like heavy metals and polycyclic aromatic hydrocarbons (PAHs). Plants are effective biomonitors because, through their large leaf surface area, intercept and also absorb air pollutants, that can cause morphological, physiological and biochemical responses. In this interdisciplinary study the concentration of airborne contaminants were correlated with plant metabolites in leaves of holm-oak (*Quercus ilex* L.). This species, a typical sclerophyllous evergreen broadleaf plant widely distributed in the Mediterranean Basin, has been used as biomonitor being an ornamental plant in parks, gardens and avenues of the city of Caserta. The concentrations of V, Cd, Cr, Pb, Ni, Cu, PAHs as well as chlorophyll contents and peroxidase activity in one year old leaves from different sites of the urban area were determined. The contents of leaf free amino acids were also measured in order to identify new indicators that could be useful to monitor air quality even at relatively low level of pollutants. The tested sites showed different contents of heavy metals and PAHs, in particular cancerogenic PAHs, as well as different amino acid patterns. Negative correlations were found between Cu and Cd contents and the peroxidase activity and between these metals and some amino acid concentrations. No relationships, instead, were found between the PAHs and heavy metal contents. All data were tested by multivariate analysis. The Principal Component Analysis (PCA), evidenced a clear grouping of the sites.

2914 ELEMENTAL COMPOSITION OF THE PM_{10} FRACTION IN THE MEZQUITAL VALLEY, MEXICO

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The Mezquital Valley has been receiving for nearly 100 years the residual waters of Mexico City to produce corn, alfalfa, oat, beans and barley among others. The information generated on the metal concentration in water, soils and agricultural products is vast. There are nevertheless limited studies of other sources of heavy metal, like atmospheric wastes. This is an important fact since the Mezquital valley is close to the Tula-Vito-Apasco industrial corridor, a zone classified as critical by the amount of polluting agents emitted to the atmosphere. In this work, we determined the composition and levels of metals present in aerosols to determine the relative importance of these sources of metals to the Mezquital Valley. Coarse PM_{10} aerosols were collected in Tlaxcoapan, Hidalgo. Polycarbonate filters were exposed every 2 days for 24 hours, from June to December 2007 (rainy season and beginning of the dry season). Elements such as Si, S, P, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Br, Sr, Hg and Pb present in the fraction PM_{10} of the filters were analyzed by PIXE using a 2.4 MeV proton beam from a Pelletron NEC-9SDH at the IFUNAM in Mexico City. Except for Zn, all elements detected in Tlaxcoapan aerosols showed higher values than those from Mexico City. Contents of Si, S, Cl and Ca are over a thousand ng/m^3, while in North Mexico these elements never exceed 600 ng/m^3. K, Ti, V, Cr and Fe show values in the order of hundreds of ng/m^3, while those from Mexico City are one order of magnitude less. Mn, Ni, Cu, Zn and Pb showed levels of a few ng/m^3 and elements such as Mn, Hg and Sr, were only occasionally detected.

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1854 PREATREATMENT OF WORN WATERS OF THE DAIRY INDUSTRY BY SAND FILTRATION PROCESS

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The problem of the environment started to become a concern with the demographic increase and the industrial and agricultural development, especially in the great agglomerations, the relative increase out of water became a major concern. Water of the factories takes care of mineral substances and organics during manufacture and constitutes one of the principal sources of contamination of the environment. The dairy industry "GIPLAIT" of Mostaganem, Algeria, is one of the industries which consume enormous quantities of water generating in parallel pollution marinates very important because of its establishment near the sea. It is in this context that the objective of our work was underlined in order to establish a technique of control making it possible to evaluate the degree of pollution of the rejections of the unit by carrying out physicochemical and microbiological analyses and consequently to fix a specific and suitable pretreatment for this type of effluent, where one carried out a sand filtration for a re-use or for a recycling of this water, knowing that unit "GIPLAIT" of Mostaganem does not have a treatment station of rejections waters. Before carrying out sand filtration we determined his porosity in order to characterize it, then, we followed the evolution of the turbidity of the filtrate according to the cumulated versed volume of elutriated worn water, where we noticed a very clear reduction of the suspended matter, which corresponds to a percentage of elimination higher than 70%. Lastly, we deduced that the sand filtration process that we applied makes it possible to reduce a large quantity of suspended matter, which makes this water clarified and consequently which can be recycled.

1855 ADSORPTION OF AN ACIDIC DYE FROM AQUEOUS SOLUTION BY SURFACTANT MODIFIED BENTONITE

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The aim of this paper is to study the adsorption of an acidic dye S.Y 4GL (i.e: Supranol yellow 4GL) from aqueous solution on inorgano-organo clay. Bentonite is a kind of natural clay with good exchanging ability. By exchanging its interlamellar cations with Cetyltrimethylammonium bromide (CTAB) and hydroxyaluminic or chromium polycations, the properties of natural bentonite can be greatly improved. Adsorption tests of Supranol yellow 4GL in batch reactors were carried out at 20°C and constant pH 6.5. Results from kinetics and isotherm experiments showed that Surfactant treated chromium intercalated bentonite, CTAB-Cr-B, (142.85 mg S.Y4GL g^{-1}substrate) had higher adsorption capacity for S.Y 4GL than chromium intercalated bentonite, Cr-B, (58.47 mg S.Y 4GL g^{-1} and b=6.22). In addition to higher capacity, CTAB-Cr-B exhibited a higher strength of adsorption (0.445 versus 0.068 L/mg) and faster uptake kinetics (5.317 10^{-3} versus 2.357 10^{-3} g mg^{-1} min^{-1}) compared to CTAB-Al-B. The presence of CTAB surfactant enhanced the adsorption ability to the intercalated bentonite towards acidic dye from aqueous solution. The adsorption affinity decreased in the following sequence: CTAB-Cr-B > CTAB-Al-B > Cr-B > Al-CTAB.

1867 BATCH SYSTEM FOR STUDY OF Cr(VI) BIOSORPTION BY DRIED WASTE ACTIVATED SLUDGE

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Activated sludge from wastewater treatment systems contains both bacteria and protozoa. The cell wall of bacteria essentially consists of various organic compounds, such as carboxyl, acidic polysaccharides, lipids, amino acids and other components. Advantages of biosorption for removal of heavy metals over chemical and physical methods are; 1) Excess sludge from wastewater treatment plants may be used as bio-sorbent, 2) Low cost, free availability and possible reuse of the biosorbent, 3) High biosorption capacity because of large surface area, 4) Selective adsorption of metal ions, 5) Operation over a broad range of environmental conditions. The biosorption of Chromium (VI) ions by dried waste activated sludge was investigated in a batch system. Dried waste activated sludge was used for removal of Chromium (VI) ions from aqueous solution by biosorption. Waste activated sludge obtained from a wastewater treatment plant in Tehran was dried, ground and sieved to a number of fractions between 50-70 mesh (0.2-0.3 mm). The maximum biosorbed chromium (VI) concentration was found to be 7.488 mg g^{-1} with initial chromium (VI) and dried waste activated sludge concentrations of 10 mg l^{-1} and 1 g l^{-1}, respectively. The effect of initial pH on the equilibrium uptake shows that the uptake of chromium (VI) decreased with increasing initial pH from 2.0 to 6.0.

1887 PHOTO-OXIDATION OF PHENOL AQUEOUS SOLUTION: TOXICITY OF INTERMEDIATE

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Phenol is one of the most abundant pollutants in industrial wastewater, i.e., chemical, petrochemical, paint, textile, pesticide plants, etc. The contamination of bodies of water with phenol is a serious problem in terms of environmental considerations and human health due to its high toxicity. The photodegradation of phenol was studied in a batch reactor system illuminated with a 400 W medium pressure mercury lamp. The effects of parameters such as pH, reaction time and initial phenol concentration on the photolytic degradation and toxicity assay have been studied. The experimental results have shown that lower pH and lower concentration of phenol favor the phenol degradation. The disappearance of phenol in each case approximately obeyed first-order kinetics with the apparent rate constants increasing with decreasing solute concentration. Bioassay tests showed that phenol was toxic to *Daphnia magna* and so resulted in quite low LC_{50} values. Comparison of toxicity units (TU) between phenol and effluent toxicity has shown that TU value for effluent was 2.18 times lower than that obtained for phenol. Thus, photolysis is able to decrease the toxicity of by-products formed during the degradation of phenol.

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2191 SPECIATION RELEVANCE ON THE REMOVAL OF CHROMIUM FROM AQUEOUS SOLUTIONS BY A LOW-COST SORBENT (LEONARDITE)

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Heavy metals are common pollutants found in many industrial effluents. The use of sorption technologies, based on ion exchange and chemical sorption for the removal of these pollutants from waste water, is especially promising. Low rank coals contain large amounts of humic acids with carboxylic and phenolic hydroxyl functional groups which can interact with metal ions. These natural materials, which are available in large quantities, may have potential as inexpensive sorbents for harmful metal ions encountered in industrial waste waters. In this work, a low-cost sorbent (leonardite) was investigated to remove Cr(III) and Cr(VI) from synthetic aqueous solutions. Batch kinetics and equilibrium experiments were performed in order to investigate the influence of pH, contact time and initial concentration of sorbate and sorbent. Adsorption test were conducted using solutions of $Cr(NO_3)_3 9H_2O$ and $K_2Cr_2O_7$ at concentrations between 1-500 ppm of chromium. The adsorbent dose was 1 g/L and the solutions were agitated in a magnetic stirrer. After the adsorption process, solutions were filtered through 0.45 μm Millipore® filters and the remaining concentration of chromium were determined by either atomic adsorption spectroscopy (total Cr) or UV- spectroscopy (Cr(VI)). pH influence studies showed that at pH<2 there is little or no Cr(III) adsorption The removal efficiency increases at pH 3 and reaches its maximum at pH 4-6. For Cr (VI) the maximum removal percentage was obtained a pH <2. This behaviour may be attributed to the combined effect of the nature of the sorbent surface and metal speciation in the aqueous solution. Kinetic studies revealed that for Cr (III), equilibrium was attained in two hours, while contact time to attain equilibrium was higher in the case of Cr(VI) sorption (72 hours). Sorption data were correlated with the Langmuir and Freundlich adsorption models. The maximum sorption capacities obtained from the Langmuir isotherm were 71.4 mg/g and 8.8 mg/g for Cr(III) and Cr(VI), respectively. Results also showed that leonardite is able to reduce Cr(VI) to Cr(III).

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2205 ELIMINATION OF THE ORGANIC POLLUTANTS BY PHOTODEGRADATION IN THE PRESENCE OF A CATALYST

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A certain number of processes of degradation which one calls AOP are currently in the process of development. They bring into play oxidizing species (OH, the ozone and hydrogen peroxide) very energy generated *in situ* oxidation by H_2O_2 and the photochemical processes make notable great strides and are appropriate rather to destroy aqueous solutions with weak concentration (worn water). It is difficult to prefer a process with another, since each one has its own advantages and disadvantages. The photocatalytic degradation received much attention these last years within the framework of the purification of water as a process of elimination of micropolluants organic (solvents, pesticides, weed killers). In this process, a semiconductor (generally TiO_2) absorbs the light and converts the photon energy into chemical energy by a redox system. The majority of the organic pollutants can thus be degraded in the presence of oxygen mineral in cash not poisons. The work of our team in this area has just begun under the green chemistry to develop then by the study of transport phenomena in materials or light and the preparation of catalysts interesting design photocatalytic reactor. As the catalyst can be excited by light in the near ultraviolet, a process solar is feasible. The group is led his laboratory work using UV fluorescent lamps, emitting at around 365 nm, in a range where the solar emission can be used, thus allowing application of research to the design of a solar photocatalytic reactor. A study is under implementation in the design of a solar reactor. Based on a literature search, an experimental measure of absorption properties and dissemination of deposition of the catalyst on a support will be undertaken on a montage original. These experimental data used to perform a simulation of the journey of light in various geometries and materials and to assist in the design of the reactor in question.

2210 MINERALIZATION AND DISCOLORATION OF TEXTILE WASTEWATER BY TIO_2 NANOPARTICLES

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The Nanophotocatalytic process using semiconductors with nanostructure, is one of the technologies used for the destructive oxidation of organic compounds such as dyes.The photocatalytic oxidation of Reactive Orange 16 aqueous solution, applied in textile industries, was assessed by UV ray irradiation in the presence of TiO_2 nanoparticles. The effect of dye initial concentration, pH, TiO_2 loading and the effect of anions' presence on dye degradation were investigated and the optimized conditions for maximum amount of degradation were determined. Analysis of the kinetic showed that the amount of dye photocatalytic degradation can be fitted with pseudo-first order model. The mineralization of RO 16 dye was reported by measuring the initial and final COD (Chemical Oxygen Demand) of the solution that was irradiated under optimized conditions.

2212 INVESTIGATING THE POTENTIAL OF USING SONOCHEMICAL REACTORS FOR DECOMPOSITION OF LAS FROM WASTEWATER

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The effectiveness of using sonochemical reactor for degradation of linear alkylbenzen sulfonates (LAS) from aqueous solution has been investigated. LAS are anionic surfactants, which found in relatively high amounts in domestic and industrial wastewaters. In this study, experiments of LAS solution were performed using methylene blue active substances (MBAS) method. The effectiveness of acoustical processor reactor for LAS degradation is evaluated with emphasis on the effect of treatment time and initial LAS concentration. Experiments were performed at initial concentrations of 0.2 mg/L, 0.5 mg/L, 0.8 mg/L and 1 mg/L, acoustic frequency of 130 kHz, applied power value of 500 W. At the conditions in question, LAS degradation was found to increase with increasing sonochemical time. In addition, as the concentration is increased, the LAS degradation rate is decreased in acoustical processor reactor.

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2249 USE OF POLYGLUTAMIC ACID PGA α21 IN THE TREATMENT OF VINASSE FROM AN ALCOHOL DISTILLERY

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Vinasse is the residue generated from ethyl alcohol production via fermentation and subsequent distillation. Its high organic content makes it an extremely polluting effluent. Vinasse has an acid pH (3.5-4) and is characterized by an elevated chemical oxygen demand (COD) in the range of 10,000 to 50,000 mg/L. It is common practice for these effluents to be discharged into public sewers or poured into evaporation tanks or even used in irrigation water in agricultural fields. However, these procedures cause stenches and pollution of surface or underground water. With the rise of ever stricter environmental norms concerning discharge of industrial waste, interest in the development of new technological alternatives to clean up waste water has increased. This study evaluated the efficiency of an organic coagulant for the depuration of alcohol distillery waste (vinasse). Materials and Methods. Vinasse was treated by a physicochemical process, involving coagulation in the first stage and oxidation ($NaClO_3$) in the second, for COD removal. Vinasse samples (500 mL) were treated as follows: a) Flocculation with a polymer of organic origin (polyglutamic acid); b) Addition of a dose of $NaClO_3$; c) treatment with activated carbon. Variables such as pH, temperature, centrifugation velocity were studied. The following parameters were analyzed: COD, turbidity (in NTU), oxidant concentration. An integrated flocculation/oxidation process, adding a natural based polymer (polyglutamic acid) as precipitant, was used in the present study to treat vinasse from an alcohol distillery. Results indicate that efficiency in organic matter removal was considerable at an acid pH and at a temperature of no more than 60°C Under the conditions tested, COD went from 40,000 to 8,200 mg/L (79.5%), turbidity was reduced from 440 to 134 NTU (69.5%) and sedimentable solids were reduced to 30 mg/L of sample. $NaClO_3$ use was only 1.2%. Polyglutamic acid supposes a decrease in toxic compound bioaccumulation risk in ecosystems, as happens with aluminum sulfate and polyacrilamide. In this study the positive effect of polyglutamic acid was observed on the rate of organic matter removal and consequently of COD in tequila vinasse.

2263 THE USE OF ALGERIAN CLAY MATERIALS FOR THE SORPTION OF HEAVY METALS FROM AQUEOUS SOLUTIONS

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The present study investigates the adsorption of Pb^{2+}, Cu^{2+} and Ni^{2+} onto Algerian bentonite clay from aqueous solutions at different initial concentration of metal ions, amount of clay, solution pH, shaking time at room temperature. The uptake of Pb^{2+}, Cu^{2+} and Ni^{2+} was dependent on increasing pH of the medium. The amounts of metallic ions remaining in solution could be affected by pH and insoluble hydroxides $M(OH)_2$ must precipitate. The results showed that the removal efficiency of Pb^{2+}, Cu^{2+} and Ni^{2+} by Algerian bentonite clay could reach 92, 90 and 75 %, respectively, when the initial concentration of metal ions is 10 mg / L and shaking time 2h. Two isotherm equations due to Langmuir and Freundlich showed good fits with the experimental data. The results have shown good potentiality for Algerian bentonite clay to be used as adsorbent for removal of heavy metals such as Pb^{2+}, Cu^{2+} and Ni^{2+} ions from aqueous solutions.

2275 EFFECT OF OZONATION ON THE REMOVAL OF COLORANTS IN VINASSE FROM BEET MOLASSES FERMENTATION

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Environmental problems in molasses fermentation factories are mainly related with the production of large quantities of vinasse, a brown colored effluent of high organic load. Color is due to the presence of non-biodegradable colorants. This work is focused on the examination of chemical pretreatment of vinasse using ozone. Ozonation pretreatment may degrade colorants, leading to an increase in the production of biogas in the further anaerobic biological treatment. The influence of ozonation pH on the color and organic matter removals was also analyzed. Vinasse was taken from a beet molasses fermentation factory. The wastewater presented the following characteristics: pH 4.5-5, COD 85000 mg/L, BOD_5 76000 mg/L, TOC 30000 mg/L, color 7-7.5 absorbance units (a.u) measured at 475 nm. The experimental system was based on a 2 liter jacketed stirred tank reactor, equipped with a Sander 301 ozone generator producing ozone from dry air by electrical discharge. Experiments were conducted in batch mode feeding 4.5 g O_3/h per liter of vinasse. The ozonation of vinasse led to a reduction in color of about 76% after 30 min reaction time. Decolorization was measured as reduction of absorbance at 475 nm. A further increase in the reaction time up to 50 min increased the decolorization percentage to 83%. The reduction in the organic matter content was considerably lower. The COD reduction was about 10% and the percentage of TOC removed was about 9% after 50 min reaction time. As ozonation efficiencies may be strongly dependent on the pH of the wastewater, the influence of this parameter on the oxidation treatment was tested. The pH was varied between 4 and 11. However, the experimental results were almost independent of the pH. Color and COD removals between 74-81% and 8-13% were obtained, respectively. Although the efficiencies were slightly higher at strong basic pH, from the experimental results it can be concluded that direct oxidation reactions between olefinic structures of colorants and ozone molecules were more predominant. The ozonation process may be able to transform the refractory compounds of high molecular weight in vinasse into simpler molecules more readily assimilated by anaerobic microorganisms. Anaerobic degradation tests were performed, showing that the ozonation pretreatment led to an increase in the volume of methane produced of about 10-15%.

2276 DETOXIFICATION AND BIODEGRADABILITY ENHANCEMENT OF AQUEOUS SOLUTIONS OF FOUR COMMERCIAL PESTICIDES ALONG A PHOTO-FENTON TREATMENT

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Photo-Fenton treatment has proven to be efficient to remove recalcitrant pollutants as commercial pesticides commonly employed in citric cultivars in the Mediterranean coast of Spain as Laition, Sevnol, Ultracid and Metasystox. However, the photo-Fenton treatment resulted to be less efficient to remove organic matter; nevertheless it could be employed as a pre-treatment to couple with biological processes, widely used in wastewater treatment. The suitable moment to couple photo-Fenton treatment with biological processes can be determined by different biological assays to measure changes in toxicity and biodegradability. Several methods are explored to measure toxicity: the inhibition of the bioluminescence of *Vibrio fischeri* and the specific respiration tax of activated sludge. Although the bioluminescence method has showed higher sensibility, the activated sludge based respirometric measurements are expected to give more information on the behavior of biological reactors. All of them show a decrease in the toxicity when the active species are removed. Changes in biodegradability have also been measured according to different assays such as the determination of short time biological oxygen demand (BODst), the BOD/COD ratio and the Zahn-Wellens test. Most of them involved activated sludge, although the period to exposure to the pollutant is different: very long for the standard Zahn-Wellens test to measure intrinsical biodegradability, intermediate time for the immediate biodegradability obtained with the ratio BOD/COD and direct biodegradability from the BODst. All of them also show an increase of the biodegradability when the active species are removed.

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2277 ORGANIC SENSITIZERS AS PHOTOCATALYSTS FOR THE TREATMENT OF THE COMMERCIAL PESTICIDE METHIDATHION

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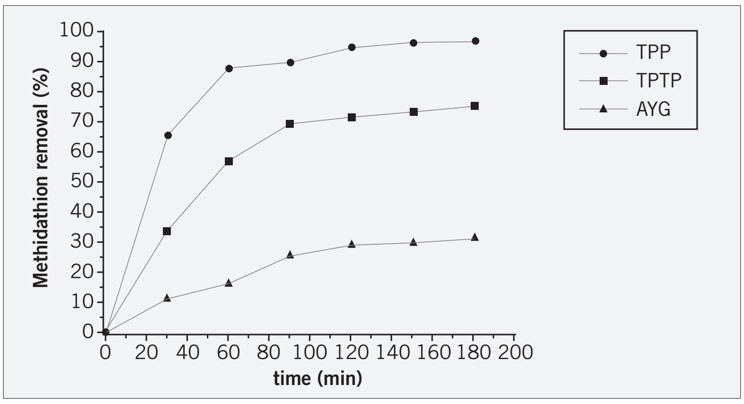
The use of sunlight to remove pollutants from industrial effluents has deserved much attention from researchers in recent years. Some chemical systems, such as the Fenton reagent or titanium dioxide have proven to be able to generate highly reactive species upon irradiation with UVA. However, it seems interesting to explore the possibility of using other species able to employ efficiently the visible fraction of the spectrum. In this context, organic photosensitizer's exhibit strong absorption bands in the visible region and thus, they might be useful for this purpose. Pyrylium (TPP) and thiopyrylium (TPTP) cations, and acridine yellow (AYG) are an example of organic compounds that can be used as photocatalyst and have demonstrated to be efficient in the removal of phenolic pollutants from wastewaters. In this study, this organic photocatalysts are used for the detoxification of aqueous solutions containing 50 mg/L of the commercial pesticide Methidathion.

Figure 1: Methidathion removal using 10 mg/L of each organic sensitizer



Complete detoxification and 90 % of toxicity abatement of the sample is achieved using 10 mg/L of thiopyrylium and pyrylium salts that arise 75 and 97 % of methidathion elimination in 3 hours (Figure 1). Samples treated with 10 mg/L of acridine yellow show a remaining toxicity (about 50 % of initial) that is in agreement with the limited removal of methidathion (ca. 30 %).

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2312 ADSORPTION OF LEAD FROM AQUEOUS SOLUTION BY GEOMATERIALS

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The contamination of wastewater and soil with toxic heavy metal ions is a complex problem. The removal of this contamination has received much attention in recent years. From an environmental protection point of view, heavy metal ions should be removed at the source in order to avoid pollution of natural waters and subsequent metal accumulation in the food chain. Lead is an environmentally common pollutant, bordering on ubiquitous. When lead is found in aqueous media, unlike most organic contaminants, it does not undergo biological degradation but accumulates through its association with inorganic and organic matter, e.g., by adsorption processes, formation of complexes, or chemical combinations. As a result, lead levels in natural water are at the microgram per cubic decimetre level. This fact is a matter of current concern as lead is a heavy metal of high toxicity, its poisoning effects being cumulative. Once retained in the body, about 90% of the lead enters the bones from which it can be remobilized. Conventional methods for removal are chemical precipitation, chemical oxidation, chemical reduction, ion exchange, filtration, electrochemical treatment and evaporation. All these procedures present significant disadvantages, such as for instance incomplete removal, high-energy requirements, and production of toxic sludge or waste products also requiring disposal. These methods are often very expensive. Alternative methods for heavy metal removal were developed in the last decade. A great effort has been contributed to develop new adsorbents. In this objective, we prepared geomaterials while mixing in different operative conditions: montmorillonite-Na, cement, active coal and polymer acrylate. In this work, the adsorption of lead from aqueous solution by geomaterial adsorbents was studied. The kinetics of adsorption, effects of pH, effect of temperature, initial concentration have been examined and compared with the different adsorbents. The study of different experimental equilibrium isotherms showed clearly the high efficiency of these new adsorbents toward lead metal. On the other hand, it has been shown that the removal of the Pb depends on the nature and the quantity of the clay mineral and the active carbon.

2313 ADSORPTION OF *p*-NITROPHENOL (PNP) ON NEW ADSORBENTS PREPARED FROM DIATOMITE AND CHARCOAL

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Increasing attention has been paid to mesoporous materials with high surface area and narrow pore size distribution because of their diverse applications (e.g., adsorbents, catalysts, and host materials). Inorganic composite materials (ICM) prepared by a mixture of natural diatomite (marcroporous material) and charcoal (microporous material) in particular operative conditions. These mesoporous materials have received attention as useful materials for separation and recovery of organic compounds. In considering adsorption from the liquid phase, that from aqueous solution deserves particular attention. Solutions of this type are frequently encountered in industrial practice where of the many possible groups of impureties present the most significant are aromatic compounds, including phenols. Phenols, and phenolic compounds, are ranked as eleventh of the 126 chemicals which have been designated as polluants by the US Environmental Protection Agency. According, the adsorption of phenol and its derivatives has been the subject of numerous investigations. *p*-nitrophenol (PNP) has been one (or the principal) adsorbate in all of these studies. The present study reports the adsorption of PNP on a series of inorganic composite materials. These new adsorbents differ by their properties (surface area, porous structure, porosity, chemical nature of the surface). Langmuir and Freundlich adsorption isotherms have also been tested at various temperatures, and adsorption kinetic and some thermodynamic parameters have been determined. The study showed that the adsorption of PNP in aqueous solutions on inorganic composite materials depends on the structural properties of theirs materials (specific surface area, volume of pores) and the chemical properties.

2351 ASPECTS OF ACTIVATED CARBON CATALYSIS OF AZO DYE REDUCTION

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Electron transfer in reductive processes such as the reduction of azo dyes in anaerobic bioreactors is often rather slow but can be speeded up by using redox mediators. Continuous addition of dissolved redox mediators such as artificial quinones to bioreactors does not appear to be a cost-effective option. The use of immobilized or insoluble redox mediators such as activated carbon (AC) would therefore be an attractive alternative. With the objective to obtain better insight in the characteristics of AC-catalysis of biological and chemical reductive transfer reactions, we investigated the effect of different parameters on AC-catalysed reduction of azo dyes by sulphide and anaerobic sludge in batch assays. Among the parameters tested were: surface chemistry (different chemical and heat treatments of AC); surface area; dye-saturation; and pH. Results of the chemical assays with sulphide point at a big increase of AC's catalysing power with increasing surface area, whereas the surface chemistry has only a very small effect. Dye-saturated AC is almost as effective as virgin AC. The relative impact of AC is especially high at pH>8, where non-mediated azo dye reduction is almost negligible. Preliminary results of the biological assays with sludge suggest that biomass adaptation is necessary for biologically mediated AC-reduction.

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2394 BIOSORPTION OF ZINC (Zn) AND LEAD (Pb) BY METAL RESISTANT BACTERIAL ISOLATE FROM MINING TAIL

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The use of microbial biomass in the removal of metals in solution, mainly of low concentrations (100 mg L^{-1}), present advantages in relation to the physicochemical methods. The resistant microorganisms are potential biosorbents. The objective of the present study was the isolation, starting from mining tail, of strains with capacity of metal biosorption (Zn and Pb). For the isolation, was carried out enrichment in nutritious broth with tail sample during 48 hrs to 27°C. Later on was carried out the isolation by dilution in plate on nutritious agar with different types and concentrations of metals (Al, Cu, Pb, Zn, Ni, Se). The isolate with more resistance, Minimum Inhibitory Concentration (MIC), was selected. For the tests of adsorption in isotherm, biomass of the isolate was obtained in 400 ml of nutritious broth, it was centrifuged and washed with sterile saline solution; the cellular pellet dried off at 80°C for 24 hrs. 0.015 g of dry biomass was put in contact, under agitation, with 10 ml of metallic solution to established pH. The contact was at different time, after which the biomass was separated by centrifugation and in the supernatant was determined the metal not absorbed by Atomic Absorption Spectometry. A resistant strain to different metals was isolated, called ZnZac whose order resistance to the metals was the following one: Zn>Pb>Se>Ni>Cu=Al. The kinetics of adsorption of Zinc and Lead (initial concentrations of 23 mg/l and 50 mg/l, respectively), show that to the 40 minutes the equilibrium is reached for the Zn, while for the lead it is reached at 20 minutes, with a percentage of adsorption of 60% and 80% respectively. Both kinetic ones were adjusted to an equation of Pseudo Second Order (r^2=0.99). The maximum absorption of Pb was 0.216 mmol g L^{-1} and for Zn was 0.207 mmol g L^{-1}. For both metals, the pattern of isotherm Langmuir (r^2>0.90) fitted better to the experimental data than the pattern of Freundlich (r^2 <0.80). The value of optimal pH for Zinc uptake was 6. The capacity of adsorption of ZnZac (Zn-Pb), with concerning others bioadsorbents reported in the literature, can be considered of medium magnitude.

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2412 REMOVAL OF LEAD USING NATURAL AND SYNTHETIC SORBENTS IN AQUEOUS SOLUTION

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In recent times, the treatment of wastewaters polluted by heavy metals has become an important environmental problem. Generally, high contents of these pollutants are detected in the effluents discharged by several industrial activities. Heavy metals are important pollutants and cause severe health problems to human being, and other living organisms. Lead is a very toxic metal at low concentrations and it can damage nervous and gastrointestinal systems, kidney, liver, and brain. Removal of lead from wastewaters can be achieved by several technologies, such as chemical precipitation, reverse osmosis, ion-exchange, electrochemical treatment, and sorption. Among these treatment methods, sorption process is believed to be the simplest and most cost-effective technique. Furthermore, this method offers the possibility of use raw materials that are either abundant or wastes from industrial operations. This condition allows reducing the operational costs. In this context, the objective of our work is to apply and compare two sorbents for lead removal from water. Specifically, we report the lead sorption isotherms and kinetics obtained with bone charcoal and immobilized chicken feathers. The sorption experiments were performed at different conditions of pH, temperature and initial concentrations. Our results indicate that both sorbents are suitable and effective for lead removal from water. However, it appears that immobilized chicken feathers offer more advantages, such as low-cost and fast metal uptake. Finally, several kinetic and isotherm models were used for fitting the sorption data.

2429 ELECTROCHEMICAL GENERATION OF FENTON´S REAGENT TO TREAT SPENT CAUSTIC WASTEWATER

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An important wastewater stream from oil refineries is the spent caustic. Caustic solutions are used as scrubbing agent during the desulphurization process to eliminate sulphur and mercaptans from oil and gasses. Spent caustic is classified as D003 (reactive sulphide) hazardous waste under the US Resource Conservation and Recovery Act (RCRA). Spent caustic is a highly specific effluent. Generally, two types of spent caustic waste streams exist depending on the origin – sulphide spent caustic from scrubbing operations and phenolic spent caustic from heavy gasoline sweetening. Typically, relative small volumes (0.1 to 8 m^3/h) are discharged, and these vary depending on the refinery's size and layout. The harmful effects are considerable due to the concentration in S^{2-} and phenols. Around 550 t of of spent caustic is produced when processing 1 Mt of crude oil. An efficient treatment is the oxidation with Fenton´s reagent. Fenton's reagent is a solution of hydrogen peroxide and an iron catalyst that is used to oxidize contaminants or waste waters. Mixing iron and hydrogen peroxide in the right manner, it results in the generation of highly reactive hydroxyl radicals (.OH) or peroxide radicals (.OOH). Ferrous iron (II) is oxidized to ferric iron (III) by hydrogen peroxide to a hydroxyl radical and a hydroxyl anion. In order to have an efficient process, the procedure requires: a) adjusting the wastewater to pH 3-5, b) adding the iron catalyst (for example as a solution of FeSO4), and c) adding slowly the H_2O_2. If the pH is too high, the iron precipitates as $Fe(OH)_3$ and catalytically decomposes the H_2O_2 to oxygen. Fenton´s reagent can be produced by electrochemical oxidation of iron (0) and adding H_2O_2 simultaneously. If iron plates are used in an electrolytic cell, continuous anodic oxidation of iron will occur at the plate surface and liberate the catalyst for Fenton´s reagent. The advantages with the electrochemical *in-situ* production of Fe^{2+} are: No generation of residual products. The process does not need to be heated. Better dosage of Fe^{2+} by controlling the electric current. Less operational costs (no chemicals). In an experimental cell, the electrochemical production of Fenton´s reagent and the use in the spent caustic treatment was tested. Both batch and continuous operation was investigated. During the process different parameters were analysed such as pH, temperature, electric current density and H_2O_2 addition rate. From the results a kinetic model was suggested and validated by the experiments. The results showed that the electro assisted process to treat spent caustic was a promising alternative to wet air oxidation that operates at 25-90 bar and 200-300 ºC.

2463 TREATMENT OF PESTICIDE CONTAINING SOLUTIONS BY PHOTOCATALYSIS

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The potential health hazards of pesticides in surface water and underground water are well recognized. These pollutants enter the natural environment, generally, during crops disinfection, during pesticide industry cleaning, miss use of and miss understanding the ecological effect of such pesticides by the farmer. Pesticides do not bind well to soil. Thus, it can diffuse through the ground and enter underground water. In surface water, pesticide was detected near stations of industrial wastewaters. In Jordan, water, in all forms surface and underground, is a valuable and important source. Different effort was performed by consecutive governments to keep different water resources in Jordan clean. Moreover the implementation of world ISO 14001 certification and more stringent environmental legislation have been important drivers for these governments to invest in investigations for economical effluent treatment. Biological treatment of wastewater is often the most economical alternative when compared with other treatment options. The ability of a compound to undergo biological degradation depends on a variety of factors such as concentration, chemical structure. The pH medium or the presence of inhibitors can also affect the biological degradation. Pesticides are considered to be resistant to biodegradation. The hazardous potentialities of pesticide on bacterial strands have been widely reported. Recently, the utilisation of advanced oxidation processes (AOP's) for destruction of organic compounds has obtained considerable attention. Moreover, the integration of chemical and biological treatment processes was recommended, by different workers, as an economical means for treating biorecalcitrant organic chemical in wastewater. The chemical process would be used as a pre-treatment in order to increase the biodegradability of the wastewater. This work reports the photolysis using UV irradiation of pesticides solutions and the effect of UV light photolysis in the treatment and biodegradability of the treated solutions. The following topics are also studied: (a) biodegradability of initial and final pesticide solution, (b) inhibition of oxygen consumption by activated sludge for initial and final solution and, (c) the effect of two UV irradiation wavelengths on degradation efficiency. Results show that, UV photolysis can be used efficiently for pesticide destruction in water solution and the biodegradability, measured as BOD_5/COD ratio, can be enhanced significantly. For the moment, more than 50 % of pesticide degradation was obtained after 30 min of treatment using 254 nm UV-light. At this condition the solution BOD_5 was increased from zero to 11 mg O_2/L.

2546 WASTEWATER TREATMENT BY ADSORPTION ONTO MICRO-PARTICLES OF DRIED *Withania frutescens* PLANT AS NEW ADSORBENT

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Several industrial wastewater streams may contain heavy metals such as Cd(II), Cr(III), Cr(VI), Cu(II), Pb(II), Zn(II), etc. including the waste liquids generated by metal finishing or the mineral processing industries. The toxic metals must be effectively treated/removed from the wastewaters. If the wastewaters were discharged directly into natural waters, it will constitute a great risk for the aquatic ecosystem, whilst the direct discharge into the sewerage system may affect negatively the subsequent biological wastewater treatment. In recent years, the removal of heavy metal from sewage, industrial and mining waste effluents has been widely studied. Their presence in streams and lakes has been responsible for several types of health problems in animals, plants and human beings. Among the many methods available to reduce heavy metal concentration from wastewater, the most common ones are chemical precipitation, ion-exchange, and reverse osmosis. Most of these methods suffer from some drawbacks such as high capital and operational costs, incomplete metal removal, low selectivity, high energy requirements. The aim of the present paper is to study the efficiency of a new process for wastewater treatment by adsorption onto grinded and dried *Withania frutescens* plant using the batch equilibration technique. The results show that the micro- particles of *W. frutescens* plant present a good retention of heavy metals, nitrate and phosphate ions from real wastewater. This retention increased with increasing of contact time. The removal percentage of heavy metals from industrial wastewater by *W. frutescens* plant was 98~99 % for Pb(II), 92~93 % for Cd(II), 91~92 % for Cu(II) and 92~93 % for Zn(II). The maximum adsorption capacity was depending on the type of ions (atomic weight, ionic radius and electronegativity…). The results indicate that the chemical oxygen demand (COD) values decrease after contact with micro-particles of dry plant. The results obtained are compared with those obtained from -i) distilled water and -ii) from laboratory solutions with various concentrations.

2683 APPLICATION OF THE NATURAL CELLULOSIC SUPPORTS MODIFIED CHEMICALLY FOR THE TREATMENT OF THE INDUSTRIAL EFFLUENTS

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The process of purification and discoloration of industrial waters (and particularly effluents of the textile industry) can meet major difficulties: Certain dyes agents get through the devices of purge without being to stop. The cost of equipments and products of purification is prohibitive. Finally, in many cases, the discoloration can be only partial because waters to be treated containing mixtures of dyes of different nature, the material of purification can be effective only screw / screw of some of them. To remedy these difficulties we prepared a cellulosic material susceptible to stop to it quasi totality of the dyes contained in water of rejection of industry. The purpose of the present work is the study of the possibility of the replacement of adsorbants at present used during waste water treatments, by cellulosic materials, these last ones will be modified by the grafting of carboxylic functions on their surfaces by bringing in the anhydrides of cyclic acids and the acrylic acid. Indeed, these functions give at the same moment to the supports of the properties of heat exchanger (interchange) of ions and modify strongly their tinctoriales affinities. The study of grafting of the cotton with the carboxylic functions by the anhydride succinique and the acrylic acid is successfully realized, several parameters were optimized (the time, the temperature, the catalyst). The gain of mass of grafting reached by the values superior to 100 % for the anhydride succinique and superior to 20 % for the acrylic acid. The estimation of the acid quantity of the functions shows that the prepared supports have an important number of free acid sites; where from the interest of supports prepared in the fixation of dyes agents. Finally, the prepared supports are used for the extraction of the industrial dyes agents in intermittent system, the study is realized on two types of dyes agents the Blue of Methyl alcohol (B.M) and the Red Congo (R.C). The profits show that dyes agents are quickly extracted in pH basic. The report (mass of the support / volume of the solution of dyes agent) minimal corresponding to the maximal rate of extraction is 3.5 g/l and 10 g/l respectively for the B.M and R.C. Various studies concerning the isotherms of adsorption allow to explain the mechanisms of the interactions. The regeneration of adsorbants is also taken into account.

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CLAYS OF THE SOUAR-FORMATION IN NORTHEASTERN TUNISIA: CARACTERISATION, APPLICATION IN PESTICIDES RETENTION

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At the time of discharge of the water polluted in a natural environment and thanks to the properties of retention, adsorption and exchange of ions, clays constitute a natural barrier which will be able to limit the toxicity and the propagation of the pollutants. To contribute to the development of clays layers of Tunisia in the field of water treatments, we undertook with a mineralogical and physicochemical characterization of some inflating clays. The characteristics of these clays will be exploited for the study of the retention by adsorption of pesticides. The adsorption of pesticides to clay mineral has been reported by several investigators. The purpose of this investigation was the removal of dithiocarbamates (pesticide) from aqueous solution by raw and purified smectitic clay. A group of isotherm tests were performed to clarify specific sorption behaviors due to the pesticide concentration. Adsorption onto clay was essentially immediate and was correlated with the cationic exchange-capacity, indicating that adsorption was primary to negatively charged sites on the clay. Langmuir and Freundlich models were employed to examine the equilibrium adsorption data. The influence of several parameters on the fixing of pesticides on clay such as the factors relating to the medium of adsorption (agitation, pH, time of contact, temperature… etc) and those relating to the adsorbent (mass, granulometry, impurities… etc) was studied in order to optimize the operating conditions of adsorptions.

1837 QUANTIFICATION OF NITROUS OXIDE EMISSIONS DURING BIOLOGICAL NITROGEN REMOVAL FROM WASTE WATER - A SYSTEMS COMPARISON

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There is strong evidence that the biological removal of nitrogen from waste water emits considerable amounts of nitrous oxide (N_2O) gas. Up to 80% of removed nitrogen is emitted in the form of N_2O, which is a strong greenhouse gas and ozone depletant. This fact is well reported for treatment of agricultural wastes but not for the treatment of communal/industrial waste water. This difference may be due to the fact that, conventionally, the emission of nitrous oxide is seen to be caused by denitrifying bacteria involved in the water treatment process. Inventories of gaseous emissions from commercial treatment works thus may have missed out a potentially significant source of this gas, namely nitrification.The project investigated, under controlled conditions in a laboratory, whether the treatment of communal/ industrial waste water for nitrogen removal can principally lead to significant releases of nitrous oxide and showed that this was not the case. Nitrogen and COD removal rates during all experiments were high, indicating that the systems were operated in a way that provided appropriate conditions for biological nutrient removal. Emission rates of nitrous oxide during the various treatment schemes were negligible; they accounted for less than 0.5‰ of nitrogen removed from the wastewater, at maximum, and for a tenth of that on average. Emissions at this rate contribute only marginally to the overall anthropogenic N_2O production: the results of this project can be extrapolated to represents 0.063% of the UK's total anthropogenic N_2O production of 128 kta^{-1} in 2005. As such, the project showed that the treatment of industrial or communal waste waters does not lead to significant emissions of nitrous oxide. However, it does not provide an explanation as to why high emissions can occur in an agricultural context; this aspect still merits further investigation. Furthermore, comparison with evolving "nitrite short-cut" treatment schemes for nitrogen rich waste waters shows close parallels to agricultural waste water treatment and this particular aspect of industrial waste water treatment may benefit from closer inspection for its N_2O release potential.

1847 PHOSPHATE REMOVAL FROM WASTEWATER BY BACTERIA AND ORGANIC BENTONITE

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The aim of this study was to determine the potential of phosphate (P) removal from wastewater by P-accumulating bacteria and organic bentonite. Organic bentonite was prepared by treating bentonite clay with quaternary ammonium salt –cetyltrimetylammonium (CTA) bromide. Cation exchange capacity (CEC) of the bentonite was found to be 179.0 meq/100 g of the dry bentonite. The CTA occupied ca. 175% of the CEC. The modification of bentonite with CTA in amounts higher than 55% of the CEC resulted in the change of zeta potential of particles from negative to positive. Only in reactors containing organic bentonite samples occupied with 3.5 and 28% of the CEC the P was efficiently removed from wastewater by combined adsorption and bacterial uptake in the biomass. The organic bentonite samples with higher CTA loading (from 55 to 175% of the CEC) showed the bactericidal effect. To enhance the P removal from wastewater in the aerated biological system, the organic bentonite can be used, but the special attention should be given to the configuration and location of sorbed CTA molecules and its potential desorption.

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2218 PHOSPHATE REMOVAL FROM AQUEOUS PHASE USING IRON IMPREGNATED NATURAL CLAY: KINETIC AND EQUILIBRIUM MODELING

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Even though phosphate is a major nutrient for plants and microorganisms, its excess amount (> 1 mg/L) is often responsible for eutrophication of water bodies. Eutrophication is a phenomenon of algal booming causing high economic damage due to the death of tones of fishes. Therefore, nowadays, the removal of phosphate from aqueous solution and municipal sewage attains great concern. In the present study, iron impregnated kaolinite based natural clay (Fe-KNC) can be effectively used for the removal of phosphate from water and municipal wastewaters. Iron impregnation on kaolinite based natural clay (KNC) was carried out by drop by drop addition method. The effect of various factors such as pH, initial concentration of phosphate, contact time and adsorbent dose on phosphate adsorption was studied by batch technique. The maximum phosphate removal takes place in the pH range of 2.0-5.0. With an initial concentration of phosphate at 25 mg/L at 30 ºC and pH 3.0, its removal has been found to be 98.7 %. The effect of pH on phosphate adsorption was explained by pH_{zpc}, phosphate speciation in solution and affinity of anions towards the active sites on the adsorbent. The effectiveness of Fe-KNC for phosphate removal was examined and compared with other adsorbents in the literature. Moreover, the adsorption results showed that Fe-KNC is 4-5 times more effective than KNC. Kinetic studies revealed that the adsorption process followed à pseudo-second order kinetic model rather than a pseudo-first order kinetic model. Adsorption followed Langmuir isotherm model and the maximum adsorption capacity was found to be 78.35 mg/g at 30 ºC. Column studies were also performed to examine the efficacy of the investigated adsorbent for the removal of phosphate from continuously flowing effluents. The data obtained point towards viable adsorbents, which are effective, eco-friendly and economically attractive for phosphate removal from wastewaters. Surface modification of KNC using iron impregnation greatly enhanced phosphate removal and resulted in a product with possible commercial potential for wastewater treatment.

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2250 NUTRIENT REMOVAL FROM WASTEWATER USING AN UCT HYBRID SYSTEM WITH SUSPENDED BIOMASS AND BIOFILMS

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Over the past two decades, several biological suspended sludge configurations have been proposed to accomplish biological nitrogen and phosphorus removal, and they all include the basic stages of an anaerobic zone, followed by an anoxic and aerobic zone. From all these process the University Cape Town (UCT) or modified UCT processes are broadly used for nutrient removal in urban sewage (Ekama 1983). All the biological phosphorus removal (BPR) processes are dependent on the accumulation of bacteria capable of storing large amounts of polyphosphate inside the cells. Contrary to normal biological conversions, the BPR is not limited by a minimum solids retention time (SRT), which results from a maximum growth rate of the Phosphate Accumulating Organisms (PAO) implicated. On contrary, nitrification and thus nitrogen removal could be negatively affected if the system is operated at low SRT, as result of the wash out of nitrifying microorganisms with a low growing rate. In this sense, the use of a carrier to promote the growth of a nitrifying biofilm could be positive to improve nitrogen and phosphorus removal in the UCT system as was also observed for COD and nitrogen removal in other high capacity hybrid bioreactors (Oyanedel 2005). The aim of this research was to study the behavior of simultaneous nutrient removal in a UCT system using two different plastic packing materials: A rough granular support of plastic of around 3 mm diameter and the commercial product Kaldnes K-3 made of polyethylene with approximately 25 mm diameter and 10 mm long. These packing materials were maintained in suspension in the aerobic chamber of the UCT hybrid system. The UCT system was fed with synthetic wastewater that simulates the composition of sewage. Phosphorus, nitrogen and organic matter removal efficiency was studied at different SRT ranging from 20 to 4 days. HRT was maintained between 12 and 18 h. A high COD removal efficiency was observed, being COD in the effluent around 50 mg/L. With regard to Nutrient removal, it was observed a high phosphorus removal during a first experimental period. P in the wastewater gradually decreased from 7-10 mg/L (influent) to less than 2 mg/L (effluent). Nitrogen removal was more problematic during the first experimental days, in which only the Kaldnes K-3 was used as carrier. Ammonia removal increased as soon as the rough granular plastic support was added to the reactor. This could be a consequence of the different roughness of both packing materials. The Kaldness K-3 has a smooth surface, in which is difficult to develop a nitrifying biofilm in the presence of suspended biomass. On contrary, roughnesses of the granular product protect nitrifiers against detachment.

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2349 NUTRIENTS REMOVAL USING MOVING BEDS WITH AERATION CYCLES

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Moving Bed Biofilm Reactors (MBBR) are based on the biomass growth over a media that moves into the reactor due to aeration, mechanical agitation or recirculation. These reactors have been gaining popularity and they are employed in hundreds of plants everywhere with different treatment purposes (organic matter removal, nitrification/denitrification), both for urban and industrial wastewater. At industrial scale they have demonstrated a high potential mainly in the agro-food industry sector. The main problem of this type of reactors is diffusional limitations depending on the agitation, aeration, type of support, etc. The selection of the size and forms of the support is fundamental to overcome that problem. In this work two different media material were employed. The experimental equipment consists in two moving bed biofilm reactors with different media, plastic rings with a diameter of 11 mm, a density of 823 g/L and a porosity of 85% and polyurethane foam cubes of 1 cm with a density of 22 g/L and a porosity of 45%. The reactor volume is around 1.6 L and the aeration was intermittent (15 minutes on and 30 minutes off). Wastewater employed is a synthetic one with a COD of 500 mg/L, ammonia of 100 mg N-NH_4^+/L and 10 mg P/L. The reactors worked with organic loading rates between 0.35-0.95 kg COD $m^{-3}d^{-1}$ and nitrogen loads of 0.08-0.22 kg N_{total} $m^{-3}d^{-1}$, obtaining a high removal of organic matter (77-89%) and ammonia (55-86%). Intracellular polyphosphate aggregations were detected by microscopical analysis using Gram and Neisser reagents. The characteristics of the media play an important role in the biological process. The accumulation of biomass is lower in the plastic rings surface, favouring nitrification; while the polyurethane foam retain less quantity of air, favouring denitrification.

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2447 USE OF RESPIROMETRY FOR THE DETERMINATION OF NITRIFICATION KINETICS

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In Brazil the imposition of stricter effluent standards for discharge of treated effluents in many cases lead to the need of applying nitrification in aerobic waste water treatment systems. From literature it is known that nitrification is described by Monod kinetics but the values of the kinetic constants depend on the origin of the waste water especially in the case of industrial wastes. Nitrification kinetics has an important impact on activated sludge design and operation, so that a method is required to determine experimentally the kinetic constants. The most important kinetic constant is the maximum specific growth rate constant of nitrifiers. Traditionally this constant is determined by operating an activated sludge system and gradually reducing the sludge age and observing nitrification efficiency. In this paper respirometry is used for the determination of nitrification constant. The method consists of recording the oxygen uptake rate (OUR) of a sludge batch obtained by operating a nitrifying activated sludge system and determine the OUR profile of OUR when substrate (ammonium chloride) is added. The OUR profile can be converted in a nitrification rate profile by applying the appropriate stoichiometric proportional constant (4.56 mg O/mg N). The profile of the nitrification rate can be used for the determination of two important kinetic nitrification constants: (1) the maximum growth rate constant and (2) the ammonium half saturation constant of nitrifiers. By repeating the test at different levels of dissolved oxygen also a third constant: the oxygen half saturation constant can be determined, leaving only one constant, the decay constant, which is of minor importance and can be assumed to have the value reported in literature. Thus the kinetic constants of nitrification are known for the waste water that is being treated and can be used for a design and operational optimization. The method was applied to assess nitrification by an activated sludge system operated with effluents from a large petrochemical complex at Camaçari in Brazil. Pilot scale activated sludge systems were operated at different DO levels and the generated sludges were used for respirometric determinations of nitrification kinetics. The method was found to be rapid and precise and was applied to optimize nitrification at full scale.

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1851 SOFTWARE SENSORS DESIGN FOR THE PHENOLS CONTAMINATED WATER TREATMENT BY THE COMBINATION OF OZONATION AND BIODEGRADATION

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Phenol and chlorophenols are strongly recalcitrant. Wastewaters containing phenols have been treated by several methods as adsorption, chemical oxidation and biological treatment. It is worth to notice that the best results have been obtained when the combination of chemical oxidation and biological treatment instead of only one of them is employed. In this sense, the control and possible optimization of the combined process requires enough information about the involved variables at each stage. Nevertheless, many of these variables are not on line measurable. It means there are not specific sensors and only discrete measures by some analytical technique are available; this situation constrains the possibilities to improve the behavior of the process. For this reason, many algorithmic approaches have been developed to obtain some numerical approximation of the entire set of variables, taking into account the on line measurable information. This technique is globally known as: state estimation or software sensors design. Differential Neural Networks (DNN) have the capacity to approximate functions with a high accuracy even in presence of noisy information. It allows us to design enough robust software sensors based on this kind of structures, using a simplified mathematical model for the DNNO training. In the present study a DNN-Observer (DNNO) (software sensor) is designed for the variable reconstruction of the treatment of the ground water contaminated with 4-Chlorofenol. The treatment is based on the combination of ozonation and an aerobic bioremediation, the last by a previously adapted mixed microbial consortium is carried out. Ozonation reduces the biological treatment time from > 10 days to 20 hr. The software sensor considers the ozone concentration in the gas phase as unique available information for the chlorophenol decomposition reconstruction. In the case of the biodegradation stage, the on line estimation of biomass and chlorophenol concentration is carry out from the dissolved oxygen (DO) data. Results obtained confirm the good performance of the proposed DNNO approach comparing with analytical techniques (HPLC and UV/VIS). Furthermore, the comparison of bioremediation with the combined ozonation-biodegradation treatment and the by-products identification by HPLC are presented.

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2145 A PILOT STUDY TO INVESTIGATE THE IMPACTS OF AGRO-FOOD DISCHARGES ON A DOMESTIC WWTP

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The approval of Directive 91/271CE, which limits the concentration of nutrients (N and P) in treated wastewater discharges, has led the majority of WWTP in Europe to work with nutrient removal processes. These WWTP for BNR are usually optimized to remove nutrients from domestic wastewater, but in some cases a significant discharge of industrial wastewater can modify the influent wastewater composition. Although such industrial discharges have scarcely been studied, they can cause many changes related to the effluent quality, mainly with regard to phosphorus and nitrogen removal. In this context, the aim of this work was to study the influence of long term agro-food wastewater discharges on a Pilot-Scale Virginia Initiative Plant (VIP) process that was optimized for the treatment of domestic wastewater. The effect of the industrial discharges coming from milk bottling industries, cheese factories and wineries on the effluent quality was evaluated. The experiments were carried out in a Pilot-Scale plant located at the Ciudad Real WWTP. The influent wastewater to the pilot plant was prepared by mixing real wastewater taken from the primary clarifiers of the full-scale WWTP and a 300 mg COD/l of one of the agro-food industrial effluents. Samples were taken every day from the influent, anaerobic, anoxic and aerobic compartments as well as from the effluent, these results were statistically analyzed and the mean values and the standard deviations were used to study the impact caused by the different discharges on the performance of the Pilot-Scale WWTP. The main results observed were the following: A positive influence on the nitrogen removal when winery wastewater was discharged. A negligible influence of the agro-food industrial discharges on the COD removal. A negative influence on the phosphorus removal, mainly observed in the experiments when industrial wastewaters from milk bottling or cheese industries were discharged. Taking into account the benefits caused by the discharge of winery wastewaters on the nitrogen removal and paying attention to the opposite effects caused on the nitrogen and phosphorus removal, the feasibility of the addition of winery wastewater to enhance the nutrient removal addition in the full-scale WWTP was evaluated.

2174 EVALUATION OF BIODEGRADABILITY AND TOXICITY OF PHENOL BY RESPIROMETRY

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Phenol presence in wastewaters can affect efficiency of activated sludge process in a wastewater treatment plant because of its possible toxic character. Respirometry can be a tool to evaluate biodegradability and toxicity of different compounds by measurements of the oxygen consumption of activated sludge.The aim of this study is to evaluate the ability of microorganisms present in activated sludge to biodegrade phenol and to know its toxicity by respirometric measurements. The activated sludge used in the study was collected from the industrial line of a wastewater treatment plant were biological treatment consists of an oxidation ditch. Before testing in the respirometer, the sludge was aerated during 24 hours until endogenous conditions were reached.The experiments were performed in a laboratory respirometer, BMT model from SURCIS, S.L. Operation is based on a closed batch circuit in which the oxygen measurements of activated sludge and combined samples are continuously monitored in a unique, optimally designed reactor vessel. Two operation modes were used: static and dynamic. From tests performed in static mode, oxygen uptake rate (OUR) was measured. Total OUR was calculated for different concentrations of phenol and it was found a relationship between OUR values and substrate concentration, based on Monod equation. Biodegradable fraction of chemical oxygen demand (BCOD) was calculated in dynamic mode tests by measuring consumed oxygen (CO, mgO_2/l). COD was measured by dichromate oxidation standard method and the ratio COD/BCOD was calculated. This allows to asses the biodegradable character of the sample under an approach of the activated sludge activity. The results obtained show that at low concentrations of phenol, biodegradability is high.Toxicity of phenol was evaluated by progressive increase in the substrate concentration in a test carried out in dynamic mode. Acetate solution was added to activated sludge in order to create a maximum respiration rate and set the reference level. Once this level was reached, doses of substrate were added in order to progressively increase substrate concentration in the reactor. In case of toxicity the respiration rates in the respirogram decrease progressively. It has been observed that it is necessary a high concentration of phenol (189 mg/l) to cause total inhibition of activated sludge activity.

2175 AN INNOVATIVE TECHNOLOGY FOR WASTEWATER TREATMENT: A PILOT-SCALE STUDY

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A wastewater treatment pilot plant of an innovative technology was constructed at the University of Murcia to test its success in achieving treated wastewater with adequate quality for reuse as garden irrigation. The cited technology, named “symbiotic plant”, is composed of two well-distinguished areas: the depuration area and the cultivation area. The depuration area consists of a gravel bed, 120 cm thick, in which the wastewater is applied by means of a network of underground drippers located inside of perforated pipelines, so that wastewater percolates through it. The cultivation area, ≅ 30 cm of sandy substrate, is situated over the depuration area. This substrate allows air entry into the bed and depuration takes place in aerobic conditions. The whole depuration process consists of four symbiotic stages in series with a previous pretreatment step. The pretreatment consists of a grid followed by a 125 μm mesh ring filter. Pretreated wastewater enters the first symbiotic column through the drippers falling by gravity through the gravel bed until it reaches the bottom waterproof layer and it is pumping to the next stage. The effluent of each column is filtered through another filter of the same size mesh, as mentioned above, before entering the next column. This pilot plant can treat 8 l/h. Physico-chemical analyses were made of samples collected from: influent and effluent of the filter and outlet of each one of the four treatment stages. In spite of the great variation in the characteristic of the wastewater generated from various university installations, which composition is comparable to a domestic wastewater typical of a town with high strength, a great homogeneity in the effluent quality is achieved. The results obtained during about one year of experimentation show the following mean removal rates: total suspended solids (TSS) 98%, chemical oxygen demand (COD) 96%, and biochemical oxygen demand (BOD_5) 99%, total kjeldahl nitrogen 90% and total phosphorus 55%. Besides, an increasing in dissolved oxygen is obtained along the stages ensuring enough oxygen level to enhance nitrification. In fact, the decomposition by microorganism of organic nitrogen to ammonia and then the complete oxidation to nitrates has been observed along the stages. The pilot plant effluent complies with the Directive 91/271/EEC effluent requirements of ≤ 35mg/l TSS, ≤ 125mg/l COD and ≤ 25mg/l BOD_5. So, in view of the results this technology may be considered suitable for the treatment of urban wastewaters.

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2176 USE OF RESPIROMETRIC METHODS FOR ESTIMATION OF HEAVY METAL TOXICITY AND INHIBITION ON ACTIVATED SLUDGE ACTIVITY

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Heavy metals are present in wastewater effluents from different industries. These substances are not only potential human health hazards but also to another life forms. The objective of this study was to apply respirometric methods to determine the toxic character of two heavy metals (cadmium and nickel) to microorganisms present in activated sludge and their inhibitory effect on the biological process.The activated sludge used in the study was collected from the industrial line of a wastewater treatment plant were biological treatment consists of an oxidation ditch. The experiments were performed in a laboratory respirometer, BMT model from SURCIS, S.L. The dynamic operation mode was used. This mode is based on a closed batch circuit in which the endogenous respiration in activated sludge is fixed as dissolved oxygen base line and then a certain amount of sample is added to be analyzed. In the respirogram, continuous measurements of respiration rate, R_s (mg/(l·h)) are showed. The inhibitory effect on the activated sludge activity was determined by comparison with a reference level, $R_{s\ max,ref}$, that corresponds to the maximum respiration rate obtained with a solution of sodium acetate (0.5 g acetate/g volatile suspended solids). A series of tests were carried out, by adding samples with the same concentration of sodium acetate and different concentrations of heavy metals. Once the corresponding respirograms are obtained maximum respiration rate of each sample was calculated ($R_{s\ max,\ m}$). Percentage of inhibition was calculated as follows:

$$I(\%)=\left(1-\frac{R_{s\ max,ref}}{R_{s\ max,ref}}\right)$$

Experimental data for both heavy metals indicate a good logarithmic correlation between inhibition percentage and concentration. When cadmium was added, an inhibitory effect was observed. The inhibition percentage increased with cadmium concentration. 90 % inhibition was achieved when cadmium concentration was 36 mg/l. Nickel showed a lower inhibitory effect on the respiration rate of microorganisms; 900 mg/l in the reactor produced a 50% inhibition. Toxicity of heavy metals was evaluated by progressive increase in the substrate concentration in a test carried out in dynamic mode. Acetate solution was added to activated sludge in order to create a maximum respiration rate and set the reference level. Once this level was reached, doses of substrate were added in order to progressively increase substrate concentration in the reactor. In case of toxicity the respiration rates in the respirogram decrease progressively. It was observed higher toxic effect for cadmium than for nickel.

2201 USING NATURAL ZEOLITES TO IMPROVE ANAEROBIC ABATTOIR WASTEWATER TREATMENT

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Slaughterhouse wastewaters have high concentrations of soluble and insoluble organics which represents environmental troubles, e.g. de-oxygenation of rivers, underground water contamination. Anaerobic digestion is an efficient process for wastewater treatment. Performances are increased using microorganisms supported on porous solids. Zeolites are crystalline microporous solids with a three-dimensional structure that have numerous applications in catalysis, adsorption and wastewater treatment. Synthetic zeolites are extensive used in industrial processes, but natural zeolites have few applications even though they are abundant. In this paper a Mexican natural zeolite as support material in an anaerobic batch reactor for slaughterhouse treatment (laboratory scale) is studied. Natural Mexican zeolite, previously characterized was used as biofilms support evaluating the effect of particle size. Reactors were inoculated with a sludge obtained from a brewery wastewater treatment plant and they are operated at 36 °C. Total chemical oxygen demand (COD) is ranged from 4500 to 9000 mg/L, approximately 33% is in the form of suspended solids. During the digestion, CH_4 production and COD were measured at 72 h intervals. Three kinds of experiments are performed: with biofilms previously formed during 30 days, 60 days and without biofilm. The COD rate remotion was around 50% in 72 h. Methane production in 12 days was 2 g/L. The particle size does not affect COD removal. The assays with biofilm presented bigger methane production. The results are not as expected due to the microbial consortium used which is partially inactive. Now, new experiments are performed with a completely active consortium. An accelerated COD degradation and then higher methane production is expected with the new consortium. Preliminary data from these experiments show that the natural Mexican zeolites have excellent properties for biofilms formation and then a high potential to be used as support for anaerobic microorganisms in wastewater treatment processes. An immediate future work is to carry out experiments with more critic operating conditions at bigger scales in batch and continuous reactors.

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2208 FLUID BED POROSITY EQUATION FOR AN INVERSE FLUIDIZED BED BIOREACTOR WITH PARTICLES GROWING BIOFILM

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Fluid Bed Bioreactor performance is strongly affected by bed void fraction or bed porosity fluctuations. Particle size enlargement due to biofilm growth is an important factor that is involved in these variations and until now there are no mathematical equations that consider biofilm growth. In this work a mathematical equation is proposed to calculate bed void fraction in an inverse fluid bed bioreactor. This equation considers the variation of particle physical properties, such as diameter and density due to biofilm growth. The average bed particle size during biofilm growth is calculated by means of a Monod kinetics model for the *Lactobacillus ac.*, used as particle attached microorganism. The experiments were done in an inversed fluidized bed bioreactor using polypropylene particles (dp = 4 mm, ρ_p = 808 kg/m^3) impregnated with a *Lactobacillus ac.* inoculum and a MRS culture medium (Man-Rogosa-Sharpe) was used as fluid in a temperature and pH controlled media. Bed height was measured at different flow rates, starting from the minimum fluidization velocity to 0.73 bed porosity. The existence of the *Lactobacillus ac.* was confirmed by gram staining and scanning electron microscopy (SEM). Particle average biofilm thickness (77 μm) was measured by optical microscopy and verified with SEM's micrographies. The proposed equation calculates bed void fraction as a function of Reynolds (Re) and Archimedes (Ar) numbers, using the approach of a balance of forces that exerts on an isolated particle that includes the drag force and extending it to the particles bed. Reynolds numbers are smaller than 200. The calculated values give a standard deviation of 3.98% for all the experimental data, and this is better than those calculated by other equations published in the literature. The use of dimensionless numbers that includes easy measurable physical properties makes this equation of easy application for design and scale-up of fluid bed bioreactors.

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2215 HEXAVALENT CHROMIUM REDUCTION BY A *Hypocrea tawa* FUNGAL STRAIN

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Microbial transformation of the highly toxic, water-soluble and mobile hexavalent chromium [Cr(VI)], to the less toxic, insoluble and immobile trivalent chromium [Cr(III)], is an economically feasible alternative for the treatment of wastewaters contaminated with Cr(VI). The main purpose of this work was to isolate, identify and characterize a microbial strain capable of reducing Cr(VI). A fungal strain with high Cr(VI) reduction potential was isolated from contaminated surface water by batch enrichment culture techniques, and further identified as *Hypocrea tawa* by its D1/D2 domain sequence of the 26S rRNA gene with 99.44% similarity. The Cr(VI) reduction capability of *H. tawa* was characterized in batch cultures conducted at initial Cr(VI) concentrations ranging from 0.59 to 4.13 mM under aerobic conditions. Residual Cr(VI) concentrations decreased as incubation progressed, until no measurable concentration of Cr(VI) could be detected in the culture media. Therefore, overall Cr(VI) removal efficiency attained in all batch cultures was 100% and consequently, this parameter was not affected by initial chromate concentration. In contrast, total chromium concentration remained practically constant throughout the incubation time (more than 98% of the chromium initially added to the culture media was always present in solution), and the fungal cells did not accumulate a measurable amount of chromium. These results indicate that the fungal strain was capable of reducing Cr(VI) to Cr(III). Higher volumetric [1.75 mg Cr(VI)/L˙h] and specific rates [0.67 mg Cr(VI)/g biomass˙h] as well as a greater capacity [77 mg Cr(VI)/g biomass] to reduce Cr(VI) were obtained with higher initial Cr(VI) concentrations (4.13 mM), which suggests that *H. tawa* has the biotechnological potential to be used in remediation processes for industrial wastewaters contaminated with Cr(VI).

2216 HEXAVALENT CHROMIUM REDUCTION BY *Trichoderma inhamatum*

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Reduction of hexavalent chromium [Cr(VI)] to trivalent chromium [Cr(III)] is a useful and attractive process for remediation of ecosystems and industrial effluents contaminated with Cr(VI). Cr(VI) reduction to Cr(III) can be achieved by both chemical and biological methods; however, the biological reduction is more convenient than the chemical one since costs are lower, and sludge is generated in smaller amounts. The objective of this work was to isolate, identify and characterize a microbial strain with high level Cr(VI) resistance and reduction potential. A fungal strain capable of removing Cr(VI) was isolated from tannery wastewater and further identified as *Trichoderma inhamatum* by molecular biology techniques. The Cr(VI) reduction process catalyzed by *T. inhamatum* was characterized in batch cultures conducted at initial Cr(VI) concentrations ranging from 0.83 to 2.43 mM. Experimental results indicate that the fungus is capable of transforming Cr(VI) to Cr(III); a transformation of a highly toxic contaminant to a low toxic form. The fungal strain completely reduced 0.83, 1.3, and 2.43 mM Cr(VI); thus, overall efficiency of Cr(VI) reduction (100%) was not affected by initial Cr(VI) concentration. Nevertheless, the time required to reduce 95% of the Cr(VI) initially present in the culture media increased linearly as the initial Cr(VI) concentration increased. The specific and volumetric rates, as well as the capacity for Cr(VI) reduction decreased as the initial Cr(VI) concentration increased, which may have been due to the strong oxidizing power of Cr(VI). Despite of this, *T. inhamatum* was capable of tolerating and reducing very high Cr(VI) concentrations without supplement of chemicals such as flocculants, reducing agents, acid or alkali. Thus, the characteristics exhibited by *T. inhamatum* suggest that this fungus could be useful for detoxification of Cr(VI)-contaminated wastewaters.

2247 A COMPARATIVE STUDY OF WASTEWATER TREATMENT, FROM A FISH CANNING FACTORY, IN A GRANULAR AND A FLOCCULENT SLUDGE SBR

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Sequencing Batch Reactors (SBR) process utilizes a fill and draw reactor with complete mixing during the reaction stage and where subsequent stages of aeration and clarification occur in the same tank. These systems often operates with flocculent sludge with a relatively low sludge volume index, around 100 ml/g-TSS, and sludge settling rate of around 1 m/h. SBR with flocculent biomass are used for treating low volumes of either industrial or urban wastewater. In recent years, the use of granular sludge has been proposed as an alternative, for treating wastewater in high capacity SBR systems. The basis of granulation is the continuous selection of sludge particles that occurs inside the reactors. The part of the biomass, which does not settle fast enough, will be washed out with the effluent (Beun 1999). Thus, the selection of the granules from a biomass mixture in an SBR can be easily performed using the difference in settling velocity between the granules (fast settling biomass) and the flocs (slow settling biomass) (Arrojo 2004). The main objective of this study was to compare the operation of two similar SBR: A granular sludge SBR and a flocculent sludge SBR system. Both reactors were used for treating the wastewater generated in a fish canning factory. COD of the wastewater was between 700 and 1100 mg/L, total nitrogen concentration was between 110 and 180 mg N/L and total phosphorus ranged around 110 mg P/L. Both reactors operated under similar conditions, in terms of organic loading rate and average hydraulic retention time. COD removal efficiencies above 90 % were obtained in both the Granular and Flocculent sludge SBRs. In the granule SBR, granule formation was observed after the first experimental days.In the flocculent SBR, full ammonia nitrification and around 70% nitrogen removal was obtained. However, only a very low amount of ammonia was nitrified in the granular sludge SBR. This was a consequence of the intrinsic characteristics of this reactor. In this system the volumetric exchange ratio of 50% every feeding cycle of only 3 min, complicate nitrification due to inhibition by free ammonia concentration. Organic nitrogen and ammonia of the influent wastewater is very high, and 3 min feeding time complicated ammonia removal during the first minutes of the cycle. In the flocculent SBR the same exchange ratio was maintained, but the feeding cycle lasted around 60 min, which avoided any ammonia accumulation in the flocculent sludge SBR. Compared with flocs, granular sludge had excellent settling properties. However, the maximum TSS observed in both SBR was similar, around 8-10 g-TSS/L.

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2251 POST-TREATMENT OF ANAEROBICALLY DIGESTED SWINE SLURRY IN ALGAL-BACTERIAL ENCLOSED PHOTOBIOREACTORS

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The treatment of livestock effluents is receiving an increasing attention in Europe due to the recent shift from extensive to intensive farming and to the high organic and nutrient concentration of these wastewaters. Despite constituting one of most cost-effective treatment technologies for the removal of the organic matter, the application of anaerobic processes to the treatment of livestock effluents is still limited by the poor nutrient removal efficiencies achieved, which usually requires a further post treatment (nitrification+denitrification or enhanced biological phosphorous removal). In this context, the application of microalgal systems for the reclamation of anaerobically treated livestock effluents provides both an *in-situ* O_2 supply via photosynthesis and nutrients recycling via N, P assimilation into the algal-bacterial biomass. The work herein presented evaluated the potential of a symbiotic algal-bacterial consortium (the microalga *Chlorella sorokiniana* and a swine slurry degrading activated sludge) as a final polishing step of anaerobically treated swine slurries. The anaerobically digested effluent (obtained from a full scale swine slurry anaerobic digester operated at 35 °C) was centrifuged for 20 minutes at 9000 rpm prior to use. Glass bottles of 1250 ml were filled with 200 ml of undiluted, 2, 4 and 8 times diluted anaerobically pretreated swine slurry and inoculated with activated sludge and *C. sorokiniana*. The bottles were then filled with helium, closed with butyl septa and sealed with plastic caps in order to ensure that pollutant oxidation occurred exclusively driven by photosynthetic oxygenation. No significant variation in total organic carbon (TOC) and N-NH_4^+ concentration was recorded in tests 1×, 2×, and 4× likely due to the severe inhibitory effects imposed by its high NH_4^+ concentrations. The biodegradation of the anaerobic effluent evaluated was thus only feasible in systems 8×. TOC biodegradation rates and NH_4^+ removal efficiencies of 55 ± 6 g C $m^{-3}d^{-1}$ and 93 % were recorded in systems 8×, respectively. In addition, acetic, butyric, and isobutyric acid, the major VFA components in anaerobically digested slurry, were totally depleted after 125 h of cultivation in these flasks. Therefore, this study confirmed the potential of algal-bacterial processes as a polishing step for the treatment of anaerobic swine slurry effluents.

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2255 EFFECT OF A REACTIVATION STRATEGY BASED ON PARTIAL BIOCATALYST REPLACEMENT ON THE PERFORMANCE OF A FUNGAL FLUIDIZED BED BIOREACTOR

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The Mexican pulp and paper industry discharges approximately 12 % of the annual industrial discharges and holds a second position in the ranking of main water industrial polluters in Mexico. Their wastewaters are characteristically recalcitrant and toxic. The objective of this work was to evaluate the effectiveness of two operational strategies on the performance of two fungal fluidized bed reactor (FBR) for the post-treatment of anaerobically-pretreated weak black liquor systems (AP-WBL) without supplementation of soluble carbohydrates, i.e. Strategy 1 (continuous operation with the same original, fungal biocatalysts and eventual spikes of protease inhibitor and glucose), and Strategy 2 (operation with partial exchange of biocatalysts. Biocatalysts consisted of pellets formed with mycelium of ligninolytic fungus *Trametes versicolor* immobilized on a core mixture of sawdust and powdered activated carbon. The strategy of partial replacement of biocatalysts was implemented by replacing 25% of the existing pellets with fresh pellets every 60 days of continuous operation of bioreactors. Lab scale fungal fluidized bed reactors were operated at room temperature at 5 d and 2.5 d hydraulic retention time (HRT). The influent to reactors was an anaerobically-pretreated weak black liquor typical of Kraft process, named here as AP-WB, that was conditioned to pH 4.5. At day 60 of continuous operation occurred the first exchange of biocatalysts (strategy 2). It was observed a transient decrease of pollutant removal efficiencies (color, ligninoids, and COD) for 5 to 10 days. The second exchange (strategy 2) of biocatalysts was performed at day 130. This time, pre-conditioned fresh pellets were used. Their pre-conditioning consisted of batch incubation with AnE in flasks, for 10 days. After loading pre-conditioned pellets into the bioreactors, no negative transients of removal efficiencies were observed. In general, the main advantage of strategy 2 over strategy 1 relied on a more extended period of active operation. Indeed, RLF under strategy 2 was able to actively remove pollutants for 180 days or continuous operation, whereas RLF with strategy 1 showed a drastic decrease of pollutant removals at 90 d. In regard to removal efficiencies, results of strategies were mixed. For instance, conventional removals achieved with strategy 2 were superior to those of strategy 1, whereas unit net removals of COD in strategy 1 at 5 d HRT were higher than those with strategy 2. Interestingly, average protease strategy in strategy 2 was lower than with strategy 1, which was consistent with a longer active operational period of strategy 2.

2258 BIOFILM FORMATION ON MEMBRANES USED FOR MEMBRANE AERATED BIOLOGICAL REACTORS, UNDER DIFFERENT STRESS CONDITIONS

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Bacterial biofilms play an important role in wastewater treatment processes, and have been optimized in the membrane aerated biofilm reactors (MABR). In MABR, a hydrophobic membrane is used as support for the formation of biofilms, and supplements enough aeration to assure an aerobic process. The aim of this work was to analyze biofilm formation by bacterial strains isolated from wastewater, on a hydrophobic membrane under different hydrodynamic conditions. Biofilm formation was studied under static hydrodynamic condition without aeration; with aeration and under turbulence condition, as well as in a membrane aerated biofilm reactor. An artificial sterile wastewater was used as influent with a COD of 720 mg/L. Biofilms were extracted from the membranes by vortex during 2 minutes for enumeration of aerobic mesophilic bacteria by plate count. Another part of the membranes was fixed on glutaraldehide (2.5%) during 10 hrs and prepared for scanning electron microscopy (SEM). Extraction of EPS was done by heat treatment, and carbohydrate content in the extract was determined using the Dubois method, with glucose as standard. Seven bacterial strains, selected on the basis of EPS formation and degradation capacities were studied under static conditions. From the seven strains, two were selected for further studies, under turbulence and in the MABR unit. Under static conditions, bacterial counts were on the range of <10.0 UFC/cm^2 to 4.16 X 10^6 UFC/cm^2, while EPS (carbohydrate concentration) varied from 3.5 to 143 µg/cm^2. There was no correlation between those two measurements, and electron microscopy demonstrated that those differences were due to differences on the type and structure of EPS and micro-colonies. Overall, microbial viable count was a more reliable method for determination of biofilm formation. Results under turbulence and in the MBAR unit were similar, and showed a microbial biofilm different for each bacterial strain. The techniques used shown that the microbial adhesion is dependent on the system conditions and the microorganism. These techniques can be used as a rapid method for an adequate selection of membranes to employ in different membrane reactors, by determination of the presence and quantity of biofilms formed on the membranes.

2264 OPERATION OF A MEMBRANE BIOREACTOR WITH A FLAT SHEET MEMBRANE TREATING URBAN WATER

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Membrane Bioreactors (MBR) consist of a biological activated sludge unit in which the secondary settler has been replaced by a microfiltration or a ultrafiltration membrane, with nominal pore sizes ranging from around 0.01 to 0.4 µm. This makes feasible a good control of solids retention time (SRT) and hydraulic retention time (HRT) in the system. MBRs are not affected by the settling properties of the sludge and the use of filtration membranes makes the concentration of suspended solids in the effluent negligible (Judd 2006, Artiga 2007). A 500 L volume submerged MBR with a Kubota flat sheet membrane of 2 m^2 was used to treat urban water that was pretreated in an aerated grit and grease removal system. This pilot plant was located in the urban water treatment plant of Bertamiráns (Galicia, Spain). The pilot scale MBR consist of three chambers connected in series: The first chamber was an anoxic chamber of 200 L, the second an aerobic chamber of 200 L in which the membrane module was submerged, and a third an aerobic chamber of 100 L volume. Sludge from the third chamber was recycled to the anoxic chamber in order to reduce nitrate concentration in the permeate (effluent). The characteristics of the pretreated urban water were those typically found in for moderate or diluted urban waters: COD concentration typically ranged from 200 to 500 mg/L, Ammonia was around 20-30 mg N/L and orthophosphate concentration was 5-8 mg P/L. HRT was gradually diminished from 100 h to 20 h. The low HRT was a consequence of the very low permeability of the membrane observed during the first experimental days, of only 20-30 L/m^2·h·bar. In order to increase the permeability of the membrane this was chemical cleaned and hydrophilised at the operating day 18. After that, permeability increased till 500 L/m^2·h·bar, and from day 18 on HRT was diminished gradually from 30 h to 20 h. The strong variation of the HRT did not affect the quality of the permeate. COD concentration was between 20 and 50 mg/L, Ammonia concentration was between 0.5 and 4 mg N/L, nitrate concentration was around 10 mg N/L and orthophosphate concentration was lower than 1 mg P/L.

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2278 BIOAEROSOL GENERATION AT WASTEWATER TREATMENT PLANTS: IDENTIFICATION OF MAIN BIOAEROSOL SOURCES

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Typical operations taking place at wastewater treatment plants, especially those involving aeration and mechanical agitation of raw wastewater, represent one of the main sources of bioaerosols that, if inhaled, could pose a biologic hazard to site workers and local residents. Six different wastewater treatment plants from southeast Spain were monitored in order to identify the main bioaerosol sources and to evaluate the airborne microorganisms levels to which workers may be exposed to. Air samples were taken from selected locations by using a single stage impactor. Total count of mesophilic bacteria was used as a monitoring parameter. The highest concentrations of airborne bacteria were recorded in the raw wastewater pre-treatment building (levels varying from 140 to 1787 CFU/m^3), the biological reactor (from 22 to 4580 CFU/m^3, depending on the aeration system) and the sludge dehydration unit (from 53 to 3103 CFU/m^3). The levels of airborne bacteria recorded at these locations were significantly higher than those registered in background locations, unaffected by the activities taking place in the wastewater treatment plant, usually lower than 100 CFU/m^3. Rotative sieves and grit tanks used for the pre-treatment of raw wastewater, alongside with the aeration systems involving mechanical agitation of the wastewater, such as horizontal rotors and surface turbines, were identified as the main bioaerosol sources during wastewater treatment. Simple operational measures such as the enclosement of these operational activities, the treatment of the exhaust air by the use of biofilters, and the use of air diffusers as an aeration system for the biological treatment would significantly limit the biological hazard that wastewater treatment plant workers may be exposed to.

2288 PHYTOPLANKTON AS BIOINDICATOR FOR WASTE STABILIZATION PONDS

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Waste stabilization ponds are usually the most appropriate method of domestic and municipal onsite wastewater treatment. It is a low-cost technology, requires low maintenance, highly efficient, entirely natural and highly sustainable. Most of the energy they use is solar energy, and need only a few electromechanical equipment. In facultative ponds, the maintenance of a healthy algal population is very important. The objective of this work is to determine the pattern of microalgae in the facultative and maturation ponds to get information for the operation and maintenance work. The important parameters for the phytoplankton are the organic load, temperature and nutrients on water, which are also measured in this study. Methodology consists in: phytoplankton and zooplankton taxonomic determination, abundance through counting with the inverted microscope, and analysis of the main water parameters at the same time. Results show that cyanobacteria are present in under-loaded conditions and Chlamydomonas when the pond is overloaded. Our aim is to find a year round pattern to use the phytoplankton as a bioindicator of the conditions of the pond. We also measure chlorophyll-a concentration changes for the different stages, as well as de dissolved oxygen concentration and pH. Our conclusion is that the weekly phytoplankton determination is essential to know the performance of the pond under determinate conditions.

2296 POST-TREATMENT OF ANAEROBICALLY DIGESTED DAIRY MANURE TOGETHER WITH MUNICIPAL WASTEWATER

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Dairy manure presents high levels of organic matter and ammonia as well as high solids content, many of them being colloidal. The use of manure as fertilizer is limited by the edafoclimatic conditions of the place where it is produced. Intensive livestock farming must also consider the land availability for fertilizing. A possible initial treatment consists in separating solid and liquid fractions by means of coagulation-flocculation processes. Liquid fraction can be treated in high load anaerobic reactors, such as UASB, where the organic load would be reduced. The chemical oxygen demand (COD) of the UASB effluent is mainly refractory. In addition, UASB effluents present high ammonia levels (N_{ammon}) and solids content significantly lower than those contained in raw manure. The characteristics of UASB effluents do not allow its discharge into public waters. In order to reach discharge conditions it is possible to remove ammonia nitrogen by struvite precipitation. The aim of this work is to study the possibility of the co-treatment of municipal wastewaters (MWW) together with treated liquid fraction of dairy manure (TLF) in an activated sludge (AS) system process slightly modified, so that the final effluent characteristics meet the requirements of the environmental legislation to observe the discharge limits. That includes denitrification processes inside the reactor. The use of municipal wastewaters has a double purpose: employing its COD as external carbon source in the denitrification process and diluting the refractory COD of the treated manure, since the biodegradable organic matter was previously removed in the anaerobic reactor. An AS lab scale system has been employed with reactor aeration and an air lift device for sludge recirculation. So, there are two flows of air that can be controlled to create oxidation and anoxic stages. If reactor aeration and sludge recirculation are suitable combined it is possible to reach such conditions that permit the oxidation of ammonia nitrogen and the subsequent denitrification stage. This way, a final effluent has been achieved with concentrations of COD, solids, ammonia nitrogen, nitrites and nitrates below the legal limits to be discharged.

2297 METHANIZATION OF 2 CHLOROPHENOL (2CP) IN PRESENCE OF OXYGEN

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Chlorophenols, very toxic organic compounds, are widely distributed in soils and water. These substances are related to cellular damage as they have mutagenic and carcinogenic characteristics. Aromatic compounds have been eliminated from wastewater under methanogenic conditions; however, in most of the cases the elimination rates are low and some toxic intermediates might be accumulated. In this sense, the presence of small quantities of oxygen under methanogenic conditions could enhance both a complete elimination of toxic compounds as well an increase in its consumption rate. Therefore, this work was focused in evaluating in batch culture under methanogenic conditions the effect of: (i) different oxygen concentrations on the consumption and methanization of 2 chlorophenol (2CP), and (ii) the sludge acclimation on 2CP consumption rate. Batch assays were conducted by triplicate in 60 ml serologic bottles at 35°C. Using a N_2 atmosphere, 50 mg 2-CP/L was added and four oxygen concentrations were evaluated (mg O_2/L): (0.5, 1.0, 1.5 and 2.0). A methanogenic assay with no oxygen addition was also conducted as control. At first, methanogenic sludge in steady state was used as inoculums. After 50 days, when 2CP was depleted, 50 mg 2-CP/L was fed again. 2CP consumption efficiency (82 %) and 2CP consumption rate values (0.0026 mg 2CP/g VSS·L) obtained at the different oxygen concentrations were very similar among them and to those obtained in the methanogenic control without oxygen. Therefore, the presence of oxygen did not increase these parameters. However, at the highest oxygen concentration, methane yield values (Y_{CH4}) and phenol production decreased in 50 and 42%, respectively, while CO_2 yield value (Y_{CO2}) increased regarding to the methanogenic control. Likewise, the mass balance indicated that close to 26% of carbon was not present at the end of the assays, suggesting that an unidentified carbon intermediate has been produced. Results obtained in the assays with sludge previously exposed to 2CP indicated that the 2CP consumption time decreased in 50% resulting in an increase in 2CP consumption rate of 73%. However, no significant difference in consumption efficiency or consumption rate values was observed among the different oxygen concentrations if compared to the control without oxygen. Therefore, the increase in these values could be due to a sludge acclimation rather than an oxygen effect. In summary, methane and phenol production decreased whereas CO_2 production increased due to the oxygen presence in the methanogenic cultures. The sludge previous exposition for 50 days to 2CP resulted in an increased in 2CP specific consumption rate.

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2324 INFLUENCE OF COSUBSTRATES IN THE ANAEROBIC BIODEGRADATION OF 2,4,6-TRICHLOROPHENOL AND METHANOGENESIS

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Chlorophenols have been commonly used in pesticides, herbicides and dyes production. 2,4,6-Trichlorophenol (246TCP) is mainly used in the paper pulp bleaching and as wood preservative. This compound can't be biodegraded by conventional biological techniques because of its recalcitrant nature.
In anaerobic conditions; 246TCP bears consecutive reductive dechlorinations until its complete mineralization. It is known that 246TCP anaerobic biodegradation needs an easily biodegradable substrate working as electron donor (codegradation). In any case, cosubstrates don't behave in the same way.
In this work, the influence of several cosubstrates in the anaerobic biodegradation of 246TCP efficiency, as well as in the metabolic pathway, is studied. The methanogenesis inhibition caused by 246TCP with every cosubstrate is also compared. With this purpose, batch assays were carried out. Anaerobic 250 mL serum bottles were inoculated with 1.5 g VSS of acclimated granular sludge from a continuous lab-scale EGSB reactor treating 2,4-dichlorophenol (24DCP). The cosubstrates tested were lactate, sucrose, volatile fatty acids (VFA) in 1:1:1 weigh ratio, ethanol, and methanol, yeast extract (YE) -4 g COD/L-, formiate and methylamine -2 g COD/L-. 246TCP concentrations tested were 80 mg/L and 113 mg/L (acetotrophic methanogenesis IC_{50}). Three feed were realised. 246TCP was added in the second feed.
The 246TCP removal efficiency was different depending on each cosubstrate. Moreover, 246TCP removal rate increased considerably after the second feed. Complete mineralization cannot be achieved because of secondary metabolites accumulation, either 4-chlorophenol (methanol, ethanol, VFA) or 24DCP (lactate and sucrose). Methylamine as well as formiate was inefficient. Monochlorophenols mineralization was achieved with YE, but both 24DCP and 26DCP were accumulated.
As far as methanogenesis is concerned, the highest specific methanogenic activity (SMA) was achieved using ethanol and methanol as cosubstrates. This means that 246TCP is better tolerated by methanobacteria with these cosubstrates. Sucrose was a good methanogenic cosubstrate because SMA was only reduced by 60% at 113 mg/L of 246TCP. In summary, it was proved that alcohols are the best cosubstrates in the anaerobic biodegradation of 246TCP. Moreover, a YE addition allows the complete monochlorophenols mineralization. In order to ensure a high SMA, sucrose can be added too.

2336 FEASIBILITY OF LEAD REMOVAL FROM INDUSTRIAL EFFLUENTS BY SULPHATE REDUCING BACTERIA

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Battery manufacturing wastewater contains high concentration of sulphate and lead at low pH values. Biological treatment by anaerobic reduction of sulphate to sulphide, with organic matter as electron donor, increases alkalinity and allow precipitating lead as sulphide. The type of organic matter, COD/SO_4 ratio and lead concentration over sulphate reduction rate are investigated in this work. Batch assays were conducted in serum bottles using granular anaerobic sludge, basal medium with sulphate and different carbon sources: synthetic (acetate and methanol) and natural (treated slaughterhouse wastewater). Periodic samples were taken from aqueous and gas phase and sulphate, sulphide, COD and lead were measured. Sulphate consumption rate and sulphide production rate showed a linear relationship with time, from the slope of the adjusted model and sludge concentration, the specific consumption or production rate are obtained. At initial COD/SO_4 of 0.5, and initial Pb concentration of 10 ppm, the effect of carbon source was tested. Specific sulphate consumption rate decreased from 24.9, 18.5 and to 13.8 mg $S\text{-}SO_4\ g^{-1}VSS\cdot h^{-1}$ for acetate, lactate and wastewater respectively. Specific sulphide production rate decreased from 7.6, 3.2 and to 2.1 mg $S\text{-}SH_2\ g^{-1}VSS\cdot h^{-1}$. Lead removal efficiencies were higher than 97 % in all cases and remaining concentration were lower than 0.3 ppm, from preliminary results obtained in this work, the biological process based on anaerobic sulphate reduction can be applied for treatment of high sulphate and metal polluted industrial wastewaters.

2211 TREATMENT OF TEHRAN AGED LANDFILL LEACHATE BY COAGULATION-FLOCCULATION PROCESS

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Sanitary landfill leachates are considered today strong and heavily polluted industrial wastewaters, exhibiting considerable spatial and temporal variations. Special care is required for their efficient treatment. The main aim of this study was to examine the application of coagulation-flocculation for treatment of partially stabilized leachates.Jar-test experiments were employed in order to determine the optimum conditions for removal of organic matter and color, i.e.coagulant-flocculant combination, effective dosage and pH. Poly aluminum chloride (PAC), ferric chloride, ferric sulphate, ferrous sulphate and aluminum sulphate as conventional coagulants, alone and in combination with an anionic polyelectrolyte were tested. High chemical oxygen demand (COD) and color removal capacities (about) were obtained during the addition of ferric chloride to the partially stabilized leachates, whereas low COD and color reduction (i.e. lower than) were measured during the addition the addition of ferric sulphate to the samples. Optimum dosage of ferric chloride was 2.5gr/l as Iron. The physical-chemical process may be used as a useful pretreatment step prior to biological treatment, or as a post-treatment (polishing) step for aged leachates.

2380 NITROGEN REMOVAL FRON LANDFILL LEACHATE IN AN ANOXIC-OXIC SYSTEM

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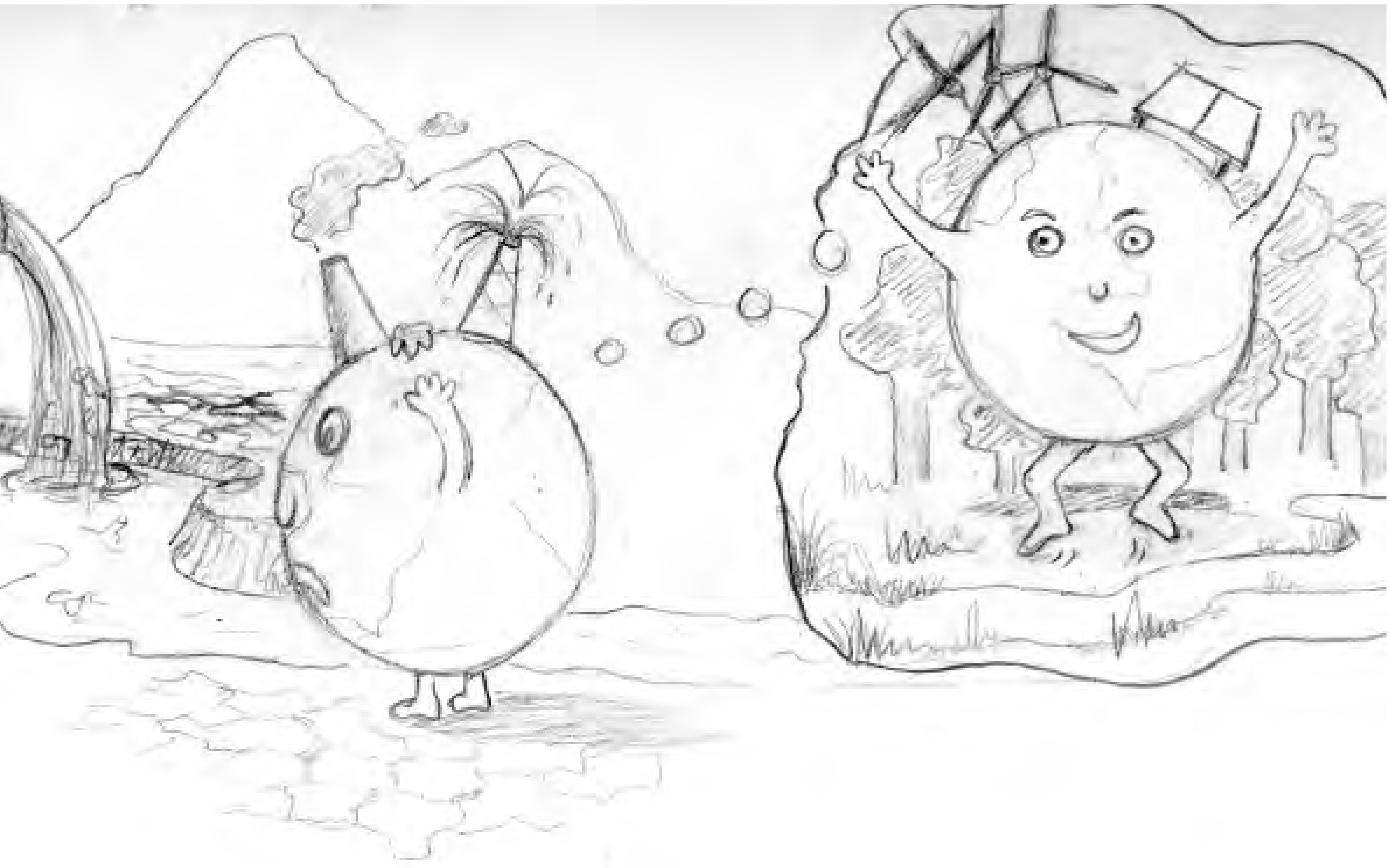
In spite of technological advances that developed several techniques of solid waste treatment, its disposal in soil is the practice more used in most countries, specially in Brazil and other developing countries. The reason for the choice of that alternative is due to low cost and simplicity of this method. Recently the environmental regulations has become more restrictive and new products like geosynthetics lead to several technological sophistication in landfill projects that made them safer to environment (Fleck, 2005). However, the leachate which results from the biological decomposition of organic matter and rainfall, is still a problem (Hamada, 1999). The objective of this work was to evaluate the ammonium nitrogen removal in a system composed of anoxic reactor followed by oxic reactor, treating leachate of a landfill in its methanic phase of decomposition. The system was operated with hydraulic retention time (HRT) of 20 days (phase I) and 13 days (phase II), during 118 and 53 days respectively. The volume of each reactor was 27 L and the volume of the settler 33 L. The oxic reactor had an automatic pH control system. The recycle ratio (Re) of the sludge varied between 0.5 and 1.0. Analyses were done according to Standard Methods for Examination of Water and Wastewater (2005). The following parameters were analyzed for the liquor and leachate before and after treatment: total and filtered COD, BOD_5, total solids, suspended solids, volatile solids and non-volatile solids (TS, TSS, TVS, VSS), total alkalinity (TA), pH, ammonium nitrogen, organic nitrogen (N-org), nitrite and nitrate nitrogen. The leachate used had a COD/BOD_5 ratio smaller than 0.1. The concentration of ammonium varried from 1110 to 373 mg N-NH_4/L. The COD removal was 70% (phase I) and 54 % (phase II). The efficiency of ammonium removal was 99% in both phases.



TUESDAY, 23 SEPTEMBER

POSTER COMMUNICATIONS

II.- Wastewater and solid waste management, treatment and reuse

III.-Strategies for the protection and remediation of natural environments

POSTER COMMUNICATION

TUESDAY 23 SEPTEMBER 2008

II WASTEWATER AND SOLID WASTE MANAGEMENT, TREATMENT AND REUSE

Wastewater Biological Treatment

Agronomic Valorization

2337 LOW-COST SWINE MANURE BIODEGRADATION USING ALGAL-BACTERIAL SYSTEMS (HRAP)

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Wastewater from livestock farming such as pig slurry can be effectively treated in High-Rate Algae Ponds (HRAPs). These systems are shallow (20-50 cm) open photobioreactors in which bacteria use the oxygen released by microalgae for the oxidation of organic matter and in turn provide both CO_2 and nutrients for microalgal activity. Apart from being cheaper (no need for intensive aeration to meet the high BOD of piggery wastewaters), photosynthetic oxygenation allows nutrients recycling and CO_2 mitigations. The operation of these photobioreactors depends on temperature and solar irradiation. Two 30 cm deep HRAPs of 462 litres mixed by a paddle wheel system were operated from January till March in an inner cold zone of Spain. The HRAPs were fed using diluted pig slurry from a primary treatment (screening followed by sedimentation). Both reactors were operated at a residence time of 10 days, the first with 1:10 dilution of manure and the second with 1:20. COD removal efficiencies of 75% and 60% were achieved in the first and second bioreactor, respectively, while the amoniacal nitrogen was almost completely eliminated in both bioreactors. Nitrogen assimilation into algal-bacterial biomass accounted for 17% of the total nitrogen removed, while nitrification and NH_3 stripping were responsible of 23% and 60%, respectively, regardless the swine slurry loading applied. Soluble phosphorous removal of 50 and 30% were recorded in the first and second photobioreactor, respectively. Dry matter biomass achieved 300 mg/l in both reactors during the period of optimum growth. Culture pH remained constant at approx. 8 while dissolved oxygen ranging from 12 to 4 mg/l. An average temperature of 3.7 ºC was measured during the period of experimentation. This study demonstrated the potential of HRAPs for swine manure wastewater degradation in cold climate.

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2339 METAL REMOVAL AND ORGANIC COMPOUND DEGRADATION BY BIOMASS OF *Aspergillus niger*

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Environmental problems are being faced by humankind as an everyday issue. Residue contaminants processing is a major concern, being a priority the use of techniques with high efficiency and lower cost. Biotechnological processes represent a good alternative to other methods, such as physicochemical, due to their low consume of energy. In this work, *Aspergillus niger* containing high chromium biosorption and phenol degradation efficiency was used for simultaneous metal removal and organic compounds degradation. 1 L of modified Czapek broth additioned with chromium(VI) (Cr(VI)) and phenol, as an electron donor, passed through biosorbente columns (height 15 cm, internal diameter 2 cm), each containing 1 g pellets of *A. niger*. The flow rate was adjusted to 39.08 ml min^{-1} using a programmable peristaltic pump. The effluents coming out from the columns were analyzed at regular intervals (1.5 hr) for residual metal and phenol content by reaction with diphenylcarbazide, and HPLC respectively. Phenol and Cr(VI) concentrations were fixed according to prior standardization. The results demonstrated that when 100 $mg.L^{-1}$ Cr(VI) were added to the modified Czapek broth, a removal of 100% of the metal was showed after 9 hours of exposure. When concentration was increased to 500 mg L^{-1} Cr(VI) obtained removal was 60%. In the case of phenol, degradation values were 95.5% and 81.6% with concentrations of 300 mg L^{-1} and 800 mg L^{-1}, respectively. Values obtained under these conditions demonstrate that *Aspergillus niger* is able and efficient in degrading high concentrations of phenol and also removes heavy metals, due to its high surface/volume rate in the system, and to its intense metabolic activity. In this system, phenol acts like the unique carbon source for the microorganism metabolism, as well as electron donor, to remove Cr(VI). Reductions in removal and degradation values when higher concentrations were proved may be due to toxic effect on the microorganism.

2346 STABILITY OF "ANAEROBIC REACTORS" UNDER MICRO-AERATION CONDITIONS

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Oxidation of sulphide in anaerobic bioreactors by introducing limited amounts of oxygen provides a relatively simple strategy for reducing the levels of sulphide in anaerobic digesters (biogas and effluent). The introduction of limited amounts of air is a general practice in agricultural anaerobic digesters, it is estimated that worldwide over 3,000 units are operated under such conditions (A. Wellinger, pers. comm.) and semi-commercial reports are founded about this topic. In spite of this, the scientific literature about micro-aerophilic reactor operation for sulphide removal is rather limited. All the studies found are for lab-scale systems. Despite the toxicity exerted by oxygen against obligate anaerobes like methanogens, its moderate introduction to anaerobic bioreactors is not expected to have a harmful impact to the process, mainly due to the limited penetration depth of oxygen in biofilms. The objective of this paper is to demonstrate the stability of the process under micro-aeration conditions and provide scientific information about micro-aerophilic operation at pilot-plant scale. Research is carried out in two continuous-flow stirred-tank digesters, at 35 ± 0,5 ºC, volume 200 L and HRT of 20 days. Reactors are fed with waste sludge from WWTP. Micro-aerophilic conditions are kept in one of the digesters introducing continuous oxygen flow controlled in the sludge recirculation with gas mass flow controller. Reactor performance is evaluated by conventional anaerobic parameters:

	Anaerobic	Micro-aerophilic
O_2 (mL/min)	0	2,5
pH	7,18	7,19
ORP (mV)	-503 / -504	-500 / -502
alkalinity (mg CaCO3/L)	6.500	6.530
% VS removed	62%	60%
productivity (mL biogas/g VS removed)	510	495
% CODSoluble removed	62%	65%
% VFA removed	98%	99%
Biogas flow (L/d)	170	150
% CH_4	63%	62%
% CO_2	34%	34%
% H_2S	1,2-1,3%	0%

Anaerobic process is stable under micro-aerated conditions which do not affect organic removal, biogas production or biogas composition (%CH_4).

2347 THE DAIRY WASTEWATER EXPERIMENTAL CONSTRUCTED WETLAND IN A COLD CLIMATE

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The increasing need to preserve nature but, at the same time, to continue to use its resources, has supported the development of new low-cost technologies which don't compromise the environment, with the aim to pursue a sustainable improvement of men's activities. Phytoremediation is considered one of the most efficient and promising techniques to depollute contaminated waters. The aim of this paper is to analyze the first activity period of an experimental wetland system placed in a cold climate region. Designed and realized with the most advanced technologies actually available, our intention is to demonstrate how well a system like this could be useful in a typical reality as the one of mountain cheese factory. This project is a part of the Rurecotec Interreg IIIA program realized under the aegis of U.E., and approved with Valle d'Aosta Regional counsil deliberation n° 964 on 7/4/2006. The constructed wetland for the diary "Laiterie Cooperative Valdigne" in Morgex (Valle d'Aosta, Italy) was built both to respect the environmental resources and to give a contribute to the development of such a recent technology. It consists on a fat-removal unit and a basin for the storage and the distribution of the wastewater which precede three pythoremediation beds: the first two are parallel and they work as submerged vertical flow wetland with gravel medium for a total area of 180 m^2, the last is a submerged horizontal flow wetland with sand medium and a total area of 360 m^2. These beds are planted with *Phragmites australis* (CAV.) Trin. Ex Steud., with a density of about 4 plants/m^2. After several months of monitoring we checked the health of the plants and how efficiently the system works on the reduction of several parameters, before and after the contribution of the plants.

2356 MECHANICALLY STIRRED ANSBBR APPLIED TO ORGANIC MATTER AND SULFATE REMOVAL: EFFECT OF FEED STRATEGY AND SULFATE LOAD

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A mechanically stirred AnSBBR treating synthetic wastewater (500 mgCOD.L^{-1}) at COD/[SO_4^{2-}] ratios of 1.34, 0.67 and 0.34, i.e., [SO_4^{2-}] concentrations of 373, 746 and 1493 mgSO_4^{2-}/L, respectively, was submitted to different feed strategies (10-min batch and 3-h and 6-h fed-batch). In nine assays the effect of each one of these parameters on system performance was investigated, i.e., feed time and COD/[SO_4^{2-}] ratio. The reactor operated at 400 rpm with total volume of 4.0 L and treated 2.0 L synthetic wastewater in 8-h cycles at 30 ºC. The results showed that the 3-h fed-batch operation presented the best conditions for organic matter removal (89%). Regarding sulfate removal highest efficiency (71%) with 0.55 gSO_4^{2}.$(L.d)^{-1}$ was obtained at a COD/[SO_4^{2-}] ratio of 1.34 and a fill time of 6h. It should be mentioned that removed sulfate load increased linearly at increasing sulfate concentration in the influent. It was therefore concluded that during the batch and 6-h fed-batch assays the removed organic load decreased as sulfate concentration in the influent increased. However, in the 3-h fed-batch assays the increase in sulfate concentration improved organic matter removal. In the batch assays the removed sulfate load increased significantly when the COD/[SO_4^{2-}] ratio was reduced from 1.34 to 0.67 and remained at this level in the assay with COD/[SO_4^{2-}] ratio of 0.34. In addition, the sulfate reducing bacteria showed difficulty to adapt to this feed strategy. In all assays the reactor presented operational stability in relation to the monitored process variables like volatile acids, bicarbonate alkalinity and solids, showing the potential to apply this technology to conjoint removal of organic matter and sulfate.

2358 MICROBIAL ORGANIC MATTER USE AND NUTRIENT REMOVAL IN A CONSTRUCTED WETLAND SYSTEM

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A Wetland System in Empuriabrava (North-East of Spain) was established in 1998 to improve the quality of secondary effluent from a sewage treatment plant, especially to diminish nutrient concentration. Significant reduction of inorganic nutrients, especially phosphorus, was reported but little is known about the microbial processes occurring at the system and its role on organic matter use and decomposition throughout the wetland. This study aimed to evaluate the efficiency of the system for the use of organic matter and the role of the microbial communities in suspension and attached to macrophytes and the sediment on the functioning of the wetland system. For that reason, influent and effluent water at inlet, outlet and middle areas of the three replicate wetlands were sampled monthly during six months. At one sampling date, the microbial community developing on macrophytes and sediment was further sampled. Inorganic and organic nutrients in water (nitrate and phosphate, dissolved organic carbon – DOC -, and biodegradable dissolved organic carbon - BDOC) were analyzed. Algal biomass (chlorophyll), bacterial biomass (DAPI counting) and total suspended solids were analyzed to control the development of the microbial community. Heterotrophic organic matter use was estimated by the measurement of extracellular enzyme activities related to the use of polysaccharides (β-glucosidase), organic phosphorus compounds (phosphatase) and peptides (leucine-aminopeptidase). Significant differences between influent and effluent water from the Wetland System were found. Enzyme activity showed a significant increase in polysaccharide decomposition at the middle of the system as indicated by the high β-glucosidase activity in water and especially at the community attached to macrophytes. Decrease in β-glucosidase at the outflow was only significant at some sampling periods. In contrast, relevant peptidase decomposition were measured throughout all the system but significantly decreased at the outflow water, indicating a decrease in available organic nitrogenous compounds. The results suggest that the microbial community developing at this Wetland System, both planktonic and benthic (attached to macrophytes and sediment), might be responsible for C and especially N organic compounds decomposition.

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2361 BIODEGRADATION OF PHENOL USING AN ANAEROBIC EGSB REACTOR

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Phenol is a compound found naturally in domestic and industrial waste waters and should be removed since in high concentrations it proves to be fatal. The present investigation was undertaken to evaluate the anaerobic biodegradability of the phenol in the wastewaters supplementing sulphates in the form of $CaSO_4$, to increment the COD_{Total} removal value. Experimental part was carried out in a laboratory anaerobic EGSB reactor with a 2.6 L volume, with an operating temperature of 35 ± 2 °C, Hydraulic Residence Time (HRT) of 1.73 days and was fed with 866 mL organic anaerobic granular sludge. Influent wastewater contained 100 ppm of C_6H_5OH, 25 mM of CH_3COONa and an additional source of sulfate in the form of $CaSO_4$ in a concentration of 100 ppm. The results indicated that the effluent pH was maintained at an average of 8.68, meanwhile the influent pH was in average 8.43 giving an alkalinity relation (α) of 0.8, which might be due to interaction of the anaerobic sludge. After experimenting with anaerobic reactor EGSB which contained the low volumetric loading rate (1 kg DQO $m^{-3}d^{-1}$), COD_{Total} was decreased by 45% when included any sulphate supplement. Otherwise, adding additional contain of sulfate in the form of $CaSO_4$, COD_{Total} was removed an average of 60% and achieved a maximum removal value of 75%. Supplementing sulphates interacting with sulphate-reducing bacteria (sludge bacterial population) might increase the amount of molecular bond breakings over organic molecules such as phenol. Anaerobic Reactor EGSB when is supplemented with an additional source of sulfate in the form of $CaSO_4$ in a concentration of 100 ppm is a feasible option, due to obtained COD_{Total} removal efficiency value (over 70%). This technology could find application in industrial wastewater treatment which contains toxic pollutants as phenol in order to accomplish the normativity and could be an alternative to make reusable this water in agricultural and ground water injection systems.

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2398 STUDY OF A NITRIFYING SEQUENCING BATCH REACTOR IN PRESENCE OF *m*-CRESOL

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The process of the nitrification has been studied scantly in presence of phenolic compounds such as *m*-cresol. The aim of this study was evaluate the tolerance of a nitrifying SBR (Sequencing Batch Reactor) to *m*-cresol and the ability of the sludge to consume this phenolic compound. Nitrification is the process of oxidation of ammonia to nitrite and nitrate by lithoautotrophic ammonia- and nitrite-oxidizing bacteria. The nitrifying bacteria are sensitive to a number of environmental factors, such as the presence of organic compounds. Phenolic compounds and ammonia can be found at high concentrations in petrochemical and industrial effluents. Thus, it is of interest to carry out investigations in dynamic systems, such as SBR, in presence of *m*-cresol. A laboratory scale SBR fed with 50 mg NH_4^+-N/ld with a working volume of 1.5 l was operated with cycles of 12 h. Each cycle consisted of the four following periods: fill (15 min, corresponding to mixed and aerated feeding); aerated react (11 h including 15 min during the filling phase, and stirred of 300 rpm), settle (30 min) and draw (15 min). The *m*-cresol was added to the reactor at 33 ml/min for 15 min and at initial concentrations ranging from 12.5 to 150 mg/l. The sludge used for inoculating the SBR was obtained from 3l continuous reactor under steady-state nitrification. The nitrifying SBR achieving simultaneously the complete ammonium oxidation and the total consumption of *m*-cresol. *m*-Cresol induced a significant decrease in the values for specific rates of ammonium consumption (39 to 86 percent) and nitrite oxidation (45 to 61 percent). After 10 months of operation in SBR, the specific rates of NH_4^+-N oxidation, and NO_3^--N formation, were 0.5 g NH_4^+-N/g microbial protein-Nh, 0.2 g NO_3^--N/g microbial protein-Nh respectively. The oxidation of *m*-cresol was carried out faster throughout the cycles and nitrification inhibition decreased with the number of cycles. Results indicated that the SBR system allowed the metabolic adaptation of the microorganisms responsible for *m*-cresol oxidation as shown by the increasing specific rates removal of this compound with the increase of the number cycles, and consequently a decrease for nitrification inhibition.

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2457 AEROBIC BIOLOGICAL TREATMENT OF MUNICIPAL WASTEWATERS AND PIG SLURRY AND THE ASSOCIATED BACTERIOLOGICAL AND PARASITOLOGICAL RISKS

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The aim of the present study was to investigate the bacteriological and parasitological risk associated with the products of aerobic treatment of pig slurry and municipal sewage. We focused on the quality of effluents and on sewage sludge and pig slurry solids from two wastewater treatment plants (pig slurry WWTP-1; municipal wastewater WWTP-2) with regard to plate counts of selected groups of bacteria (mesophilic, coliform, faecal coliform) and the efficiency of their removal. The efficiency of removal ranged between 86.5 and 95.7 % for WWTP-1 and between 79.8 and 97.9% for WTP-2. Parasitological examination showed that no helminth eggs (*Ascaris* spp., *Trichuris* spp. Were found in effluents from both wastewater treatment plants despite their presence in the effluents. One sample of sludge (out of 40) contained 1 egg of *Ascaris* spp. and additional one only 1 one egg of *Trichuris* spp. This indicates that the current sludge treatment cannot guarantee complete devitalisation of helminth stages and additional processes are required to ensure safety of sludge before it is applied to agricultural soil.

2512 PRODUCTION OF MANGANESE PEROXIDASE (MNP) FROM *Anthracophyllum discolor* FOR LIGNIN DEGRADATION

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In Chile, millions of tons per year of pulp and paper are produced, generating large quantities of toxic and intensely colored wastewater, causing severe water pollution. The primary contributors to the color and toxicity are the lignin and its derivative compounds. The color is responsible for problems such as reduction in the light penetration depth of the receiving waters, which may affect aquatic life. A biological process that has attracted substantial interest is the use of white rot fungi, which may remove or reduce a wide variety of color generating compounds. The ability of white rot fungi to degrade lignin is due to its extracellular enzyme system composed of laccases, lignin peroxidases (LiP) and manganese peroxidases (MnP). In previous studies, *Anthracophyllum discolor* was isolated from templated forests of southern Chile, and has demonstrated high activity of MnP. However, the optimal culture conditions for the production of the enzyme have not been yet determined. In this context, the aim of this investigation was to evaluate the effect of different culture conditions on the production of MnP from *A. discolor*.The fungus was incubated at 25 ºC and 150 rpm on liquid medium containing glucose as carbon and peptone as nitrogen source.The culture condition of pH (3.0 - 6.0), differents concentrations of inductor Mn^{2+} ($MnSO_4$ 100-1000 μM) and Tween 80 (0.05 - 2%) were evaluated. At optimal operational condition, the degradation of different lignin concentrations (100-2000 mgL^{-1}) was evaluated. All factors influence strongly MnP production. Best MnP accumulative production (450 UL^{-1}) was achieved at pH 4.5 before of 20 days of incubation. The $MnSO_4$ addition increased MnP activity, obtaining an accumulative activity of 2800 UL^{-1} when 250 μM of $MnSO_4$ was added. On the other hand, the addition of Tween 80 (0.05%) increased 2-fold the MnP activity. The lignin concentration (>400 mgL^{-1}) increased MnP production, that contributed to the removal of colour (70-90%), phenolic compounds (60-80%) and chemical oxygen demand removal (80-95%). These results show the potential use of *Anthracopyllum discolor* for the degradation of wastewaters containing lignin compounds, as well as for the treatment of colored effluents.

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2519 USE OF 4-AMINO-NAPHTALEN SULFONIC ACID AS A THE SOLE CARBON, NITROGEN AND SULPHUR SOURCE, BY A MICROBIAL COMMUNITY

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Sulphonated aromatic amines are toxic compounds widely used in chemical synthesis of pharmaceuticals, pesticides and azo.dyes. They are also generated by degradation of many azo compounds. Sulphonated amines are readily soluble in water, thus causing damage when discharged to aquatic environments. From effluent's samples obtained from textile industries, a microbial community able to use 4-aminonaphtalen sulfonic acid (ASA) as the sole carbon and energy source was isolated. The main objective of this work was to evaluate if this community could grow on this compound with the minimal addition of K_2HPO_4, $MgCl_2$ and $CaCl_2$, in spite of their C:N:S unbalance.
Experiments were carried out in a packed bed reactor, using pebbles as support material for the microbial community attachment. Hydraulic retention times (HRT) were varied from 10 to 59 hours, determining the effluent's COD and ASA concentrations. The higher ASA volumetric removal rate ($R_{V,ASA}$ = 2.0 mg L^{-1} h^{-1}) was obtained at HRT of 12.5 h, with removal efficiencies of 100% for ASA determined by HPLC and 97% when determined as COD. When HRT diminished to 10 h, the ASA removal efficiency dropped to 65%, determined by HPLC, and 40% when determined as COD. Under these circumstances, it was observed ASA accumulation and diminution of metabolic intermediaries.
Five bacterial strains constituted the microbial community. Three of them were identified by PCR-DNA amplification, sequencing and comparison with the GenBank sequence data base. These were: *Gordonia polyisponivorans* (EU124551.1), *Acinetobacter sp*. (EU252078.1) and *Acinetobacter lwoffi* (EU240445.1).
We can conclude that the isolated microbial community was able to use 4-aminonaphtalen sulfonic acid as the sole carbon, nitrogen and sulphur source, with high removal efficiency.

2524 EXPERIMENTAL DESIGN OF COPPER BIOSORPTION FROM AQUEOUS SOLUTION BY *Aspergillus terreus*

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The heavy metals releases to environment are dangerous because of their high toxicity, not biodegradability and accumulation in living organism. The biosorption has been showed as an economical, ecofriendly and high performance process to remove heavy metals. In this study, an experimental design has been used to evaluate and optimize the process of copper biosorption from aqueous solution using *Aspergillus terreus* as biosorbent. The percentage of metal removal was studied in a batch system by a full 2^4 factorial design with seven central points. The four independent variables considered, at two different levels, were agitation (50 - 150 rpm), temperature (30 - 50 ºC), pH (4 - 6) and biosorbent dose (0.01 - 0.175 g). The results indicate that a curvature of the responses was not detected. The not-significant value of lack of fit showed that the linear model was valid. The linear model with four variables and their interaction for prediction the maximum point was established. The regression was significant and the adjusted linear coefficient of determination was very good (= 0.9617). The main effects of pH and biomass were the most significant for copper biosorption from aqueous solution. It was substantiated by the fact that the effect of interaction between pH and biomass was very significant; it is followed by the effect of agitation. The best conditions for copper biosorption (69%) for the present study were pH 6, biosorbent dose 0.01 g, agitation 50 rpm and temperature 50 ºC.

2534 EFFECT OF TEMPERATURE ON THE ANAEROBIC DEGRADATION OF PHENOL AND THE MICROBIAL COMMUNITY

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The residue produced during anaerobic digestion of organic waste is rich in nutrient and can be used as fertiliser. However, one concern is the content of organic pollutants, as these may influence the soil fertility negatively and should therefore only occur at low levels. In this study, the effect of the process temperature on the anaerobic degradation of different phenolic compounds was investigated. Phenols have been shown to have a negative impact on soil microbial activity and can appear in anaerobic bioreactors both as components of the in-going substrate, and as intermediates during degradation of different complex aromatic compounds. As the degree of phenol degradation strongly depends on the microbial degrading capacity, another objective was to investigate the structure of microbial communities in different digestion processes.
The impact of temperature was investigated with two laboratory-scale bioreactors operating at 37 ºC and 55 ºC. These reactors have treated the same type of organic municipal household waste for many years. The degradation of phenols (*o-, p-, m-*cresol and phenol) was investigated by analysis of the digestion residue and by addition of phenols to small-scale batch cultures (118 ml). The batch cultures were incubated at 20 - 55 ºC and 37 - 60 ºC with inoculums from the mesophilic and the thermophilic bioreactor, respectively. The effect of temperature on the microbial populations in the bioreactors was investigated by phylogenetic analysis with 16S rRNA specific primers for archaea and bacteria.
The capacity to degrade phenols in the two laboratory-scale bioreactors, analysed in batch cultures, was found to be less efficient at 55 ºC as compared to the lower temperature. This result was also confirmed by chemical analysis of the residue, demonstrating a comparably higher content of phenols in the bioreactor operating at thermophilic temperature. Although the biological degradation generally is faster at the higher temperature, a lowering in temperature below 49 ºC in the thermophilic system triggered the degradation of phenol. The difference in degradation may be due to different microbial communities being active at the different temperatures. Both the community structure and the diversity differed at the two process temperatures, with a significant lower diversity at the higher temperature.

2537 CONTINUOUS BIODEGRADATION OF SULFANILIC ACID IN A MULTI-STAGE PACKED BED REACTOR

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Sulfanilic acid SA is an aromatic amine derived from the azo-bond cleavage of several textile dyes. Recalcitrance and toxicity of this amine is high, and with frequency it could be found as an aquatic contaminant; thus, biodegradation process for its removal are justified. Although the SA molecule contains carbon, nitrogen and sulphur, its C:N:S proportion is unbalanced for microbial growth in a biodegradation process; thus nutrient complementation should be necessary for its complete removal.
The main purpose of this work was to use sulfanilic acid as carbon and nitrogen source or as carbon, nitrogen and sulphur source by a microbial community isolated from textile industries discharges. For the continuous SA biodegradation process, two mineral salts media were formulated. Medium A contained, in g L^{-1}, SA, 0.05; K_2HPO_4, 0.20; $MgSO_4$, 0.1; $CaCl_2$, 0.02. In medium B, $MgSO_4$ was substituted by $MgCl_2$, 0.1 mg L^{-1}.
The SA biodegradation process was carried out in a laboratory scale multi-stage reactor,. Each stage was packed with small river stones, and separated by a sintered glass plate. For both culture media, liquid flow rates were varied to obtain hydraulic retention times HRT ranging from 3.5 to 23 h. Residual amine at each stage was spectrophotometrically (248 nm) and chromatographically quantified (HPLC). Residual COD was also determined. For HRTs varying from 6 to 23 h, the overall SA removal rates in both culture media rounded 100%, determined by HPLC, and about 90%, when determined by COD. The lesser COD removal could be explained by a slight accumulation of degradation byproducts observed by liquid chromatography. At lower HRTs, the SA removal efficiencies, determined by HPLC and COD, diminished for both media, although they were slightly higher in medium B, with values of 86 and 82%, respectively. SA removal efficiencies at intermediary stages were obviously inferior to that obtained at the last stage. Two of the five microbial strains constituting the microbial community have been identified 16S rDNA amplification as: *Methylobacterium radiotolerans* (AB17564.1) and *Dyella japónica* (AB110498).
Finally, it can be concluded that this microbial community is able to use sulfanilic acid as the sole carbon, nitrogen and sulphur source.

2542 AZO-DYES MIXTURE DEGRADATION IN A FIXED BED BIOFILM REACTOR PACKED WITH VOLCANIC POROUS ROCK

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Textile industries discharge great amounts of dyes and dyeing-process auxiliaries, which pollute streams and water bodies. Several dyes, especially the ones containing the azo group, can cause harmful effects to different organisms including humans. Through bacterial and mammalian tests, azo dyes or their derived aromatic amines have shown cell genotoxicity. The purpose of this work was to evaluate the effect of air flow rate on azo-dyes mixture biodegradation by a microbial community immobilized in a packed bed reactor. The azo dyes, acid orange 7 and acid red 88 were quantified by high performance liquid chromatography (HPLC). The same technique was used for determination of the aromatic amines formed by the azo bond cleavage. As a measure of mineralization, COD removal was also evaluated. The bioreactor, with an operation volume of 0.51 liters, was packed with volcanic porous rocks and the mixture was fed at a constant dilution rate of 0.019 h^{-1} with a mineral salt medium containing 50 mg of each dye per liter. The bacterial community was immobilized on the support by flooding the packed bed reactor with an inoculum of the microbial community. Air flow rates ranging from 0.016 to 3.5 VVM were tested. It was observed that the highest azo-dye degradation and COD removal efficiencies; > 90% and > 80%, respectively, were reached at low air flow rates. One of the aromatic amines, sulfanilic acid, was almost totally removed. Instead, 4-aminonaphtalenesulphonic acid showed some accumulation. This fact could be explained by the relative recalcitrance differences of both azo-derivatives, related to differences in their structural complexity.

2604 DEGRADATION OF A MONOSULFONATED AZO DYE BY AN INTEGRATED BIOSORPTION AND ANAEROBIC SYSTEM

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A simulated textile effluent containing a monosulphonated azo dye was fed to an anaerobic bioreactor in which a natural adsorbent, spent brewery grains (SBG), was incorporated. SBG is a by-product of the brewing industry and could act as adsorbent as well as an electron shuttle (lignin fraction) in the dye degradation mechanism. Furthermore, it can also work as a conditioner for the anaerobic biomass. The influence of the dye (Acid Orange 7, AO7) concentration (60 and 150 mg/L) and the presence of SBG in the performance of upflow anaerobic sludge blanket reactors (UASB) was evaluated. Mesophilic reactors were operated at a hydraulic retention time (HRT) of 14 h and 7 h. Results indicate for anaerobic reactors with SBG a decrease on COD (chemical oxygen demand) removal with the fed dye concentration (from 93% for 0 mg AO7/L to 54% for 150 mg AO7/L). However, the percentage of colour removal was in average of 98 ± 3%, higher that those found in conventional anaerobic bioreactors (93 ± 3% for 60 mg AO7/L). This could be due to an improvement on dye adsorption along with an increase of the azo bond reduction rate, possibly related with the presence of SBG. Ultraviolet-visible spectra and HPLC chromatograms of the dye and its anaerobic metabolites display marked alterations in both systems, with and without SBG. A nearly total dye reduction occurs, since the main characteristic peak of the dye is not observed in the outlet samples, which is in agreement with the high colour removal values attained. Aromatic amines generated by dye anaerobic reduction, sulfanilic acid (SA) and 1-amino 2-naphtol (1A2N) are still present in the discharge of the conventional anaerobic reactor. Nevertheless, HPLC chromatograms of samples gathered from the system with SBG did not display the presence of 1A2N, suggesting that this aromatic amine is bioeliminated. As sulphanilic acid could be degraded under aerobic conditions, a complete dye removal is then possible. Beside an improvement of colour removal, biogas production was higher in the bioreactor with SBG, which could also be useful for the feasibility of this type of process.

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2606 BIOLOGICAL ANALYSIS OF ENDOCRINE DISRUPTING COMPOUNDS IN TUNISIAN SEWAGE TREATMENT PLANTS

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The endocrine disrupting compounds (EDCs) are frequently found in sewage treatment plant (STPs) works. Natural and synthetic hormones have been identified as the major contributors to the estrogenic activity in sewage. Dosing and identification of EDCs are certainly of great interest and can lead to the improvement of chemicals treatments. With reporter cell lines developed in the laboratory and allowing the detection of nuclear receptor activities, we characterized the endocrine disrupting profile of water, particulate matter and sludge from three Tunisian sewage treatment plants (STPs). These reporter cell lines enabled us to detect in these samples ligands of the estrogens, androgen, dioxin and pregnant X receptors. STPs water samples exhibited estrogenic and androgenic activities. Particulate matter and sediment samples showed estrogenic activity but also aryl hydrocarbon (AhR) and and pregnant X (PXR) receptor activities. No (ant) agonistic activity of glucocorticoid, mineral corticoid and progesterone receptors was detected in the samples indicating that environmental compounds have a limited spectrum of activities. By performing competition experiments with recombinant ER, we demonstrated that the estrogenic activity present in water mainly contained compounds of strong affinity while compounds present in sludge showed a weaker affinity for ER. 8h and 16 hours reporter cell line exposure allowed us to state that the compounds present in STPs particulate matter and sludge and able to activate dioxin receptor are more readily degraded than chlorinated compounds and are probably polycyclic aromatic hydrocarbons (PAHs).

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2611 EFFECT OF TEMPERATURE INCREMENTS IN SEPTIC TANK EFFICIENCY

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Septic tanks are the main sewage disposal system used in Yucatán, Mexico. Septic tank content is stabilized under anaerobic conditions and is considered the temperature has a significant effect on the efficiency. This work was developed in order asses the feasibility to improve communal septic tanks efficiency by increasing content temperature. Temperatures inside the tank were increased using a hybrid heater system (solar and electricity). An experiment was carried out in a sewage treatment plant using a 2.8 m^3 septic tank and a hybrid heater system (solar and electricity) to increase temperature inside the tank. Three different temperature values were tested (30, 35 and 40 ºC) and their effect on chemical oxygen demand (total and dissolved), suspended solids, organic nitrogen and ammonia Nitrogen in septic tank effluents. The hybrid heater system helped to maintain the temperature inside the septic tank contents efficiently. The septic tank effluent characteristics for the parameters tested demonstrated that there was not a significant difference among the results for the three different temperatures. However dissolved COD showed a slightly reduction with temperature increments.

2686 STUDY OF THE SLUDGE SEDIMENTATION DYNAMICS BY MEANS OF AN OPTICAL SYSTEM

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Nowadays, the problem of water contamination causes the optimization of the processes carried out in wastewater treatment plants to improve the pollutants removal efficiency. In this work, an optical system was implemented to measure the sedimentation speed of sludge in wastewaters, this parameter is very important for designing mechanisms that deposit the sludge in the sediment lower levels of treatment plants. The results obtained with the optical system proposed agree with those obtained using graduate test tube techniques, and provide higher sedimentation speed accuracy.
A technique was carried out to measure the speed of sedimentation of sludge with 4 lasers and from a graph of voltage against time, and 3 graphs of intensity against time, from this information could be known in which time the main sludge level goes through the lasers, later this information was used to elaborate a graph of distance against time, which would provide the speed of sedimentation finally.
In order to try to obtain equivalence between the results obtained with the conventional method of test-tube and the optical system, a graph was made of speed of sedimentation of the graduated test-tube against speed of sedimentation of the optical system. As we can observe, the correlation between both systems is good so that a factor of R^2 is had = 0.8444, is to say, that the 84,44% of the points are on the straight line or closely of her. The curve that better adjusts to these points that show an increasing tendency, is a straight line, whose equation is a y = 0.9431x.
With base in the results found in this work, it is possible to be concluded that is feasible to obtain the speed of wastewater sludge sedimentation by means of an optical system, due to the small differences between the values of the conventional and optical method.

2882 EFFECT OF DISCONTINUING SUCROSE IN THE FEED ON THE PERFORMANCE OF A BIOREACTOR TREATING A MIXTURE OF 2,4,6-TRICHLOROPHENOL AND PHENOL

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Previous recent work of our research group has demonstrated the excellent performance of a partially-aerated methanogenic (PAM) fluidized bed bioreactor (FBBR) for the removal of a mixture of 2,4,6-triclorophenol (TCP) and phenol (Phe) when continuously supplemented with 1000 mg COD-sucrose/L. However, concern has arised regarding bioreactor performance without supplementation of a degradable carbon source (co-substrate sucrose). Thus, the objective of our work was to evaluate the effect of discontinuing the supplementation of sucrose to the reactor influent on the performance of a PAM FBBR. A lab scale was loaded with biocatalysts (a bed of bioparticles, each bioparticle had a core of granular activated carbon colonized by an undefined microbial consortium with anaerobic, facultative and aerobic microorganisms) and aerated with air at a rate 2vvm and operated a 1-day hydraulic retention time, 35 ºC, with continuous effluent recirculation to expand the bed of bioparticles. Two operational stages were distinguished: in the first stage the influent contained a mixture of 120 / 30 / 1000 mg/L of TCP / Phe / COD sucrose; in the second stage only the xenobiotic concentrations were the same 120/30 TCP/Phe whereas sucrose addition was discontinued. Bioreactor performance was evaluated in terms of removal efficiencies of TCP (η_{TCP}), Phe (η_{Phe}) and COD (η_{COD}), concentrations of chlorinated intermediates, as well as other response variables. Removal efficiencies of both stages were very high and close, η_{TCP}: 99.9 and 99.9%; η_{Phe}: 99.9 and 99.9%; η_{COD} = 96.46 and 97.48% for stage 1 and stage 2, respectively. Low traces of 2,4 di-chlorophenol (0.05 mg/L) and 4-chlorophenol (0.07 - 0.26 mg/L) were found during the first 5 days of operation of the second stage, probably due to the adaptation to the lack of cosubstrate sucrose. Moreover, GC-MS analysis confirmed the absence of chlorinated organic intermediates typcial of aerobic degradation pathways of TCP. Net increase of chloride anion in effluent ranged between 59.5 - 61.5 mg Cl^-/L; these values were very close to maximum theoretical concentration of 62.8 mg Cl^-/L. Moreover, chlorinated organics adsorbed onto bed bioparticles of the reactor and volatilized with biogas were found negligible compared to the mass inflow of TCP. We could not find evidence of methane in biogas, and specific methanogenic activity of bioparticles was below the detection level of the assay, although PCR-DGGE analysis of biomass showed bands of methanogenic archaea. Aerobic specific respiration rate of stage 2 was slightly superior to that in stage. Concerning xenobiotics removal and their presence in treated effluent, our results indicate that bioreactor performance was not negatively affected by discontinuing the addition of co-substrate sucrose. Performance of the PAM-FBBR in this work compared favourably with performance of other bioreactors of single environment (either methanogenic or aerobic) treating TCP or other chlorophenols reported in the international literature. Our results indicate that the application of PAM-FBBR to the treatment of groundwaters polluted with chlorophenols and characterized by the lack of degradable co-substrates, is a promising alternative for on site bioremediation.

2904 FLUIDIZED BED BIOREACTORS COUPLED TO ZERO-VALENT IRON FILTERS FOR REMOVAL OF HIGH CONCENTRATIONS OF PERCHLOROETHYLENE

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The aim of this work was to evaluate the effect of coupling continuous bioreactors with zero-valent iron filters on removal of PCE. Two types of reactors with simultaneous electron acceptors were used: partially aerated methanogenic (PAM) and methanogenic-denitrifying (MD). Lab-scale fluidized-bed reactors (FBBR) were operated as follows: PAM at λ = 135 g COD/g O_2 and M-D at λ = 9 g COD/g $N\text{-}NO^{3-}$ with 80 mg/L of PCE in the influent. It is worth highlighting that this PCE concentration is very near the saturation concentration of PCE at 35 ºC (90 mg/L). Two periods of operation were run, namely Period 1 and Period 2, where the only difference consisted of the coupling of bioreactors to zero-valent iron filters fitted in the recirculation line. Both reactors were fed with 1 g COD methanol/L as carbon source in the two periods. Bioreactors were operated at 1-day hydraulic retention time and 35 ºC. Each reactor was fitted with an activated carbon trap in the biogas exit line in order to capture and determine volatilized CACs. Average performances of both bioreactors were very close, and no significant differences were found between periods. PCE removal was high and similar in both reactors (99.58 and 99.69 for PAM and M-D respectively) and both Periods. COD removal averaged between periods was slightly higher in M-D (92.3%) than in PAM (89.9%). Trichloethylene (TCE) concentration in effluents was low in both reactors in both Periods and concentrations of dichloroethylene (DCE) and vinyl chloride (VC) in influent were below levels of detection of the method. Traces of etylene were found in biogas. Concentrations of PCE, TCE, DCE and VC were periodically determined in bed bioparticles as well as in the activated carbon of a biogas trap and were found to be very low; abiotic removal of PCE and intermediates was negligible compared to the overall removal, strongly suggesting biological degradation as the main driving force in our FBBRs. It can be concluded that there were no significant differences between biochemical performances of the two systems between periods. In fact, removal efficiencies of PCE without ZVI were already so high that there was little room for improvement by the addition of the ZVI filters. Fitting ZVI filters to our simultaneous electron acceptor bioreactors should be conceived more as a safety factor for dealing with negative transients caused PCE surges or hydraulic rate shocks rather than an improvement of their background performance.

2909 BIOLOGICAL TREATMENT SYSTEMS APPLIED TO THE DEGRADATION OF CHLORINATED ORGANIC COMPOUNDS: REVIEW OF ADVANCES AND PERSPECTIVES

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Biological treatment of municipal and industrial wastewater it is a field well developed, very well known technologies, and of great application for control of water contamination. Biological treatment of wastewater has been carried out mainly by sole aerobic and anaerobic treatment. Nevertheless, the chlorinated organics compounds usually are recalcitrant to the degradation in biorreactores with a single acceptor of electrons: O_2 in aerobic, CO_2 in methanogenic, NO^{3-} in denitrifying systems, and $SO_4^{=}$ in sulfate-reducing systems. In the last 15 years a particular approach to biological wastewater treatment has attracted considerable attention: bioreactors possessing several electron acceptors in the same vessel (simultaneous electron acceptors, SEA). The objective of this work is to review the state of the art on the biological treatment of chlorinated compounds with dominant environmental (aerobic, anaerobic, combinative systems in series) and SEA bioreactors. Aerobic bioreactors are efficient for the removal of clorinated compounds with low and intermediate levels of chlorination (for example Dichlorophenols and Chlorophenols), whereas studies reporting the application of these systems to higher substituted chlorine organic substances are scarce and discouraging. Anaerobic biological treatment has been widely used for removal of chlorinated xenobiotics and highly persistent pollutants. Particularly, for chlorophenols, they mainly emphasize the use of bioreactors of immobilized biomass, such as the Unflow Anaerobic Sludge Bioreactors (UASB), anaerobic filters (packed beds) and more recently anaerobic fluidized bed bioreactors (FBBR). Anaerobic treatment of chlorinated compounds such as chlorophenols, has been shown to be very efficient for the dechlorination and degradation of halogenated organics with high degree of chlorine substitution in the aromatic ring. Yet, very often there exists accumulation of compounds with lower levels of chlorination. This becomes a serious disadvantage as far as the fulfillment of discharge regulations and treatment cost. Methanogenic-Aerobic Systems (MAS), where simultaneous aerobic and anaerobic metabolisms coexist in the bioreactor, can be easily accomplished in immobilized biomass bioreactors. In effect, microbial aggregates distribution like bioparticles in FBBR, allows for oxygen cosnumption by facultative bacteria at the outer layers of the biofilm, whereas strict anaerobic bacteria and methanogenic archaea can grow under anaerobic conditions in the inner layers of biofilm. Several works have shown the tolerance of methanogenic consortia to O_2. MAS has several advantages over sole anaerobic or aerobic treatment systems, e.g. (i) it can have both aerobic and anaerobic degradative pathways, (ii) slow biomass growth like in anaerobic reactors may be achived with related savings in sludge disposal, (iii) since we have two metabolisms in the same vessel, operational costs are reduced compared to 2-stage series biological processes. Without any doubt, the evolution of methanogenic reactors to SEA bioreactors, is a consequence of the success and wide acceptance of the anaerobic digestion as a wastewater treatment option. Finally, MAS is a promising alternative for the efficient removal of toxic and persistent substances in wastewater and polluted groundwater, particularly for the class of xenobióticos that cannot be degraded, or they are only degraded of incomplete form in biorreactores with one electron acceptor.

1866 STUDY ON THE COLLECTION AND DISPOSAL OF HOSPITAL SOLID WASTES IN KARAJ CITY (IRAN)

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Hospitals and other health care institutions generate waste day in and day out which may be a potential health hazard to the health care workers, the general public and, the flora and fauna of that area. Safe and effective management of hospital waste is not only a legal necessity but also a social responsibility. Many of hospitals in Iran neither have a satisfactory waste disposal system nor a waste management and disposal policy. The main objective of this research is study on the collection and disposal of hospital waste in Karaj city (Iran). This study was presented in four major hospitals of Karaj city in Iran, namely, Kasra, Alborz, Imam and Ghaem. Results showed that in most hospitals about 2.5 to 3 kg of waste was generated per bed per day. In the all of hospitals, infectious wastes were less than 15% to 20% of the wastes and were collected separately, also the collection box were disinfected after anytime of discharge. There was one incinerator in Alborz hospital and one autoclave in Imam hospital for waste sterilization and there was not any treatment activities for sterilization of wastes in two others hospitals. The most hospitals in this study are not efficient in waste management when compared to those in developed countries. Improvement of waste management in the hospitals is urgently needed both for combating occupational health hazards as well as to safeguard the environment.

2196 BIODEGRATION STUDIES OF OIL SLUDGE CONTAINING HIGH HYDROCARBONS CONCENTRATION

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Oil industry has a significant impact on environment due to the emission of, dust, gases, waste water and solids generated during oil production all the way to basic petrochemical product manufacturing stages. The aim of this work was to evaluate the biodegradation of sludge containing high hydrocarbon concentration originated by a petroleum facility.
A sludge sampling was done at the oil residuals pool (ORP) on a gas processing center. This work comprises physicochemical and microbiological characterization of the sludge by determining: total hydrocarbons (TPHs), metals, sulfur, nitrogen, carbon, phosphorous, heterotrophic and hydrocarbon degradation microorganisms. Biodegradation assays were performed at a microcosmos level based on a Taguchi L_9 experimental design involving 4 variables and 3 levels: moisture, nitrogen source, surfactant concentration and oxidizing agent. pH, CO_2 production and TPH concentration were assessed.
TPHs concentration in sludge was 280,021.4 ± 6797 mg kg^{-1} dry mass (dm). Most TPHs reported a carbon content ranging from 24 to 35 C, manifesting that heavy hydrocarbons are mainly present. In addition, high concentration of Fe and S were detected (5500 mg kg^{-1}·d.m. and 4800 mg kg^{-1} d.m., respectively), thus increasing system complexity. Both heterotrophic microorganisms (7 x 105 UFC/g·d.m.) and hydrocarbonoclastic bacteria (7 x 104 UFC/g·d.m.) were also determined. Statistical analysis of results showed that the variables with major influence on CO_2 production and TPHs biodegradation were the nitrogen source and moisture. The maximum TPHs removal was 50,879 mg kg^{-1}·d.m.

2231 EVOLUTION OF THE STABILITY PARAMETERS COMPOSTING TWO-PHASE OLIVE MILL WASTE WITH GRAPE MARC AND VINE BRANCHES

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Modern olive-oil extraction technology generates a large amount of two-phase olive mill waste (TPOMW) in Mediterranean countries, with composting being a viable alternative to the traditional disposal of these residues. Vine branches and grape marc also constitute abundant organic residues in these countries. In this study, TPOMW was composted with vine branches and grape marc as bulking agents for use as organic amendment. Two composting piles of about 5000 kg each were prepared by mixing fresh TPOMW and vine branches and TPOMW and grape marc using the windrow pile system. Temperature, moisture, organic matter, total N, pH, electrical conductivity, and phenols were monitored throughout the composting process. The resulting composts were rich in organic matter and free of phytotoxicity, and had high potassium and inorganic and organic nitrogen contents, but medium phosphorus content. At the end of composting, the NH_4^+-N/NO_3^--N ratio was less than 0.16, indicating a good degree of maturity. Maturity was also confirmed by a considerable increase in germination indices during the composting process due to decomposition of the phytotoxic organic compounds. The resulting composts had a high agronomic value, and can be used as organic fertilizers in ecological agriculture.

2235 HAZARDOUS FLY ASH: A DISCUSSION ON THE SPECIATION AND TREATMENT OF HEAVY METALS

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Fly ash is a fine grained material, with high specific surface, considered hazardous by European legislation due to its heavy metal content. However, the hazardousness of a fly ash is directly dependent on the fuel that is combusted. Normally coal fly ash is considered non-hazardous, while biomass (e.g. wood and straw) fly ash has specific regulation for its reuse in agricultural fields (Danish BEK39, 2002). Heavy metals are found in many fine grained materials in problematic concentrations. When it comes to municipal solid waste (MSW) fly ash, the hazardousness is far more accentuated. The total concentrations of heavy metals are higher in MSW fly ash. A complete analysis of these fly ashes is carried out, based on a both experimental and literature survey.
There are different studies approaching fly ash for its reuse. Some studies try to remediate them while others try solidification/stabilization (S/S) techniques. Both approaches are normally based on total heavy metal concentrations rather than on its leachability. A discussion on speciation is then necessary. The complexation of heavy metals in the fly ash matrix is a rather complex issue. It may be bond deeply into glassy particles or as soluble salts, which are easily detached and prompt to leach. A deep characterization based on the crystalline and amorphous components of fly ash is discussed, in order to evaluate the heavy metals tendency to leach.
The removal of heavy metals from fly ashes through the electrodialytic process (EDR) has been tried out. The goal has always been to remove the total amount of the heavy metal fraction. This approach has not been realistic, once fly ash is continuously forced to dissolve and solubilize heavy metals that were attached in a more strongly bond in the matrix. This work tries to evaluate this approach and establish a new one in the remediation of a highly complex and heavy metal concentrated material such as fly ash.
In parallel, S/S techniques have been widely used to retain heavy metals inside a complex and highly alkaline matrix. This is the case of concrete. The use of coal fly ash into concrete manufacture is not a novelty. However, other types of fly ash have been tested and studied for the same effect. This study will debate on the integration and complexation of heavy metal containing fly ash and its reflection on mortar characteristics.
The overall goal of such study is to debate two different approaches to heavy metal pollution regarding a hazardous material. Remediation technologies and S/S techniques are not necessarily antagonists' concepts, yet they can be complementary in the heavy metal pollution management.

2269 FUNGAL TREATMENT: A PROSPECTIVE PROCESS FOR ECO-FRIENDLY BIOREMEDIATION OF WASTEWATER SLUDGE

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None of the conventional techniques is safe and environmental friendly for wastewater/sludge disposal. A sustainable, safe and environmental friendly biological technique is a great apprehension to the relevant scientists. Since the fungal treatment was exercised to evaluate its potentiality for sustainable bioseparation and bioremediation of wastewater. Bioseparation and bioremediation of wastewater sludge by fungal inoculation implied the decreasing of biosolids, total suspended solids (TSS), turbidity, chemical oxygen demand (COD) and specific resistance to filtration (SRF) of wastewater. Prior grown fungal (*Mucor hiemalis* Wehmer) broth was used for raw sludge treatment in first study. Dry biosolids (DBS), TSS, turbidity, COD and SRF were evaluated against bioseparation and dewaterability of treated sludge. All parameters were decreased significantly ($P \leq 0.01$) over period of fungal treatments. Maximum decreasing/removal of suspended solids was noticed in decant fungal biomass [DFB, Grown in ME (Malt extract)] and fungal biomass (FB in ME broth) at 3-day but the closest results were observed thereafter in all treatments. The SRF measures the dewaterability was decreased maximum (0.26×10^{-12} mg/kg) equivalent to 95.5% decreased at 2-day in fungal biomass [FB in WF (wheat flour) broth] treatment. The removal (%) of TSS, Turbidity, COD and SRF were observed 96.0, 99.4, 92.6 and 97.6, respectively in supernatant of treated sludge at 5-day by FB in WF treatment. In second study fungal entrapped biosolids from prior study was recycled as inoculum for further bioseparation of supplemented raw sludge. Impressive results were also achieved in bioseparation and dewaterability of treated wastewater sludge. Fungal entrapped biosolids offered 98% removal of TSS at 6-day without nutrient supply. Consequently, 99 and 87% removal of turbidity and COD, respectively were achieved in supernatant of treated sludge. The lowest (1.75×10^{12} m/kg) of SRF (equivalent to 70% removal) was observed at 6-day after treatment. All results except SRF were not influenced further after WF supplementation. The present treatments offered significant ($P \leq 0.01$) improvement in all results except SRF of treated wastewater sludge. The present technique is addressing a potential avenue of probable solution for safe and environmental friendly management and disposal of wastewater sludge for future.

2292 RABBIT MANURE UNDER COMPOSTING AND VERMICOMPOSTING PROCESS: A QUEMOMETRIC QUALITY EVALUATION

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Agricultural waste such as manures can be composted and/or vermicomposted in order to obtain amendments containing mature and stable organic matter. Compost stability refers to rate of organic matter decomposition expressed as a function of microbial activity, and maturity generally refers to the degree of decomposition of phytotoxic organic substances produced during the active composting stage (Castaldi, et al., 2008; Emino and Warman, 2004). Many researches hava been conducted on the variables and conditions defining the composting process and numerous measures have been suggested for definition of compost maturity and stability (Zmora-Naum et al., 2007). Few attempsts have been made to find and define those properties, wich can be useful for composts of varying source (Tang et al., 2003; Zmora-Nahum et al., 2005). Campitelli and Ceppi 2008 have proposed a statistical methodology for classification organic amendment from different source.
The main objective of this research was to determine using, quemometrics techniques, which is the quality category of an organic amendment obtained with rabbit manure as a raw material with different stability and maturity degree.
The quality category in a process of composting-vermicomposting of rabbit manure was determined in three different phases: CE1: at the beginning of the process (first 15 days); CE2: after 75 days and VE: after 210 days of the beginning.
The following parameters was measured at each phase of the process: pH, Total Nitrogen (TN), Total Organic Carbon (TOC), Water-soluble carbon (WSC), Gemination index (GI), TOC/TN, and WSC/TN ratios were calculated according the techniques described by Campitelli and Ceppi (2008).
The multivariate technique was carried out using Discriminate Linear Analysis (DLA). The parameters measured were included in the data matrix obtained for Campitelli and Ceppi 2008. The quality categories were: A (high-quality), B (intermediate-quality) and C (deficient-quality). The materials CE2 and VE were assigned to B category, because in the process reach an adequate pH values and have a high maturity degree. The material CE1 was included in C category, due to their low stability degree and the inadequate high pH values that would be unsafe for agricultural purpose.
The materials obtained through a composting process would be classified in different quality categories using LDA.

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2348 EVOLUTION OF DAIRY MANURE IN A DUNGPIT WITH REGARD TO SOLID-LIQUID SEPARATION AND METHANE PRODUCTION DISTRIBUTION

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The recent growth of intensive dairy farming implies the industrialization of dairy farms, improving production efficiencies. However, the rise in the number of cows has led to large quantities of dairy manure, which can not be properly managed by farmers, giving place to environmental damages. There is also a concern regarding green house gases (GHG) emissions. Livestock industry is the largest source of green house gases methane (CH_4) and nitrous oxide (N_2O) in the primary sector in Europe. Anaerobic conversion of dairy manure into biogas is an attractive way of managing these kinds of wastes, providing benefits such as renewable energy, environmental protection and nutrients recovery. A renewed interest in this technology has emerged since new trends to limit carbon dioxide and other GHG emissions. The final objective of the work being developed is to obtain a liquid fraction whose COD was mainly composed of biodegradable matter so that it can be anaerobically digested with lower HRT and subsequently be easily subjected to post-treatments in order to obtain a final effluent that could be reutilised or discharged according to environmental regulations. The objective of the present work was to determine the transformations in the dairy manure regarding the distribution of the components and methane production of liquid and solid fractions. Raw dairy manure (RM) was taken from the cow house of a dairy farm and then stored in closed flasks with gas release system devices with a pressure of 5 cm column water and introduced in a thermostatic bath at 20 ºC. Each 15 days a sample was withdrawn and was separated into a solid fraction (SF) and a liquid fraction (LF) to analyse their compositions. Separation was accomplished by means of mechanical procedures, screening and centrifugation. Samples corresponding to days 0,45 and 90 (RM, LF and SF) were anaerobically digested at 35 ºC. LF corresponding to day 45 was the one with the highest mass percentage, 77%. COD_{total} of the LF augmented moderately as storing time increased, up to 50 g COD/L, whereas $COD_{soluble}$ reached 20 g COD/L. Volatile fatty acids (VFA) concentration increased until day 45, 31 g CODVFA/L; from then on it started to decrease. N-NH_4^+ and NKT concentrations grew up to 2.5 y 4.4 g N/L, respectively. Regarding methanogenic productivity, on day 0 LF and SF contributed equally to methane production (50% each fraction). On day 45 LF produced 65% of the total methane production whereas SF produced 35%. Methane production of RM was the same at days 0 and 45 of storage, 17.5 L CH_4 NCTP/kg RM. The conclusion of the present work is that RM can be stored in a controlled way with no losses in methane production enhancing the transference of biodegradable COD to the LF.

2350 ANAEROBIC DIGESTION OF SOLID SLAUGHTERHOUSE WASTE CHEMICALLY PRETREATED

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One of the mayor problems facing the industrialized world today is to solve environmental contamination and identify efficient treatments to give solution to the current problems like the generation of enormous quantities of liquid and solid wastes. The solid slaughterhouse waste, due to its elevated concentration of biodegradable organics, can be efficiently treated by anaerobic digestion although the high content of lignocellulosic materials, makes it a slowly process. With the purpose of accelerate the hydrolysis step and increases the soluble fraction, it was decided to work with chemically pretreated wastes. the alkaline pretreatment consisted in the addition of NaOH in concentrations ranging from 0.1 to 0.8 g per g of VSS and the solubilization rate was calculated as follows: the ratio between the soluble chemical oxygen demand (sCOD) measured after pretreatment and the total chemical oxygen demand (tCOD) measured after pretreatment (CODs/CODt).
A comparative between anaerobic digestion on mesophilic and psicrophilic phase of pretreated waste was made. The first set of experiments was performed with two laboratory scale digesters, R1 and R2, each having a working volume of 6 L, R1 operated at room temperature and R2, operated at controlled temperature of 35 ± 2 ºC. The hydraulic retention time (HRT) was kept constant during 30 days, resulting in a solid load of 1.6 g TS/ L-d and an organic load of 2.16 g TCOD/L-d.
The reactors were inoculated with 1000 ml of anaerobic seed sludge collected from an UASB reactor with specific methanogenic activity of 1.32 g COD/g VSS-d. Feed and effluent samples were analyzed for pH, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total and partial alkalinity, volatile fatty acids (VFA), tCOD, sCOD, and ammonia nitrogen (NH_3). The analyses were performed per standard procedures (APHA, 1995). The biogas volume generated was measured by volumetric displacement in an acidified water column to avoid the CO_2 solublilization. Firstly, the solubilization percentage after pretreatment with NaOH doses was evaluated. Results have been found to be better the 0.6 g NaOH/ g VSS dose with a 48% solubilization.
Of the comparative among the R1 and R2 reactors, TS and VS reductions were 32 and 34% for R1 while 40 and 46% was found for R2. sCOD reductions were 32 and 57% respectively. The biogas volume measurement after 30 days were 24 and 30 L from R1 and R2 respectively, obtaining yields of 12.9 y 16.56 L per kg of waste added for R1 and R2. From these results, it's concluded that: 1) The waste pretreatment is an efficiently option to accelerate the hydrolysis step as well as increase the waste soluble fraction 2) Anaerobic digestion process in mesophilic phase is more efficient that psicrophilic phase since the biogas production and COD reductions are higher, and 3) Anaerobic digestion with pretreated wastes in mesophilic phase fulfills with the actual Mexican Standards for sludge and biosolids.

2359 KINETICS OF FORCED AERATED BIODEGRADATION OF DIGESTED SEWAGE SLUDGE-REED MIXTURES AT DIFFERENT TEMPERATURES

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This work presents a kinetic analysis of the aerobic biodegradation of mixtures of anaerobically digested sewage sludge and dried reed, at different temperatures. The batch experiments were carried out in laboratory scale reactors with T control and forced aeration of the solid mixture. The biowaste mixture was treated during 90 days at four different temperatures: 25º, 40º, 50º and 60 ºC, maintaining moisture and taking weekly samples for C and VS measurements. Two different kinetic models were used to fit the C mineralization curves: the 2C model that considers two organic fractions (biodegradable and inert), and the 3C model that considers three fractions (easily biodegradable, slowly biodegradable and inert). In both cases, the kinetic rate constants were calculated by mathematical fitting and compared with previously reported similar studies. Also, the temperature influence on the rate constants values was studied for both models by using a T dependent equation. Kinetic rate constants obtained agreed with previous published research, and good fitting to the experimental data was obtained with both models. Similar values of the rate constants were obtained for mineralization of the biodegradable fraction (2C model) and the easily biodegradable fraction (3C model). The rate constants for the slowly biodegradable fraction (3C model) were quite lower. A good correlation between rate constants and T was observed. Different optimum temperature values were obtained for every rate constants, depending of the different carbon fraction under degradation considered. The T dependent rate constant values obtained could be used for modelling the C mineralization of real variable T composting processes.

2363 ACID FERMENTATION OF MUNICIPAL SLUDGE: THE EFFECT OF SLUDGE TYPE AND ORIGIN ON THE PRODUCTION AND COMPOSITION OF VOLATILE FATTY ACIDS

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Due to the more stringent legislations controlling discharges of wastewater treatment plants (WWTPs) and existing problems such as high sludge production, new wastewater treatment processes resulting in considerably reduced sludge production and more effective treatment would be of great value. In this study, the feasibility of implementing acid fermentation process on different types of municipal sludge to increase soluble chemical oxygen demand (SCOD), especially short-chain volatile fatty acids (VFAs) was investigated by batch and semi-continuous experiments. The municipal sludge originate from five major treatment plants located in Denmark were used. Analytical procedures were conducted according to standard methods. COD (total, soluble) were determined by the dichromate reflux method, NH_4^+-N and NO_3^--N were determined by automated phenate method, and PO_4^{3-} – P was measured by ascorbic acid reduction method. Fermentation of primary sludge produced the highest amount of VFAs and generated significantly higher COD- and VFA-yields compared to the other sludge types regardless of which WWTP the sludge originated from. Fermentation of activated and primary sludge resulted in 1.9 – 5.6 % and 8.1 – 12.6 % COD-yields in batch experiments, respectively. The COD-yields for primary, activated and mixed sludge were 19.1, 6.5 and 21.37 %, respectively, in semi-continuous experiments operated at SRT of 5 d and temperature of 37 ºC. The results revealed that even though the VFA production of primary sludge was higher compared to activated sludge, still significant amounts of VFA can be produced by fermentation of activated sludge due to the significantly higher production of activated sludge than primary sludge in WWTPs. At the same time, the biological hydrolysis and acidification of activated sludge in municipal WWTPs instead of primary sludge also reserves primary sludge for production of biogas and thereby create additional economical gain for municipal WWTPs.

2364 EVALUATION OF BIOSOLIDS QUALITY GENERATED BY THERMOPHILIC ANAEROBIC DIGESTION

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In Mexico has been considerate that wasted activated sludge, by their own or acquired characteristics after an stabilization process, may be improvement susceptible whereas the permissible limits maxim not exceed the Mexican Official Norm for established contaminates (NOM-04-SEMARNAT-2002) or by hand, their disposal in definitive form as not wasted dangerous, pollution effect attenuated for environmental and protection for the all people.
Mesophilic anaerobic digestion (AD) is a biological process with more utilization by wasted activated sludge stabilization. AD give adequate characteristics of disposal in land to the biosolids and become the elimination of bad odor associated with them, it has been an important reason of heir used generalized. However, mesophilic AD has been replace by thermophilic AD due to permissible limits strict of pathogens, beside thermophilic AD has demonstrated perform with the pathogens norm, maintenance advantage of the mesophilic AD.
The main objective this paper was evaluated the biosolids quality obtained by thermophilic anaerobic digestion, in function on the pathogens microorganism concentration; volatiles suspend solids, vectors attractions and heavy metals.
The evaluation of the biosolids quality was realized with NOM-04-SEMARNAT-2002 by sludge and biosolids. Thermophilic anaerobic digestion was became in reactor CSTR during 150 days, using a temperature of 55 °C with constant stir at 300 rpm, hydraulic retention time of 10 days and feed with wasted activated sludge (WAS) with a concentration mean 3% of solids. The volatile suspend solids removal efficiency in the reactor was upper at 38% somewhere about 25 days of operation, reaching a maximum value of 60% at the end operation.
Waste activated sludge shown a mean concentration of 1.6×10^{6} NMP/g ST of fecal coliforms and the concentration for biosolids was 7.8×10^{5} NMP/ g ST. Heavy metals concentration for the waste activated sludge and biosolids samples was below of the permissible limits maxim. Biosolids obtained by thermophilic anaerobic digestion may be classified as excellent class due to heavy metals and B class due to fecals coliforms concentration, therefore this biosolids can be used for their recycling in urban uses without direct public contact during their application, forestry uses as improvement of the soil.

2389 SPECTROFLUORIMETRIC DETECTION OF GLUTATHIONE AND THIOL-CONTAINING COMPOUNDS IN VARIOUS ENVIRONMENTAL MATRICES

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Low-molecular-weight hydrophilic thiols (cysteine, glutathione -GSH-, thioglycolic acid, 3-mercaptopropionic acid,...) have received much attention owing to their biochemical and environmental importances. These compounds serve as a key step in the formation of high-molecular-weight organic matter fractions, as strong ligands for transition metals and are thus involved in mobilization of metals in vegetal (phytoremediation through phytochelatins synthesis, for instance), aquatic or terrestrial environments through complexation reactions. Current available methods for detection of these compounds use liquid-chromatography, including precolum derivatization protocoles, with a spectrophotometric (fluorescence or ultraviolet) detection.
Aims of this study is thus to by-pass the chromatographic step by a semi-direct spectrofluorimetric determination of derivatized thiol-based compounds in order to propose a rapid and less time-consuming method of determination of these analytes in various environmental samples. The analytical protocole developped is based on a specific derivatization of the thiol group with a fluorescent tagging reagent [o-phtaldialdehyde (OPA)] in presence of an amine, followed by a direct spectrofluorometric measurement of the corresponding isoindole fluorescent derivative; isoindole formation reaction is improved by use of a buffered solvent and fluorescent intensity of the derivative enhanced by a mixed solvent (water-methanol) or use of a surfactant.
Quantification of various thiol compouds (glutathione, cysteine, 3-mercaptoethanol, 3-mercaptopropionic acid, N-acetylcysteine, thioglyclic acid, ...) is carried out on synthetic and real samples issued from sewage sludges, soils and composts. Sum of total thiol compounds is expressed as GSH-equivalent. Optimization of the procedure includes pH values, nature and amine concentration, reaction duration, reaction solvent, fluorimetric detection conditions.
First results are obtained with 2-aminoethanol as amine reagent and a mixed solvent (H_2O-CH_3OH, 50/50 v/v) at pH 9 (in a sodium borate buffer). Detection of isoindole adducts are carrried out at λ_{ex}=335 nm and λ_{em}=450 nm. This rapid and direct determination of thiol compounds in environmental matrices seems very promising, according to our first results obtained on real samples.

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2426 USED BATTERY COLLECTION CAMPAING IN CENTRAL MEXICO: STATISTICS AND METAL CONTENT REPORT, ADVANCES IN RECYCLING TECHNOLOGY AND LEGISLATIVE/REGULATORY SITUATION ANALYSIS

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Nowadays, environmental pollution produced by the disposal of used cells and batteries is a major concern in Mexico. The regulatory law proposal (NMX-AA-104-SCFI-2006) establish a content limit 20, 7.5 and 5 fold higher in Hg, Cd and Pb, respectively, than the European directive 2006/66/CE. Furthermore, transnational companies refused to have participation on the collection/recycling process, putting forward that pollution comes only from illegal market batteries. In this work, we present a report of a collecting program in the state of Tlaxcala, followed by a study of the metal composition on used batteries from legal and illegal market, and the progress in the development of recycling technology in our group. A six month collecting campaign battery survey was statistical analyzed. 77% of the total corresponds to “AA” type batteries; 3 out of 10 are illegal, and the second more frequent trademark is also illegal. Measurements showed that 36% of the batteries had a residual voltage between 1.2 y 1.4 V, while 70% had more than 1.0 V. The 5 legal and 4 illegal more frequent trademarks were chosen to metal recovery experiments and content analysis. Ten items from each one were opened and the internal mass was reduced to dust. Extraction of the alkali, zinc and manganese were done by washes of cold distilled water, 0.7% H_2SO_4, and roasted with $(NH_4)_2SO_4$, respectively. Additionally, MnO_2 was obtained by electrolysis. From the extraction solutions the metal content was determined by ICP-OES technique. The analysis showed that the alkali lixiviate, pH between 6.60 - 13.25, had only traces of Zn and Mn; while the acid lixiviate and the roasted procedure recovered more than 90% of the total Zn and Mn, respectively. The electrolysis was made using a Zn cathode and Pt wire anode. The MnO_2 was obtained as a brown solid deposited on the bottom of the flask. Electrodeposition of Zn was also achieved by the use of H_2O_2 and a graphite anode. The analysis of Hg, Cd and Pb showed that there’s no significant difference in content between legal an illegal commercialized batteries. Furthermore, all of them obey the permissible limit levels of the Mexican legislation, and should be classified as no dangerous residues (can be thrown to the domestic rubbish), while 8 out of 9 of the selected battery trademarks should be rejected under the European directive. Finally, the necessity for legislative and regulatory updates for the Mexican normativity is stressed in order to minimize the impact on human health and the environment of this type of residues.

2439 ANAEROBIC WAS CO-DIGESTION WITH OMW AND GLYCEROL

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The main by-product of any biological wastewater treatment is waste activated sludge (WAS). Anaerobic digestion is the most common treatment technique for sludge stabilization, resulting in a reduction in the amount of volatile solids (VS) with biogas production, at the same time. On the other hand, many agro-industrial organic wastes are readily biodegradable and as a result, anaerobic co-digestion of sludge with agro-industrial wastes is being developed with potential advantages such as increased biogas production and improved nutrience balance.
In this study, the case of Olive Mill Waste (OMW) and glycerol, a by-product during biodiesel production, have been examined. More specifically, sludge mixtures were treated anaerobically in several retention times (SRTs).The laboratory experiments were carried out in a cascade of two anaerobic digesters, 40l and 65l respectively, operated at 38 ºC.
Two experimental cycles were carried out. At the first one, the co-digestion of sludge mixture (contained 30% OMW and 70% WAS) was carried out at sludge retention times (SRT) of 12.3, 14, 16.4 and 19.7 days. For these cases, volumetric methane production rate ranged between 0.35 and 0.55 L_{CH4}/Lrd following the prospective increase, as the SRT was decreased. Moreover, COD removal efficiency increased with SRT augmentation and ranged between 41 and 72%. Volatile solids reduction remained almost the same, nearly 30%. Moreover, as the SRT increased, the increase in NH4-N concentration that was observed was lower and estimated at 360%, 320%, 260% and 112% for SRT 12.3, 14, 16.4 and 19.7 days respectively.
In the second trial of experiments, anaerobic digestion of sludge mixtures (contained 20% glycerol that was ten times diluted and 80% WAS) was carried out, in the same retention times as in the first cycle. Particularly, volumetric methane production rate was 0.92, 0.81, 0.68 and 0.55 L_{CH4}/L_rd for SRT of 12.3, 14, 16.4 and 19.7 days respectively. In terms of the reduction of volatile solids (VS), the values were quite constant, nearly 30-35% and almost independent from SRT. The COD removal efficiency achieved high values and ranged from 88 to 94%. Nevertheless, the increase in NH_4-N production was constant near 200%.
A comparison between the two substrates shows a great ascendancy of glycerol mixture as it improves significantly the biogas production rate (30% higher) and the COD removal efficiency.

2449 HEAVY METAL SEQUESTRATION BY HUMIC SUBSTANCES DURING PHYTO-TREATMENT OF SEWAGE SLUDGES

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The presence of heavy metals in sludges stabilized in a reed bed system, may affect their use for agricultural purposes; however, the environmental impact of sludges depends on the availability and phytotoxicity of their heavy metal.
The aim of this paper was to determine the effectiveness of a reed bed (*Phragmites australis*) sludge treatment system in two urban wastewater treatment plants in Italy after two-year period of operation: by estimating the process of sludge stabilization, following conventional and non conventional parameters related with the evolution of organic matter quality (Water Soluble Carbon, Dehydrogenase activity, Fulvic Acids, Humic Acids, Pyrolytic indices of organic matter Mineralization and Humification); by following the heavy metal speciation bioavailability in sludges.
The Community Bureau of Reference (BCR) method for heavy metal speciation was followed.
The results showed that mineralization and stabilization of sludge over time occurred as shown by the decrease of dissolved organic carbon and re-synthesis of humic-like matter. The results also showed that the content of heavy metals was associated to the least mobile fractions of sludges.

2462 A LABORATORY EXPERIMENT ON VERMICOMPOSTING OF WINERY RESIDUES AND SEWAGE SLUGGE

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Organic waste addition to agricultural soils is proposed as a disposal strategy to improve the structural properties and organic matter content of soils. In this work, the results obtained after a vermicomposting process are reported. The process has been performed mixing rabitt crop wastes with increasing addition of either vinasse biosolids or municipal sewage sludges. For this purpose, a laboratory experiment was conducted in which both wastes were inoculated with earthworms (*Eisenia foetida*) and maintained under controlled conditions for 4 months. In our experimental condition, better results were obtained in the treatment with sewage sludges treatments than in that with vinasse biosolids.
The final vermicomposts products had higher humification rate, nutrients content and pH, lower conductivity and were not phytotoxic, fulfilling most of them the Spanish guidelines for compost.
Therefore, these vermicomposts can be used as organic amendments in field soils. This method constitutes a very effective ecotechnology for obtaining mature organic amendments. This study opens the possibility for the biotransformation of organic residues through vermicomposting processes. The transformation degree is evaluated following the growth of the inoculated earthworms and the chemical changes experimented by the residues.

2475 NONDURABLE MANAGEMENT OF HOUSEHOLD WASTE IN BATNA (ALGERIA) AND PROSPECTS FOR A SUSTAINABLE DEVELOPMENT

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The concept of sustainable development is based on the principles of use responsible for the resources of planet and of environmental protection. The question which one does put, how to save our natural resources before is too late? The management of waste implies a total control of the life cycle of the products, since their production until their elimination. The methods evaluation of life cycle (ECV) and of management of life cycle (GCV) seemed useful instruments to contribute to choose a management of solid household waste. This method requiring of the data which were collected starting from the investigations into grounds and of the statics recorded by the direction of the environment. In Algeria, the life cycle of solid household waste is very short, which generally passes by the production of waste, the regrouping in a site and the treatment which will be done is in wild discharges where an important mass of our natural resources is burned, or in center technical hiding, which does not fulfil the requirements of a controlled discharge. The town of Batna, knows a management system requiring of the important modifications, since the production of waste until elimination and that from the point of view of a sustainable development, while starting to distribute the flow of waste identified between recycling, composting, the recuperation of the energy and the medical hiding and by examining principal materials in the flow of waste (paper, glass, …etc).

2538 SOLAR DRYING IN GREENHOUSE OF MIXTURE OF OLIVE MILL WASTEWATER AND OLIVE CAKE IN MOROCCO

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Morocco is a country which produces olive oil extensively and this industry within the country is currently under huge expansion. This particular industry, which is usually realized with triphasic processes using the technique of pressing, generates tons of wastes: olive mill wastewater (OMWW)(liquid waste) and olive cake (solid waste). The OMWW which is being dumped in the environment results in serious environmental pollution while olive cake do have a proper disposal. To switch from the traditional process (three phases) to advanced technology for olive oil extraction (two phases) for Morocco is not practical due to problems related to agricultural, industrial as well as on socio-economic accounts. Hence, it is an essential need to devise a technology that could address this issue resulting in an efficient process for waste disposal and thus enhancing the olive industry in Morocco.
In the present work, a new integrated process is described to eliminate the OMWW produced by a triphasic system. This process consists in using olive cake as a sponge to absorb all the OMWW produced and then to dry the mixture in a greenhouse to get a final moisture of 10% (weight/weight). The dried mixture could be valued in the same way as the original olive cake.
The ratio of OMWW/olive cake (86 litres/100 kg) produced in this type of Moroccan mills is such that the amount of OMWW exceeds the retention capacity of olive cake (62 litres of OMWW/100 kg of olive cakes). In these conditions, the olive cakes produced by a mill are not able to absorb all the OMWW produced by the same mill. Realizing this problem, drying has been done in 2 steps. The first step was to humidify the olive cake with OMWW up to 60% (initial moisture of olive cake was 35% at exit of the press) and apply a drying process to reduce this moisture to 32%. The second step of drying was to re-humidify the mixture to 50% moisture then drying to 10%. The saturation of olive cake with OMWW at 60% moisture allowed to accelerate the first drying and the fact to limit this first drying to a final moisture of 30% allowed to incorporate much more easily the rest of OMWW in olive cake for the second drying step.
The heat gain in the greenhouse compared to a system of drying in open sun has been determined. The kinetics of drying was recorded. The effect of a forced convection was also tested in order to accelerate the process. Other parameters that influence the drying process need to be studied and optimized. Finally, a characterization of the dried mixture also need to be performed to predict its future utilization and determine whether it might be valued in the ways existing for crude olive cake. The above dried material will be used as substrate in Solid State Fermentation for culturing filamentous fungi and edible mushrooms.

2560 BIOGASIFICATION OF INDUSTRIAL BIOWASTE AND SEWAGE SLUDGE – MANAGEMENT OF BIOGAS QUALITY

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Biogasification, i.e. anaerobic digestion, is a well known sustainable option for the management of organic solid wastes and sludges. The produced biogas is a valuable biofuel to replace fossil fuels in different technical applications (like heating, electricity, transport fuel generation) which in turn determine its quality requirements. In this study, the composition of biogas was extensively analyzed from two different points of view: biogas utilization and the biogasification process control point of view. The aim of the present study was, first, to find out the effects of different process conditions on the concentration of harmful trace impurities in biogas and, secondly, to detect the gas compounds indicating process changes and upsets. To achieve this, concentration of siloxanes, sulfur compounds (like H_2S, DMS), ammonia, nitrous oxide (N_2O) and some VOC compounds in biogas were determined in transient well controlled biogasification reactor conditions. In first test run, industrial biowaste and sewage sludge were codigested in a pilot reactor (200 liters) for 4 months by stepwise increasing the organic load up to a high value of 10 kgVS/m^{3}d (Fig. 1). The physico-chemical state of the process (pH, T, alkalinity, VFA, ammonia, biogas production and its CH_4-content) was carefully monitored throughout the test period, and the gas composition, as well as abundance and population structure of methanogenic archaea were determined with molecular DNA-techniques (real-time PCR, DGGE and sequence analysis) at the loadincreasing points (increased stepwise from 2 to 10 kgVS/m^{3}d). The results showed that the process respond to load increase was very stable: the physico-chemical state remained constant and the increase in total production of biogas followed closely the increase in loading until 8 kgVS/ m^{3}d was reached (Fig. 2). The gaseous concentrations of the main sulphur compounds, siloxanes, as well as most of the VOC compounds, except ethanol, remained quite constant, showing no sensitivity to process changes. The formation of ethanol was however found to accelerate at the load-increasing points. Furthermore, a significant increase in microbiological N_2O formation was occasionally found due to some oxygen penetration in the reactor. In our paper and poster, the effects of the changing process conditions on the measured gas concentrations in biogas are presented in more detail.

Figure 1. Loading and total biogas production of the studied co-digestion process.

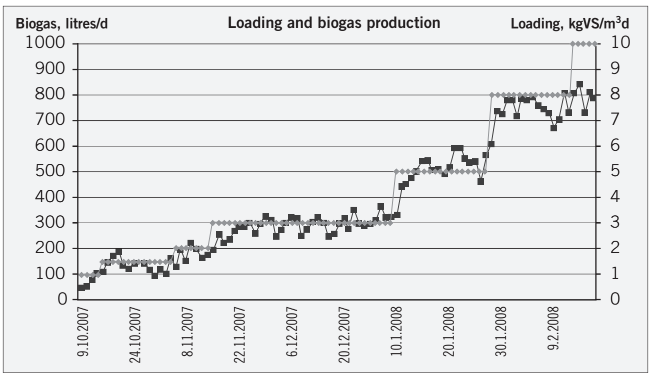
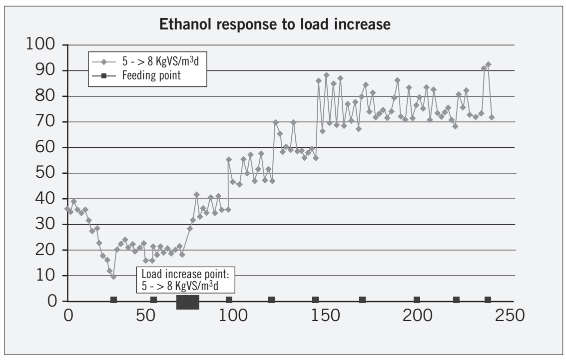


Figure 2. An example of the response of ethanol concentration inbiogas to loading increase



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2623 THE FATE OF HELMINTH EGGS IN SEWAGE TREATMENT PLANT SLUDGE ON COMPOSTING

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The sludge generated by sewage treatment plants is rich in minerals (N and P) making it an attractive fertiliser. However, it can contain organic and metallic micropollutants susceptible to cause toxicity in the soil-plant system. Recently, the works of Amir et al. (2005) and Jouraiphy et al (2007) have shown that micropollutants occur within the range of levels considered safe, opening perspectives for the recycling of the sludge in agriculture. In this light, a composting trial was run in a biodigester, with the sludge mixed with green waste. To ensure the maturity of the final product, the changes occurring in the physical, chemical and biological parameters were monitored. The study of the sanitary standards of the final product obtained included a study of the viability of *Ascaris* eggs during composing. After two months of composting, the final product presented a C/N ratio of 9.3 with 33.5% decomposition and enabled 80% germination confirming its maturity. Likewise, the number of *Ascaris* eggs was below the standards fixed by the WHO.

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2177 EFFECTS OF THERMICALLY-DRY SEWAGE SLUDGE AND MUNICIPAL WASTE COMPOST AMENDMENT ON MICROBIAL BIOMASS, DEHYDROGENASE ACTIVITY AND CO_2 FLUXES IN A DEGRADED AGRICULTURAL SOIL

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Understanding the dynamic of soil C is a key to managing soil organic matter to enhance soil fertility and ecosystem functioning, and reduce trace gas emission from soils. Our objective was to determine the influence of thermically-dry sewage sludge (TSL) and municipal waste compost and the application management on soil (mixed or on soil surface) applied at two rates of 30 t ha^{-1} and 60 t ha^{-1}, on CO_2 fluxes, microbial biomass C (MBC) and dehydrogenase activity (DH), during an incubation study. The experiment was conducted with a degraded agricultural soil (Calcic Luvisol) from the experimental farm "La Higueruela" (Santa Olalla, Toledo). During the course of the experiment samples were taken periodically during four months for monitoring the mentioned soil microbiological parameters. Temperature (28 ºC) and soil moisture (70%) were controlled and there were not lixiviation losses. Organic amendments, particularly TSL, provoked a significant increase of CO_2 fluxes, MBC and DH activity caused by a global revitalization of soil microbial metabolism. TSL on soil surface treatment showed a slightly larger increase in CO_2 fluxes, MBC and DH activity than that with the TSL mixed with soil. The results obtained during the experiment are in accordance with the hypothesis of energy optimization as ecosystems develop: values for CO_2 respiration, MBC and DH were higher during the first weeks, due to the response of the soil biota to the fresh input of organic C sources for microbial growing, especially in TSL that is a raw organic material with no stabilization treatment, followed by a decrease in the total microbiological activities as a consequence of a significant reduction of metabolic rate of soil's microorganisms, feasibly attributed to the reduction of energy sources in the system.

2190 AGRONOMICAL USE OF SEWAGE SLUDGE FROM URBAN WASTE WATER TREATMENT PLANT

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The Urban Waste Water Treatment Directive (91/271/EEC) aims to encourage the use of sludge whenever appropriate; the sewage sludge is a product of wastewater treatment which could find a reutilisation in the agricultural field. The necessity to find a solution to the sewage and biomass disposal has lead to the development of techniques based on natural reuse of agricultural soils. In this way it represents, through a closed loop system, a source of all the nutritional elements which have been remove by the agriculture. The sewage sludge application on soils represents an alternative to the traditional stock strategies; more convenient, from the economical point of view, than incineration or disposal in landfill, it also contributes to solve the sewage sludge recycling issue. The reclaim of these biomasses for fertilization could establish more benefits because it allows exploiting the fertilizer proprieties of the material, representing a strong potentiality of nutritive elements as nitrogen, phosphorus, potassium, calcium and magnesium and even more of organic matter. However, the presence of toxic elements, in particular heavy metals as zinc, copper, nickel, cadmium, lead, chrome and mercury, with high concentrations, makes their management tangled. The aim of this project is to grow up rice and maize under experimental conditions, in order to evaluate the nutrients contribution of the sewage sludge on the final production, while observing the connection between application of sewage sludge and residual heavy metals concentration in soils, leachate and in different plant parts. The project is structured over a three years time, in order to evaluate the temporal change of the environment conditions. The experimental sites is compostted by 9 manure thesis, different in doses of mineral nitrogen (urea) and the nature of sewage sludge add to the soils, from zero to two different concentration (5 – 10 ton/ha) crossed one each other to compare the significant fertilizer properties in terms of production. At the same time, the nitrogen concentration in its main forms (ammonium, nitrate and total), the organic matter (carbon), phosphorus, potassium and heavy metals concentration (lead, zinc, copper, cadmium, chrome, mercury) are studied in the following matrixes:

- soils
- leachate
- plants (grain, shaft and leaf…)

Thus, a correlation between the different manure thesis and the diffusion of elements from soil to the plant and their uptake were found. The analysis are now being performed thanks to ICP technology, FIAstar™ 5000 Analyzer Principle: Flow Injection Analysis (FIA) following the proceeding provided for the Italian law in the field of the chemical –physical analysis. This work reports the results of a two year growth. In this session of the project, we have evaluated that the sewage sludge is a valuable source of nitrogen, completed with the mineral one, enable to obtain good results (up till 15 ton of dry matter for hectare) in terms of production both for rice and maize. Through a statistic analysis of the results, we could conclude that the sewage dose has a positive effect on the plant growth even more that the urea. The critical point in their use is the accumulation of heavy metals, which were not found in dangerous concentrations inside the plants; while it was observed that zinc significantly accumulates with higher level in the plants treated with higher sewage doses.

2209 WASTEWATER RECLAIM FOR GOLF GRASS IRRIGATION

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In order to preserve the conventional available water resources and diminish the withdrawals of ground water, there is a need of alternative water supplies. The reuse of the treated wastewater to fill part of the agriculture needs in water consumption will contribute to these objectives.
The total surface of the open spaces of the Agadir city (public, private, golf courses) is estimated to be 878 ha with a need of water for irrigation reaching 8096000 m^3/year. The golf courses alone occupy an area of 268 ha (30.5% of the total Agadir open spaces area), with a water consumption of 3216000 m^3/year.The present potential of the wastewater treatment plant M'zar of Agadir, available for irrigation purpose without any restrictions (category A of WHO norms), is of about 10 000 m^3/day and will reach 50 000 m^3/day by the year 2010. This production capacity of treated wastewaters will fulfill the whole water needs for irrigation of the entire open spaces of Agadir.
This study concerns the feasibility of the reuse of the treated wastewaters, issued by the wastewater plant M'zar of Agadir, for golf grass irrigation purpose. It presents the planning, the protocol and the preliminary results of the tests that are being performed. Full scale tests are being carried out in the wastewater plant site on parcels of 25 m^2 using different variety of grass seed. The preliminary results presented in this paper, related to the monitoring during the first 21 days, show that the use of treated wastewater for irrigation of golf grass does not affect seed germination, has positive effect on the grass growth: tillering and evolution of leave length, in comparison to the uses of conventional water (ground water).

Keywords: Wastewater reuse, irrigation, golf grass, Agador - Marocco.

2222 EFFECTS OF LONG-TERM APPLICATION OF MUNICIPAL SOLID WASTE COMPOST ON SPECIATION AND AVAILABILITY OF HEAVY METALS IN SOIL

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The application of municipal solid waste compost in agriculture provides a valuable source of plant nutrients and soil fertility. Nevertheless, heavy metals accumulation may be a problem. A seven-year field study was carried out to investigate the effects of farmyard manure (40 and 120 t/ha) and municipal solid waste compost (40, 80 and 120 t/ha) application on the total content, speciation and availability of heavy metals in a calcareous Tunisian soil without vegetation.
Experimental results showed that the application of farmyard manure had no effects on the distribution and availability of heavy metals in soil. The municipal solid waste compost incorporation had no effects on the concentration of Ni and Cr in soil. However, a considerable increase of Cd, Cu, Pb and Zn was observed in the top-soil (0-20 cm) with the added rates. The acid extractable fraction (exchangeable and carbonate bound fraction) of all metals increase slightly in municipal solid waste compost treated plots. Remarkable increases in reducible fraction of Cd, Pb and Zn and in oxidisable fraction of Cu were noted in amended plots. The residual fraction showed a high percentage of Cu, Zn, Ni and Cr.
The availability of heavy metals in the soil was evaluated using EDTA solution. The results showed that the application of municipal solid waste compost contribute to a notable increase of available Cd, Cu, Pb and Zn and to a slightly increase of available Ni and Cr compared to the control soil.

Keywords: Municipal solid water compost, sequential extraction, availability, heavy metal, soil.

2228 IMPACT OF REPEATED TWO-PHASE OLIVE MILL WASTE APPLICATION ON PHOSPHORUS FRACTIONATION IN A DEGRADED OLIVE GROVE SOIL

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Loss of organic matter is one of the main forms of soil degradation in Mediterranean agricultural soils, and external sources of organic matter are required to improve soil properties. The two-phase centrifugation system in the olive-oil extraction industry produces a large amount of olive mill waste sludge (TPOMW) which can be used to add organic C to degraded soils. In order to investigate the impact of TPOMW soil amendments on P fractionation, experiments were conducted on soil samples collected from a field study on a degraded olive grove soil amended over five years with three levels of TPOMW (0, 60, and 120 Mg ha^{-1}, annually). A sequential extraction technique based on the Hedley fractionation scheme was used to evaluate the change in phosphorus forms in the amended soils.
All the P fractions were significantly raised ($P < 0.05$) by TPOMW amendment in the order: HCl-P > residual-P > $NaHCO_3$-IP > NaOH-IP > NaOH-OP > soluble-P > $NaHCO_3$-OP. The relative concentrations of H_2O-P, $NaHCO_3$-IP, NaOH-IP, and HCl-P increased, whereas $NaHCO_3$-OP, NaOH-OP, and residual-P (this fraction of P at the lowest rate of amendment) decreased, after TPOMW amendment. The proportion of soluble P in the TPOMW-amended surface soils is large, so that P loss by runoff would occur readily in these amended soil.

2252 MICROBIAL INDICATORS OF FECAL CONTAMINATION IN SOILS UNDER DIFFERENT WASTEWATER IRRIGATION PATTERNS

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The use of wastewater to irrigate produce was a common practice in some suburban areas in México. The continuous use of wastewater can increase the chance of fecal soil contamination, which can percolate in soil and finally cause groundwater contamination. A suburban area in Chihuahua, Mexico, has been traditionally irrigated with wastewater for production of agriculture goods, including produce and animal foodstuffs. The objective of this study was to determine microbial contaminants that serve as indicators of fecal contamination in different soils due to irrigation practices in a suburban area of Chihuahua, Mexico. Four soil types were evaluated; a soil with a history of irrigation with wastewater and still irrigated (S1), a soil with irrigation, but no history of using wastewater (until 2003) (S2); a soil with no irrigation history at all (S3) and a soil close to S1 and close to the river where the wastewater is transported (S11). Three soil depths were evaluated: 0-15, 15-30 and 30-50 cm. A total of 150 soil samples were analyzed for presence of fecal coliform and *Salmonella*, using the MPN technique. Out of the 150 soil samples, two were positive for the presence of *Salmonella*, one within the S1 area, and one within the S2 area, both at a 30-50 cm depth (3-4 MPN/g soil). For fecal coliforms, one sample in S2 and one in S3 were positive, but the higher number of positive samples were present in areas S1 (4 sites) and S11 (3 points). In most of the sites, contamination was located at higher depths (30-50 cm); the highest value was of 93 MPN/g soil. According to the results, fecal contamination is higher in those areas that have been in contact with wastewater for long time; therefore, irrigation with wastewater can cause soil and groundwater contamination, causing severe problems in aquifer health, as well as contamination of crops grown in the area.

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2265 HEAVY METAL RISK ASSESSEMENT IN THE USE OF URBAN WASTES FOR THE RESTORATION OF A FOREST SOIL AFFECTED BY FIRE

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Reforestation measurements after burning of forests areas are the best management practices to avoid soil erosion and for a quick recover of the vegetation cover destroyed by fire. The use of organic amendments could increase the viability and vitality of introduced plantlets and to restore soil biological activity. In this work, compost of municipal solid wastes (CMSW) was introduced with tree seedlings of *Pinus pinea* in April 2005 in a burnt forest area of *P. nigra*. Two doses of CSMW, 1.5 and 3 kg/plant, and two forms of addition, mixed with the soil (M) and in the bottom of the hole (B), and a blank without organic amendment, were introduced as experimental factors in a complete block design with five replicate plots of fifteen trees each. Five soil samples at two depths (S, 0-20 cm and P, 20-40 cm) and also a composite sample of needles were taken in each plot in the spring 2007 and analysed in terms of total and organic carbon, total nitrogen and *pseudo-total* heavy metal contents after microwave assisted digestion in concentrated nitric acid. *Pseudo-total* contents of heavy metals in soil samples, only displayed statistically significant differences between the form of treatment, mainly at the highest dose of application, for Zn and Cu at both soil depths and for Pb in the upper soil samples. In contrast, total contents of Fe, Cd, Ni and Pb in the needles displayed significant differences in treated plots with respect to the blank, mainly at the highest dose applied on the bottom of the hole. Besides the application of organic residues could increase the contents of certain heavy metals in soil, an important phytostabilization could be achieved by mean of the permanent vegetation cover.

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2322 EVALUATION OF PLANT AVAILABLE NITROGEN IN CONCENTRATED PIG SLURRY

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In Northeast Spain the expansion of the pig industry has brought as a result the production of vast amounts of pig slurry that exceeds field crops fertilization needs and consequently has contributed to the environmental deterioration of the region particularly ground water with NO_3^- pollution. Under such circumstances, it is needed to treat and/or export pig slurry. During the last years the implantation of cogeneration plants that take advantage of the surplus of energy to produce concentrate pig slurry by water evaporation that could easily transported. Although this product is already used, little is known about its possibilities as fertilizer. In order to evaluate the nitrogen fertilizer value of concentrated pig slurry an experiment was carried under laboratory conditions. The soil was pre incubated for three weeks at 30 ºC and at 55% water-filled pore space. Treatments consisted of a control (C) (the reference substrate obtained blending 1 part of soil to 1 part sand), a mineral fertilizer (MF) applied at 82.31 mg kg^{-1} of $(NH_4)_2$ HPO_4 and 24.95 mg kg^{-1} of $(NH_2)_2CO$ (equivalent to 217 kg ha^{-1} y 67 kg ha^{-1} respectively) and two doses of concentrated pig slurry, 769 mg kg^{-1} and 2310 mg kg^{-1} (CPS2 and CPS6, corresponding to 2000 kg and 6000 kg ha^{-1} in that order) with tree replicates. The plant available nitrogen (PAN) of each treatment was determined by means of the standard incubation leaching technique of Stanford and Smith (1972) using 250 ml leaching jars. For monitoring microbial respiration additional samples were kept in 103 mL closed airtight recipients, and CO_2 released was captured with NaOH 0.5 N. After the first week almost all mineral nitrogen applied (31.50 mg N kg^{-1}) to MF was lixiviated, in spite of this, total nitrogen net mineralization was significantly higher than in control plots. During the first 8 weeks the net mineralization rate of CPS6 was lower than that of CPS2, however CPS6 released more CO_2 than CPS2; therefore the increased microbial activity of CPS6 enhanced microbial nitrogen immobilization. As long as NO_3^- pollution is concerned, it seems that concentrated pig slurry could be more environmentally friendly than synthetic nitrogen fertilizers.

2331 EFFECT OF PULP MILL SLUDGE ON SOIL CHARACTERISTICS, MICROBIAL DIVERSITY AND VEGETAL PRODUCTION OF *Lollium perene*

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The Chemical properties of the sludge (high organic matter content, pH, buffer capacity, nitrogen and phosphorous level, and low concentration of trace heavy metals and organic pollutants) suggest that this material may represent a valuable resource as soil amendment, improving soil fertility. The aim of this study was to evaluate the effect of pulp mill sludge addition to volcanic soil (Andisol) on soil characteristics, microbial diversity and vegetal production of *Lollium perenne*, in field assays. Field assays were conducted in an Andisol soil (profile 0-20 cm) belonging to the Temuco Serie, located in Southern Chile (38º 42' S, 73º 35' W). Each plot was of 5 x 2 m with three replicates (totalizing 12 plots). Stabilized sludge (one year old) of pulp mill wastewater was added up a soil (0, 10, 20 and 30 ton/ha), incorporating it in the first upper 10 cm. The soil was incubated for 1 month before put de seeds. *Lolium perenne* L. cv quartet seeds were applied in each plot in a quantity equivalent to 25 kg/ha. The assay was maintained for a period of 105 days, and at days 45, 75 and 105 the plants were cut to evaluate the vegetal production (biomass). At the end of the experiment, the physico-chemical characteristics of the soil were determined. Also, the effect of sludge addition on soil microbial diversity using molecular techniques (DGGE) was evaluated. The sludge addition improved physico chemical properties of the soil. The biomass obtained in each cut increased with the increment of sludge addition. The biomass obtained of *Lollium perenne*, at the end of the experiment, was 60% more respect to the control soil (without sludge addition) when 30 ton/ha sludge was added to the soil. Organic matter content increased in the soil with increasing amounts of the pulp sludge, obtaining 35% more organic matter content with the application of 30 ton/ha of sludge than the control soil. The analysis of the microbial diversity showed that the application of the sludge does not modify the microbial community of fungi and bacteria even in the high doses applied.

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2375 COMPARISON BETWEEN SECONDARY AND TERTIARY TREATMENT OF WASTE WATERS EFFLUENTS AND THEIR DEVELOPMENTS IN IRRIGATION APPLICATIONS

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In Tunisia, the reuse of the treated waste water effluent for agricultural production, constitute a new source of irrigation water. In fact, it allows promoting the use of these waters and the fertilizing matters that they contain. This application also to safeguard the resources in water that are limited, especially in the arid and semi arid regions.
Two parcels were constructed during this study and respectively irrigated by secondary and tertiary treated water.
The tertiary treatment process by infiltration–percolation showed an effectiveness to eliminate biodegradable pollution, with a reduction that surpasses 65%. The retention of the Kjeldhal and ammoniacal nitrogen by the massive soil is remarkable and are respectively 93% and 72%. The sanitary quality of the secondary effluent and percolate was also measured. The reductions of IBFC (Indicated bacteria of fecal contamination) are respectively 2.1 Ulog and 2.5 Ulog. Statistical analyses of phosphorus content revealed the existence of a significant difference between the fruits (tomatoes, potato…) irrigated by the third treated waters (infiltration percolation) and the fruits irrigated by secondary waters. The following impact of irrigation by waste waters on the bacteriological quality of the irrigated cultures showed that these cultures were influenced by the source of water used. The reuse of waste waters, after a sufficient supplementary treatment, is therefore efficient in non restrictive irrigation.

Keywords: Irrigation, wastewater, supplementary tratment. Infiltration percolation.

2411 APPLICATION OF PIG SLURRY TO SOILS. EFFECT OF AMMONIA STRIPPING OVER NITROGEN AND TOC LIXIVIATION

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The pig slurry is usually employed as fertilizer for agricultural use. Nevertheless this farmer waste contains high levels of nitrogen which can lixiviate and contaminate water, especially once the ammonia has been transformed into nitrates. The mobility and transformation of nitrogen and organic matter from pig slurry after soil application is a subject of great interest from an environmental point of view.
In this work, two experiments were carried out applying different pig slurries (crude and treated by stripping with air at pH 9) to the top of a soil column of 1 m length and 25 cm diameter containing approx. 100 kg of soil. After application of each pig slurriy to the soil, the concentration values of ammonia, nitrites, nitrates and Total Organic Content (TOC) in the column effluent were monitored as a function of the time.
In essence, no ammonia was detected in the effluent for both types of slurry. As it could be expected, the total amount of lixiviated nitrate was higher in the experiment using crude pig slurry. Also, the nitrate concentration in the effluent was more homogenous over time after applying the stripping treated pig slurry. However, TOC concentration in the column effluent was higher in the experiment using treated pig slurry, the organic content from crude slurry being more retained in the soil column. Although this treatment reduced the risk of contamination of water by nitrates, the possible effect of increasing the organic matter concentration should be considered in field scenarios.
The breakthrough curves were modelled using the Hydrus 1D code. The adsorption of ammonia during transport in soil was an important process. Nitrite and nitrate adsorption was lower than ammonia adsorption, but could not be neglected. Nitrification chain was modelled using two sequential reactions ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$). The kinetics coefficients accounting for transformation from nitrite to nitrate were larger than those accounting for ammonium to nitrite, the transformation from ammonia to nitrite controlling the kinetics of the nitrification process. TOC adsorption was higher in crude pig slurry, which can be attributed to the organic matter solubilitation after the stripping treatment process.

2479 FOLIAR APPLICATION EFFECTS OF BEET VINASSE ON RICE YIELD AND CHEMICAL COMPOSITION

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This study presents an account of rice (*Oriza sativa* cv. Puntal) yield quality parameters as influenced by the foliar application of an industrial byproduct (beet vinasse). Beet (*Beta vulgaris* L. subsp. *vurgaris*) vinasse is a product of great agricultural interest, because of its organic matter content, N and K concentrations. Such information is desirable for finding out the suitability of renewable energy resources such as byproducts of different industries as alternatives to reduce the use of synthetic fertilizers. For this purpose, the main objective of this work was to study the effects of foliar fertilization with beet vinasse at different dose (1000, 1500 and 2000 l ha^{-1} of beet vinasse mixed in 1000 l of water, respectively) during two experimental seasons (2001, and 2002) on rice (*Oriza sativa* cv. Puntal) nutrition, yield and quality near Seville (Guadalquivir Valley, Andalusia, Spain). Macro- and micronutrients, chlorophylls, total carotenoids and soluble carbohydrate concentrations on rice leaf samples were collected for each experimental season at two growth stages to observe the influence of the foliar fertilization with this byproduct in plant nutrition. Also, grain mineral composition, percentage of full grains, protein concentration in the grain, starch concentration in the grain, and rice yield were determined to observe the influence of the foliar fertilization with byproduct in crop quality and yield. The obtained results show that the foliar fertilization with a byproduct rich in fulvic acids (but also containing macro- and micronutrients) increased the leaf concentration of micronutrients Fe, Cu, and Mn, and macronutrients N, P and K uptake by plants. Highest values of chlorophyll A and B were found in the plots foliar fertilized, which presumably favoured photosynthesis. Foliar application of the byproduct caused significant increases in grain protein of about 26%, in grain starch and full grains of about 5% and in rice yield of about 3% respect to plots without foliar fertilizer.

2498 USE OF HIGH-STABILITY COMPOSTS IN RECREATIONAL AREAS: ASSAYS ON COLD SEASON TURFGRASSES

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Recreational and sport areas, steadily increasing on number and occupied surface, show great interest as consumers of large amounts of organic products. High-quality composts could be used to improve soil properties, increasing its water-hold capacity and reducing the amounts of synthetic fertilizers needed to support the vegetal cover. In a 10-month assay using triplicated uncovered pots, the effects of four rates (5, 10, 20 and 40 t dry matter/ha) of two different organic products (green waste compost, and MSW compost aerobically-stabilized during one year) on germination and growth of seven varieties of cold season turfgrasses (*Lollium perenne* var. Pizzazz, Grand Slam and Belida*, Festuca arundinacea* var. Gold Millenium*, Poa pratensis* var. Bedazz*, P. pratensis/P. arundinacea* var. Fire *&* Ice and *F. ovina* var. Ridu) have been studied. Colour, ground cover, and general aspect were assessed in winter, spring and summer by means of standard procedures, and the vegetal mass generated during ten months after sowing was weighted. Even with rates as high as 40 t/ha, no effects of the compost applications were found to suggest any kind of phytotoxicity on the cold season turfgrasses assayed, although some hints about N immovilization by the highest rates of green waste compost were observed in some turfgrasses. These results show the interest of starting new trials using reduced irrigation and mineral fertilization to study the potential water and fertilizer saves.

2501 USE OF HYDROPONICS CULTURE TO ASSESS NUTRIENT SUPPLY BY TREATED WASTEWATER

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The use of treated wastewater for irrigation is increasing, especially in those areas where water resources are limited. Treated wastewaters contain nutrients that are useful for plant growth and help to reduce fertilizers needs. Nutrient content of these waters depends on the treatment system. Nutrient supply by a treated wastewater from a conventional treatment plant (CWW) and a lagooned wastewater from the campus of the University of Balearic Islands (LWW) was tested in an experiment in hydroponics conditions. Half-strength Hoagland nutrient solution (HNS) was used as a control. N, P and K concentrations were lower in LWW than in CWW, while HNS had the highest nutrient concentration. Barley seedlings were grown in 4 L containers filled with the three types of water. Four weeks after planting, barley was harvest and root and shoot biomass was measured. N, P, K, Ca, Mg, Na and Fe contents were determined in both tissues and heavy metal concentration was analysed in shoots. Dry weight barley production was reduced in CWW and LWW treatments to 42% and 12%, respectively, comparing to HNS. However, to a lesser extent, reduction was found in shoot and root N content. Treated wastewater increased Na content in shoots and roots of barley and Ca and Cr content in shoots. However, heavy metal content was lower than toxic levels, in all the cases. Although treated wastewater is an interesting water resource, additional fertilization is needed to maintain a high productivity in barley seedlings.

2557 HUMUS APPLICATION, PRODUCED BY ORGANIC WASTE TRANSFORMATION IN GROWING LANDS

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Every year in Mexico are produced more tons of garbage (19 874 259 tons) than tons of corn (18 309 000 tons). Fifty percent of domestic garbage is constituted by organic remainders. In December 2005 began de project "Biotransformation of Organic Remainders in Humus for its Application in Growing Lands" with the purpose to prove the methods of: 1) Natural outdoors transformation, 2) Accelerated fermentation with thermophilic and mesophilic microorganisms, and 3) Transformation with microorganisms such as californian red worm, *Eisenia andrei*, to transform efficiently fruits and vegetables remainders. The obtained humus has been applied in the growing of vegetables such as: green rind tomatoes, broccoli and beetroot, as well as in nine tests constituted for over 300 corn hybrids (white, yellow and blue), of the CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo) that were established in the School of Agricultural Sciences. Observing that the application of humus has a considerable beneficial effect in the crop fields investigated until now.

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2878 EFFECTS OF SEWAGE SLUDGE COMPOST APPLICATION ON SOIL BIOLOGICAL RESPIRATION IN A *Festuca arundinacea* CROP

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Application of compost in agricultural soils have obvious effects on physical, chemical and biological properties of soil, therefore it has effects on crops grown on them. Furthermore, in a particular soil, environmental conditions, especially temperature and rain, have important effects on the microorganism's behaviour. The present study shows, on the one hand, sewage sludge compost application effects over particular soil biological parameters: soil basal respiration (SBR), soil microbial respiration (SMR) and soil microbial biomass carbon (SMBC) as well as their relationship with soil moisture and grass harvest. On the other hand, it has been studied how annual climatologic changes have affected these soil biological parameters. The experiment was conducted during one year and eight months in a Calcaric Luvisol in which was grown a perennial crop of *Festuca arundinacea* Schreber. Six treatments were established: no amendment (control), 65 t/ha sewage sludge compost (CL), fractionated mineral fertilization (F) and CL+F, each treatment in irrigation conditions and, in addition, control and C unirrigated. During all the experiment, the highest levels of SBR, SMR and SMBC were found in compost treatments. Not only soil moisture but also crop yield was higher in every treatment where compost was applied to soil in comparison with treatments with no compost application. Throughout the year, SBR (measured at 25 ºC) was higher in heavy rain periods while lowest SBR values were found in periods with high temperatures, low precipitation and low soil moisture. Nevertheless, SMR (measured at ambient temperature in every period) showed a opposite trend to SBR so SMR showed maximum values in periods with high ambient temperatures and modest precipitations and minimum values in months with lowest ambient temperature. SMBC maximum values coincided with periods with moderate ambient temperature and high precipitations.

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2379 PHENOL AND 4-CHLOROPHENOL REMOVAL BY *Comamonas testosteroni*: COMETABOLISM

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The biological degradation of phenol and 4-chlorophenol (4-CP) by *Comamonas testosteroni* CECT 326T was studied. Whereas phenol was completely removed, *C. testosteroni* was not able to grow on 4-CP as a sole carbon and energy source. Phenol and glucose were added as growth substrates for cometabolic transformation of 4-CP, since the presence of cosubstrates can enhance the toxic compound removal by pure cultures. Phenol and glucose can induce the enzymes required for 4-CP degradation. High efficiencies were obtained in all the experiments carried out in presence of both cosubstrates. In spite of the addition of glucose reduces the lag phase of 4-CP removal, lower phenol concentrations are required in order to obtain the same degradation efficiencies. The cometabolic degradation of 4-CP was proportional to the rate of phenol oxidation, which provides the electrons for the initial monooxygenase reaction. The cometabolic 4-CP uptake rate in the presence of phenol can be further enhanced by using glucose as an additional carbon and energy source. No significant influence of glucose concentration on 4-CP removal was observed.

2405 MICROBIAL ASSESSMENT FOR THE TREATMENT OF Ni-V CONTAINING WASTES

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The application of microbial removal of metals is a relatively recent practice that takes advantage of microbial capacity to sequester, adsorb, and accumulate metallic components present in liquid and solid samples. There is scarce information about Ni and V pollution; nevertheless, it is well known the negative effect on environment of these metals mainly those related to their presence in industrial wastes, soils and sediments. The aim of the present work was to obtain microorganisms able to tolerate and remove Ni and V to afterwards evaluate them for their ability to remove metals in liquid and solid media. Microorganisms were obtained from mine wastes and effluents that came from Guanajuato, México. Samples were characterized by their metal content and physical, chemical and microbiological parameters. Selective cultures were prepared using PHG-II media added with the target metals (Ni, V and Ni/V at 3, 1 and 3/1 mM, respectively). Afterwards, obtained isolates were screened for their ability to grow and remove Ni, V and Ni/V at three different concentrations, in PHG-II solid media. Positive metal removal in plates was evidenced by the formation of a clear halo around the colonies after exposure to H_2S vapours. Only those isolates able to form a clear halo around the colony/remove metal(s) were subjected to following studies a) CMI evaluation and b) metal removal from Ni-V containing wastes, in liquid media. Thirteen isolates were obtained from samples 7 from V media, 2 from Ni media and 4 from Ni/V. Most of them (92%) corresponding to Gram negative bacilli. Sample characterization showed that their composition was variable, non appropriate microbial growth conditions were observed, but microbial counts were enough to get isolates. Results also showed that only 5 isolates were able to form a clear halo, mainly in presence of V and Ni/V salts at the three evaluated concentrations. No significant differences were observed both in microbial growth and halo at the three evaluated concentrations. Results of CMI and metal removal in liquid culture indicated the potential application of the obtained isolates for the treatment of Ni and V containing wastes, although more studies are needed.

1840 MTBE AND BTEX'S MIXTURE DECOMPOSITION BY OZONE IN GAS PHASE

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Presence of methyl-terbutyl-ether (MTBE), benzene, ethylbenzene, toluene and *m-p*-xylenes (BTEX) in water and soil represent a major environmental problem due their affects on live organisms. There are some treatments for these contaminants, but they just can mobilize the volatiles to other phase (vapor extraction from soil for example), venting, etc. However, there exists the problem of the treatment of ground water, residual water and contaminated soil with high content of volatile organics compounds (VOC's), because of their possible stripping to atmosphere surrounding the contaminated water and soil. The last can provoke the air contamination in the case of the incomplete decomposition of VOC's in the liquid and solid phases. One possible alternative to avoid the VOC's liberalization to the gas phase is to apply a treatment by ozone. This study deals with the decomposition of model of VOC's (MTBE and benzene) and their mixture by ozone in gas phase that were previously dissolved in water. The experiments in a tubular reactor with fixed length (1.6 m) were realized. These experiments were conducted in two stages. In the *first* one, organics mixture was ventilated by oxygen flow to liberate VOC's to the gas phase. Second stage deals with the liberated VOC's decomposition by ozone in the gas phase (in the tubular reactor). The VOC's decomposition efficiency was determined by the input and output analysis of pollutants concentration. The obtained results confirm the possibility to use ozone for VOC's decomposition in gas phase.

2340 TREATMENT OF A GASEOUS EMISSION FROM A LEATHER INDUSTRY IN A SUSPENDED-GROWTH BIOREACTOR

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Industrial and manufacturing operations release, on a large scale, Volatile Organic Compounds (VOCs) to the air. VOCs are of significant environmental concern as some contribute to the photochemical ozone creation potential, ozone depletion potential, global warming potential, toxicity, carcinogenicity and local nuisance from odour. Suspended growth bioreactors (SGB) have been recently the subject of study for the treatment of gas streams containing VOCs. An SGB removes VOCs by bubbling the contaminated air through an aqueous suspension of active microorganisms, with some potential advantages such as absence of plugging and easier biomass and nutrient control. The objective of this study was to establish a SGB for the treatment of a gaseous emission generated at a leather industry located in the centre region of Portugal. The SGB was operated for 210 days mimicking the operating conditions found *in situ*. A microbial consortium able to degrade the main volatile organic compounds (VOCs) constituting the gaseous emissions of this industry was enriched and used as inoculum for the SGB. The SGB was exposed to organic loads up to 350 $g.h^{-1}.m^{-3}$. A maximum total VOCs elimination capacity of 290 $g.h^{-1}.m^{-3}$ was achieved. Gas recycling proved to improve the SGB treatment performance. Overall, the SGB was shown to be robust under the experimental conditions tested, including night and weekend shutdown periods.

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2518 ADSORPTION OF VAPOR-PHASE TOLUENE ON ORGANIC CLAY (OC-CPC) AND ITS REGENERATION CONDITIONS

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Main air pollutants include gases such as carbon monoxide, nitrogen oxides, sulfur oxides, partially burned hydrocarbons and volatile organic compounds (VOC´s). Most VOC´s emissions come from small sources, such as cars, nail enamel bottles, paint containers, solvents, etc. These represent a human health risk at high concentrations, mostly in indoor environments, either at home or in work environments, where pollution levels are higher and hazardous than outdoors. The reduction of the concentration of these gases and vapors to acceptable levels, can be carried out by a number of methods, including adsorption processes which use adsorbents like activated carbon but its adsorption is affected by the relative humidity of water vapor in the environment. A number of studies, have demonstrated that clay materials having a laminar structure, like bentonite, can be chemically modified, changing their physicochemical characteristics such as interlaminar distance, surface area, pore size and polarity or chemical affinity. Some studies have focused mainly on obtaining modified clays to be used for the adsorption of organic and inorganic contaminants, which depends on both the nature of the mineral surface and the compound intercalated in the clay's interlayer space. In the present work the potential use of modified clays in the adsorption of vapor-phase toluene present in air and its regeneration process were investigated. A mexican bentonite was used to prepare an organoclay (OC-CPC) by intercalating cetylpyridinium chloride (CPC). The infrared and thermogravimetric analyses enabled to differentiate the structures obtained. Interlayer distance was assessed through X-ray diffraction. The adsorption studies showed a great affinity of toluene in gas phase for OC-CPC due to the hydrophobic character that resulted, and also to the increase in the interlaminar distance. The OC-CPC used in toluene adsorption experiments, was thermically treated in order to regenerate its adsorption capacity. The recovery ratio was of 90%. The results showed that organ clays can be used as an alternative to activated carbon for VOC's adsorption on enclosed working environments with the advantage that can be easily regenerated and that it is not affected by the presence of water vapor as activated carbon is.

2199 STUDY BY DYNAMIC LIGHT SCATTERING OF AN O/W EMULSION OF AN EPOXI RESIN DISPERSED IN WATER BY MEAN S OF A TRIBLOCK COPOLYMER OF TYPE PEO-PPO-PEO

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The curing epoxy resins are widely used in various fields of chemical industry, such as adhesives, automotive, coatings, etc. The process operation consisting of flow and mixing of epoxy resins become difficult due to their high viscosity. One solution is to dissolve the epoxy resin in volatile organic solvents (VOS) such as toluene, xylene or benzene. However, the use of VOC is not only expensive but harmful to the environment. In order to contribute to reduce the cost of operation of dilution with a inexpensive solvent and to protect the atmosphere of VOC vapors, the use water as a means of dispersion of the epoxy resyn appears to be a friendly environmental alternative. This work concerns the use of water as a solvent to disperse the epoxy resin in emulsion of the type O/W, the interfacial surface of the resin micro-droplets and water is stabilized by means of a copolymer triblock PEO-PPO-PEO. So the main objective of this work is on the one hand, to determine the extent of resine/water concentration to attaion a stable dispersion of resin, on the other hand, to study physicochemical aspects such as particle size of the O/W emulsion for a bisphenol A epoxy resin through the use of surfactant Synperonic. One of the suitable techniques to measure the particle size is the dynamic light scattering (DLS), such a technique allows to determine the hydrodynamic radius of the particles in a sample. The dynamic light scattering is widely used in the study of emulsions, suspensions and in the area of polymers. The study by DLS took place both for the system of copolymer micellar triblock Synperonic PE/F108 as to the O/W emulsion, in order to analyze the variation of the particle size from one system to another. Through this study, it was possible to obtain an estimate of hydrodynamic radius and structures present. In the micellar system was found two different morphologies, attributed to monomers copolymer micelles in aqueous solution and copolymer with hydrodynamic radii of 1 and 5.9 nm, respectively. In the case of the emulsion O / W, identified three morphologies, which are attributed to the presence of monomers, micelles and aggregates complex (clusters). The sizes of these structures were 3 nm to monomers, 39 nm for micelles and 1664 nm for clusters. Comparing the results of the micellar system with those of the O/W emulsion, there has been an increase in the size of the monomers and the micelles. The hydrodynamic radii of the structures found in the micellar system are smaller than in the case of the emulsion. This can be attributed to a change in the conformation of the molecules triblock copolymer for the case of monomers, and the variation of size of the micelles is attributed to the swelling of there to interact with the molecules of resin. On the one hand it can be concluded that the use of water as a means of dispersion in an O/W emulsion of an epoxy resin is effective for processing and desirable to eliminate the environmental problems that are generated by evaporation of volatile organic solvents did environment. Moreover, the study of dynamic light scattering enabled an analysis of the morphology of the O/W emulsion and the particle size of the structures present in the system.

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2353 SELECTION OF BIOREACTOR OPERATING POLICIES FOR AN OPTIMAL PRODUCTION OF MICROBIAL POLY (β-HYDROXYBUTYRATE)

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Currently, both the considerable environmental pollution increase and the reduction of the world petroleum reserves are the two principal problems associated to the use of synthetic polymers. In spite of these factors, almost the totality plastics produced around the world are of petrochemical origin. Also, this kind of industries approximately produced 245 million tones in 2006 which after of its use, 40 % is discarded into landfills. Also, several hundred thousand tonnes of plastics are discarded into marine environments every year and accumulated in oceanic regions. The biopolymers are devised as the solution to this environmental problem, being the polyhydroxyalkanoates (PHAs) and specially the poly (β-hydroxybutyrate) (PHB) the most actually studied. PHAs are synthesized as intracellular reserve material by a wide variety of bacteria from at least 75 different genera. These biopolyesters are produced under unbalanced growth conditions, i.e. excess of a carbon source and nitrogen, phosphorous, sulphur or oxygen limitation. PHB is a crystalline thermoplastic with a melting point around 175 ºC. It is a non xenobiotic plastic, being then totally degradable. Actually the production of PHAs at an industrial level is much smaller than the production of plastics of petrochemical origin, basically because the PHAs are much more expensive to produce in comparison with petrochemical plastics. The two more relevant stages of PHB manufacture are fermentation and extraction process. In the present work, a complete analysis of two different operating policies for producing PHB has been developed. Fed-batch and continuous operation modes have been studied and compared in order to obtain the most convenient operation scheme which maximizes the PHB productivity. Dynamic optimization techniques were applied to optimization both the feeding profiles in a fed-batch bioreactor and the unstable state operation in a chemostat. The optimization results obtained clearly demonstrate that the optimal operation of a continuous bioreactor with a flow divisor and a continuous separation stage is more adequate than the optimal solution found for a fed-batch bioreactor. The PHB productivities reached for optimal fed-batch and optimal continuous bioreactor were 3.08 and 4.04 g PHB/L-h, respectively.

2481 A TECHNOLOGY TO MITIGATE THE PESTICIDE CONTAMINATION INTO THE WATER

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Although plant protection products are already regulated in Europe under Directive 91/414/EEC, there is increasing concern about the pollution of ground and surface water caused by point sources of pesticides, such as tank filling, spillages, faulty equipment, washing, waste disposal, and direct contamination. One tool for the reduction of pesticide point source contamination is a biological system where chemicals are bound and biologically degraded: an offset lined system where wastewaters containing pesticide residues leach through a biomix. A pump system is provided to pump the water onto the surface of the biomix and allow it to drain under gravity, keeping the biomix wet. The biobed was installed in a vineyard farm in province of Piacenza in 2003 and, since then, the functioning of the system has been monitored every year with sampling of water and biomix. This monitoring program has been done in order to verify the efficiency of the mitigation system during five years of experimentation in real field conditions, where loadings of organic and inorganic pesticides remained in the waste water and reached the biobed. In the system, the water was pumped on the biomix in cycles of 15 minutes every 4 hours. The pesticide residues were analysed by HPLC-DAD or GC-MS, depending on their chemical characteristics. The analysis of some pesticide residues in water and biomix inside the system showed the biobed to function well, reaching a water decontamination greater than 90% for example for metalaxyl, penconazole, fludioxonil, cyprodinil. At the same time, some studies were conducted at a lab scale developing a prototype system where it is easier to test different biomass compositions (beginning from the original Swedish biomixture) and to determine which the best performance was. Water and biomix were sampled and analysed by HPLC-DAD, and some results were quite promising, representing a useful starting-line to improve the knowledge of the biomix used in biopurification installations. New studies on hydraulical improvements and on biomix composition are in progress, developing some biofilters at laboratory and field scale.

Acknowledgements: This research and its future implement

2500 STRATEGIES OF BIOREMEDIATION OF A CONTAMINATED COASTAL ECOSYSTEM (BOLMON LAGOON, SOUTH-EASTER MEDITERRANEAN COAST)

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Bolmon ecosystem (Bouches du Rhône, South-easter France) is a coastal mediterranean lagoon. This ecosystem presents a great interes in terms of ecology, economy and cultural aspects. Bolmon is connected to the salty Berre pond, itself connected to Mediterranee sea, via tiny artificial channels and a main one (Rove channel) that also bounds it to the South. It also receives fresh water from the small river La Cadiere (with a mean annual inflow of 1 m^3/s). This lagoon is hyper-shallow (1 m to 1.8 m depth) and contains a large amount of soft, muddy bottom sediments. The resuspension of sediments, due in particular to the wind, creates an important and quasi-permanent turbidity. As a consequence of the urbanisation of the close environment Bolmon lagoon is strongly contaminated by different polluants arriving from its drainage bassin. The impacts result in supra-eutrofication with a drastic decrease of the animal and vegetal bio-diversity; in the same time, phytoplanktonic blooms with high growth of cyanobacterias (one of these potentially toxic) were recurrent. Because from the hydrodynamic point of view this hydro-system is controlled mainly by the wind forcings, it occurs a strong competition between salty and fresh waters through the channels.The increase of the salinity induced by recent hydrolic managments appeared to favour the funccionning of Bolmon ecosystem. In association with différent Scientific and regional Institutions, a plan of remediation of Bolmon laggon had been elaborate. Two types of actions will be considered: (a) active control of hydric exchanges with adjacent hydrosystems (with higher salinity); (b) active control of turbidity by orienting the loaded water to suspension traps: creation of islets (playing the role of artificial wetlands). The respectif impacts of these perspectives of Bolmon laggon remediation will be studied on different planktonic and benthic biological compartments, on the composition and on the state of the sediment and on the organic matter.

2309 DEGRADATION OF CHLORINATED COMPOUNDS IN AN ANAEROBIC-AEROBIC PROCESS

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Remediation technologies that involve gas transport (e.g., soil vapor extraction and air sparging of groundwater) cause the emission of gases contaminated with chlorinated solvents. Under anaerobic conditions, reductive dechlorination of trichloroethylene (TCE) proceeds via the formation of cis and trans dichloroethenes (DCEs) and vinyl chloride (VC) as intermediates. Since the dechlorination decreases with a decreasing extent of chlorination, accumulation of these intermediates is frequent. On the contrary, the rate of aerobic degradation increases with a decrease in the number of chlorines per molecule, particularly in the transformation of DCEs, and VC. For this reason, the main objective of this work is to use a combination of an initial anaerobic process with an aerobic process to improve the overall degradation efficiency of TCE. Experiments developed under sulfate reducing conditions inoculated with fresh sediments obtained from hydrothermal vents showed the transformation of approximately 70% of TCE (initial concentration 200 μM) to DCEs , VC and in a minor extent to ethene (up to 3 μM). Some new research was conducted to use an aerobic system to completely degrade the volatile intermediates produced during the anaerobic process. A bacterial consortium previously used to eliminate chlorophenols was adapted to different concentrations of TCE and dichloromethane (DCM) during 3 months. Preliminary results on the aerobic experiments show that under gradual addition of TCE and DCM during microcosms experiments, the consortium is still viable up to a concentration of 10 μM and 40 μM of TCE and DCM respectively. With an initial biomass of 7.26×10^4 CFU/ml in the presence of 2 μM TCE and 10 μM DCM after a week of incubation, the biomass increased up to 5.67×10^6 CFU/ml for TCE cultures and 1.99×10^7 CFU/ml for DCM cultures. Further incubation showed that the consortium was still viable after a month. The microbial growth obtained with these pollutants as substrates demonstrates that the microorganisms efficiently degrade DCM, but they are not efficient for the degradation of TCE. Aerobic microcosms experiments were also performed for the degradation of DCM, DCEs and VC showing that the bacterial population was able to degrade the pollutants in different rates and extents.

2310 VITAMIN B_{12} IN ENRICHMENTS OF HYDROTHERMAL VENTS SEDIMENTS SUPPLEMENTED WITH COBALT AND METHANOL.

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Vitamin B_{12} (VB12) is an important cofactor present in methanogens and acetogenic bacterium. Biosynthesis of cyanocobalamin under different anaerobic and aerobic conditions has been reported for sewage, granular sludge and pure cultures. Corrinoids containing cobalt have also been identified as components in at least one dehalogenase belonging to *Dehalococcoides* bacteria. Several reports support the use of this vitamin as an efficient catalyst during the biodegradation of some chlorinated compounds. The objective of this work was to increase the microbial population containing cobalamins in hydrothermal vents sediments in order to enhance the biodegradation of chloromethanes. Mexican hydrothermal vents sediments were chosen due to the large microbial biodiversity that they may represent. In some previous experiments, these sediments were capable of degrading trichloroethene (TCE) under sulfate reducing conditions. Two sets of sediments taken from two close but morphologically different vents were used for the experiments. Two types of controls were prepared: (1) sediments amended with cobalt at a final concentration of 0.05 mg/l and (2) sediments without cobalt but with methanol (20.8 mM) as a substrate. Treatments consisted of sediments (2 g wet sediment; 0.68 g vss/g wet sediment (type 1) and 0.34 g vss/g wet sediment (type 2)) amended with cobalt and methanol. The VB12 content in the sediments was determined by capilar electrophoresis. In general, preliminary results of VB12 content in the cultured sediments showed that for sediment type 2 the VB12 content was approximately 0.5 mg/g vss in the cultures amended with cobalt after two weeks and over time its content increased to 4 mg /g vss (6 weeks). For both sediments, the combination of methanol and cobalt showed that VB12 was produced (detection was more evident) over time (ranging from 3.5 mg/ g vss in 2 weeks to 6 mg/ g vss in 4 weeks). Some other experiments demonstrated no methanogenic activity without substrate. Experiments including dichloromethane in the treatments were conducted to evaluate the biodegradation rates of the compound in the presence of these cultures. Concluding results on methanogenic activity, VB12 content and biodegradation rates of dichloromethane under these conditions will be presented.

2156 BIODEGRADATION OF AN OIL-HYDROCARBON CONTAMINATED SOIL, ENHANCED BY SURFACTANTS: EFFECT OF THE TYPE AND DOSE OF SURFACTANT

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The aim of this work was to study the effect of different parameters, such as surfactant type and dose, soil initial hydrocarbons concetration, and soil granulometry, over the total petroleum hydrocarbons TPH degradation, as well as over the microbial count (as colony formating units CFU/g soil) along the process. Four points were sampled in a contaminated site, with different TPH concnetrations (point A 19.571 mg/kg; point B 13.838 mg/kg, point C 18.086 mg/kg and point D 18.706 mg/kg). Due to the similarity between C and D, it was decided to take oly three points (A, B, and D).Samples were passed through mesh 10 (2 mm). Sample D was also used without the meshing procedure. A seto f micocosmos assessments were carried out, where soil and three different surfactants (SDS, TW80 y Emulgin 600) and concentrations (4 mg/kg; 40 mg/kg; y 400 mg/kg) were added. Humidity and phosphorus and nitrogen were fixed to reach a C:N:P ratio of 100:10:1. Microcosms were incubated for three months at 28 ± 2 °C. Higher TPH renoval was observed for point A (82.68%), employing 400 mg/kg of Tween 80. Final microbial biomass was of $12x10^8$ CFU/g soil. For point B, best TPH renoval was of 64.84%, using 4 mg/kg of Emulgin 600 (final biomass load was $51x10^3$ CFU/g soil). For point D, using 4 mg/kg of SDS, best result was 54.14% (fianl biomass count was $31x10^5$ CFU/g soil. Regarding point without meshing process, it was observed that higher removal was 38.81%, when using 400 mg/lg of SDS (final biomass load was of $83x10^4$ CFU/g soil). In conclusion, TPH renoval was very dependen on the initial TPH concentration, the surfactant type and dose, as well as of the particle size distribution. All this information is very usefull when designing a remediation process.

2236 SCREENING OF BACTERIA FOR PAHS DEGRADATIVE GENES AND BIOSURFACTANT PRODUCTION

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Polycyclic aromatic hydrocarbons (PAHs) are widespread pollutants and many of them have toxic, mutagenic or carcinogenic properties. Due to their high hydrophobicity, PAHs tend to interact with non-aqueous phases and soil organic matter; as a consequence, they are potentially unavailable for microbial degradation. It is therefore important to find bacteria able to degrade the PAHs together with bacteria able to increase the bioavailability of the contaminant. Rhizosphere is a rich-nutrient environment in which bacteria are metabolically more active than in bulk soil. The starting hypothesis behind this work is that because plants can benefit from the elimination of the contaminant, they will favour the presence of degradative phenotypes in their rhizosphere (Siciliano et al., 2001). To test this hypothesis in the context of PAHs degradation, we have started a screening to identify bacteria able to degrade and solubilize PAHs. Soil samples from rhizospheric and from bulk soil were taken from two different areas, near a petrol station in Cenes de la Vega and near a burned forest in Madrid. We were able to isolate phenanthrene degrading bacteria from all the samples, indicating that both rhizospheric and bulk soil contained degradative genotypes. By 16S rDNA analysis we have classified them among the genus *Pseudomonas, Sphingomonas, Novosphingobium, Mycobacterium, Arthrobacter* and *Burkholderia*. Our next step will be to identify the genes responsible for the phenanthrene degradation and once we get them we will use them as probes to analyze the degradative populations in rhizosphere and in bulk soil to determine the prevalence of each phenotype in the different type of environments. To test bacteria for biosurfactant production, we will use the drop-collapse assay in several media and we will proceed as above to identify genes and analyzed the populations in soils.

2272 REMEDIATION OF SOILS CONTAMINATED BY PAHS USING A SEQUENTIAL METHOD: DESORPTION WITH SURFACTANT - ELECTRO-CHEMICAL DEGRADATION

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Polycyclic aromatic hydrocarbons (PAHs) cause a high environmental impact when released into the environment. Many PAHs can have detrimental effects on both the flora and fauna of affected habitats through uptake and accumulation in food chains, and in some instances, pose serious health problems and/or genetic defects in humans. PAHs are hydrophobic compounds and their persistence in the environment is mainly due to their low water solubility. In addition, environmental factors, such as soil type and structure, pH, temperature and the association with co-pollutants, such as other hydrocarbons and heavy metals, can prolong their time in the environment.
The objective of this study was to evaluate the capacity of decontamination of polluted soils with PAHs using the sequence desorption - electrochemical treatment: desorption of PAHs from the soil followed by electrochemical degradation of the liquid collected. Several PAHs (Anthracene, Benzo[a]pyrene, Phenanthrene, Benzanthracene, Fluoranthene and Pyrene) have been used as model compounds since they are found in high concentrations in contaminated environmental samples. Due to their hydrophobicity, soil desorption has been limited. In this work, it has been evaluated the use of six surfactants, Brij 35, Merpol, Tergitol, Tween 20, Tween 80 and Tyloxapol, on the PAHs desorption from a model soil such as kaolinite.
Furthermore, the electrochemical degradation of PAHs was investigated, with the surfactant that gave the best results. The surfactants selected were Tyloxapol, Brij 35 and Tween 80 for Anthracene, Benzo[a]pyrene and Phenanthrene, respectively. The electrochemical treatment of these solutions was carried out in two electrochemical cells with different working volumes (0.4 or 1.5 L), and electrode material (graphite or titanium). Near complete degradation was attained for all the experiments in both electrochemical cells.

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2274 REMEDIATION TECHNOLOGIES FOR TREATMENT OF PAH CONTAMINATED SOIL AND STRATEGIES TO ENHANCE PROCESS EFFICIENCY

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The presence of carcinogenic polycyclic aromatic hydrocarbons (PAHs) in soils poses a potential threat to human health. The removal of these contaminants presents a challenge to scientists and engineers. PAHs are characterized by their palpable hydrophobic nature. Consequently, these species tend to be adsorbed on solid particulates, especially on the organic fraction of the solids. Accordingly, developing techniques intended to remediate contaminated soils have to consider all the features associated to the adsorption-desorption stages and availability of PAHs to added chemicals. In the present study, two treatments have been developed: bioremediation and electrochemical treatment.
An innovative process that combines soil electrokinetic remediation and liquid electrochemical oxidation for the total degradation of organic compounds present in a polluted soil has been developed and evaluated, using several PAHs such as phenanthrene and benzo[a]pyrene. In order to increase the PAHs solubility the addition of a co-solvent (i.e. ethanol) or surfactant as processing fluid is required. The influence of the desorption agent employed on PAHs remediation from the soil matrix has been demonstrated. Thus, in benzo[a]pyrene-spiked soil, there is no presence of contaminant in the extracted fluid if the processing fluid is a solution of ethanol. However; when a solution of surfactant Brij 35 is used, benzo[a]pyrene was transported towards the cathode chamber where it was collected. Moreover, the extent of this recovery depends on the pH profile on the soil. When no pH control was used, the percentage of recovery reaches a value of 11%; however when pH control was applied, the recovery of benzo[a]pyrene on the cathode chamber depends on the pH selected, but it ranges from 28% to 66%, varying from pH 4.5 to 7.0. In order to obtain the total degradation of mobilized PAHs from the contaminated soil, electrochemical degradation of PAHs was investigated, using an electrochemical cell with a working volume of 0.4 L, and graphite as electrode material. The results showed a direct relationship between the ionization potential of PAHs and the degradation rate. Moreover, higher PAHs degradation has been obtained by bioremediation enhanced by bioaugmentation and biostimulation.

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2299 PRODUCTION OF BIOSURFACTANT BY *Rhodoccus sp.* AND EFFECT OF SHAKING ON BACTERIAL GROWTH AND BIOSURFACTANT YIELD

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Nowadays, remediation of soils contaminated with hydrocarbons is environmentally important, and among restoration techniques, bioremediation has proven to be an economical and ecologically viable technique. In many cases, bioremediation involves the application of microbial biosurfactants, with excellent results in improved bioavailability and biodegradation. Up to now, the study of microbial biosurfactants has focused on the study of Gram negative bacteria, and on the optimization of nutrients in growth media. The aim of this work was to determine biomass and biosurfactant production by a *Rhodococcus* strain isolated from a hydrocarbon-contaminated soil. The kinetic parameters obtained, are important to suggest the best shaking conditions for biomass and/or biosurfactant production. *Rhodococcus* sp was grown in a minimal medium (without thiamine) added with kerosene (1%) as only carbon source, and growth was followed for nine days, under three shaking conditions: 125, 180 and 240 rpm. Bacterial growth was followed by OD and bacterial count; residual kerosene was determined by gravimetric Total Petroleum Hydrocarbons (TPH) and biosurfactant production was followed by surface tension and trehalolipid concentration, using cell-free supernatant and cell extract. Cells grew more rapidly at 180 rpm (μmax 0.0906 hr^{-1}), while cells at 125 rpm were slower (μmax 0.0653 hr^{-1}) and at 240 rpm, μmax was 0.0489 hr^{-1}. However, there was a higher yield of biosurfactant production in the cell-free supernatant at 125 rpm and 240 rpm (0.468 and 0.365 mg biosurfactant/mg kerosene respectively), giving almost 10.55 times more than at 180 rpm (0.0443 mg biosurfactant/mg kerosene). Water surface tension (68 dynes/cm) was reduced to 28.36 dynes/cm (125 rpm) and 39 dynes/cm (180 rpm) by the supernant and up to 27 dynes/cm by the cell extract, results were similar at 125 rpm and 240 rpm. As inoculum, a 38 h culture grown in kerosene was used, where cells were bacilli in morphology; but when subjected to 240 rpm, the cells died. Cells grown on TSA are cocci, and were used as inoculum at a concentration of $1X10^5$ cells/ml. Production of biosurfactant by *Rhodococcus* can be carried out at 125 rpm for soluble biosurfactant production, while at 240 rpm, a higher biomass is obtained.

2300 DEGRADATION OF PHENANTHRENE BY A CHILEAN WHITE ROT FUNGUS *Anthracophyllum discolor*

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Anthracophyllum discolor, a white rot fungus of southern Chile, has been reported to be an efficient degrader of clorophenols and azo dyes. This fungus produces ligninolytic enzymes being manganese peroxidase (MnP) the major one produced. The main purpose of this study was to evaluate the effect of phenanthrene concentration on ligninolytic activity of *A. discolor* measured by poly R-478 decolorization, and to evaluate the potential of this fungus for degrading phenanthrene in liquid media. Decolorization assays were performed in flasks containing Kirk culture medium inoculated by *A. discolor*. After seven days of incubation, phenanthrene solution in acetone was added in five flasks in order to obtain different concentrations (0, 10, 25, 50 and 100 mg/L, respectively). The Poly R-478 dye was added to each flask to give a concentration of 0.2 g/L in culture medium. Decolorization of the dye was monitored during one week as percentage reduction in the absorbance ratio at 520 and 350 nm in comparison with the dye control. For the phenanthrene degradation assays, flasks containing Kirk culture medium inoculated by *A. discolor* were incubated (30 °C, 7 days). After the incubation, phenanthrene solution was added to give a concentration of 50 mg/L in culture medium and incubated (30 °C, 28 days). Periodically, samples were collected in order to measure the degradation of phenanthrene and MnP activity. The fungus was capable to decolorize the PolyR-478 dye in presence of phenanthrene, indicating ligninolytic activity under these conditions. However, the decolorization of Poly R-478 decreased with the increment of phenanthrene concentration affecting the ligninolytic activity of the fungus. At 100 mg/L of phenanthrene, the decolorization rate was reduced in a 40% in comparison with the control (0 mg/L). Respect to phenanthrene degradation, *A. discolor* was capable to degrade about 70% of the contaminant in liquid media after 28 days of incubation and its MnP activity increased until 6-fold in presence of this contaminant. Further studies will be conducted to evaluate the phenanthrene or other polycyclic aromatic hydrocarbons (PAHs) degradation in soil by *A. discolor*, in order to optimize the potential of this strain for an effective bioremediation of contaminated soils.

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2318 LINDANE CONTAMINATED SOIL BIOSTIMULATION WITH VEGETABLE ORGANIC NITROGENATED EXTRACTS: EFFECTS ON SOIL BIOCHEMISTRY

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1,2,3,4,5,6-Hexachlorocyclohexane (HCH) was one of the most extensively used organochloride insecticides. Technical mixture of HCH cosists of eight steric isomers but only the *γ*-isomer, known as lindane, is insecticidal and it is commercial. Despite the fact that most countries have prohibited the production and use of the toxic lindane (Voldner et al, 1995), many contaminated soils remain because of the long persistence of lindane (MacRae et al, 1984), and, as a result, it cause environmental disease.
Soil biostimulant, are organic products composed of peptides, amino acids, polysaccharides, peptides, humic acids and/or phytohormones, fat etc. They are also claimed to work through a series of widely varying mechanisms including activation of soil microbial activity, and promotion or augmentation of the activities of critical soil enzymes.
There is experimental evidence that soil biostimulants can increase process of biodegradation polluted areas via the activities of indigenous xenobiotic degrading microorganisms.
The main objective of this study was to know the influence of different soil biostimulants (BS), organic products composed of peptides, amino acids, polysaccharides, peptides, humic acids and/or phytohormones, fat, etc, those are claimed to work through a series of widely varying mechanisms including activation of soil microbial activity, and promotion or augmentation of the activities of critical soil enzymes on lindane contaminated soil microcosms. Furthermore, we have characterized the influence of soil biostimulants on the microbial dynamics of lindane contaminated microcosms.
The application of biostimulants increase strongly soil biochemical activity of lindane contaminated soils, thus, we have measured as biochemical soil parameters some enzymatic activities as dehydrogenase and acid phosphatase, and in both cases it was showed an increase several times higher in lindane biostimulated soils.
Also, in this work it was studied with molecular techniques as DGGE the effect of these organic nutrients on the composition of microbial communities in a contaminated soil, because the natural attenuation of HCH is attributed to microbial activity and, as a result, bioremediation is considered a potencial strategy of soil bioremediation.
Thus, the use of biostimulants that increased the metabolic activity (dehydrogenase, acid phosphatase) was linked to the disappearance of lindane.

2401 EFFECT OF TWO PHYTOHORMONE PRODUCER RHIZOBACTERIA ON THE BERMUDA GRASS GROWTH RESPONSE AND TOLERANCE TO PHENANTHRENE

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Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria that have the ability to relieve environmental stress in plants, increasing the plant growth potential. Of importance to phytoremediation, PGPR stimulate plant root development and enhance root growth.
This study evaluated the growth response and the tolerance to phenanthrene of Bermuda grass: *Cynodon dactylon* inoculated with two phytohormone producer rhizobacteria: strains II and III, isolated from a contaminated soil with petroleum hydrocarbons.
Seeds of *C. dactylon* were surfce-sterilized with 10% sodium hypochlorite and then thoroughly rinsed with sterile distilled water, these sterilized seeds were incubated for 30 minutes at room temperature either in sterile distilled water as a blank control or in a bacterial suspension in distilled water of the strains II and III, adjusted to an absorbance of 0.5 (5×10^7 CFU/mL) at 600nm. Twenty seeds of this species treated and non treated with bacteria, were placed separately in baby food flasks with Magenta SIGMA caps, containing mineral medium and 6% bacteriological agar, supplemented with 100 and 200 mg/L of phenanthrene concentrations and the control experiments were considerate without the addition of the contaminant. All the experiments were performed by triplicate and maintained al 30 ºC in a growth chamber with photoperiod of 12:12, for 28 days. The growth of plants were evaluated by the measure of their root and shoot length and obtained the tolerance index (TI), expressed as the ratio of the shoot and root lengths of plants grown in the presence and absence of phenanthrene.
The root growth of *C. dactylon* species were favoured by the presence of both strains. The results of TI obtained to phenanthrene for the two concentrations, showed that the development of plants inoculated with the strain III was better, with a growth percentage between 80 and 110% compared with the growth plants without the contaminant and bacteria. Thus, this kind of rhizobacteria promoted the root elongation and the plant growth giving tolerance to phenanthrene in this species.

2409 REMOVAL OF PAH FROM CLAY SOIL CONTAMINATED WITH DIESEL OIL BY BIOREMEDIATION TREATMENTS

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Diesel oil is one of the most common soil organic pollutants, as a consequence of spilling of storage tank spills and accidental leaks. In Pernambuco State, Northeast part of Brazil, there are several evidences of soil contamination by petroleum derivates due to gas station leakings. There is also a growing concern because of the development of a new petrochemical industry pool headed by the construction of an oil refinery with capacity of 200,000 barrels per day, 60% of which will be diesel oil (expected operation in 2011). Clay soil is extensively used as a natural sealant in gas station and dumping industrial areas. However, there are few studies on bioremediation treatment of clay soil, especially under tropical climate. The objective of this work was to evaluate the removal of total polyaromatic hydrocarbons (PAH) and oil and grease (O&G) from a clay soil experimentally contaminated with diesel oil, by comparing different bioremediation techniques. The treatments used were landfarming (LF), bioestimulation (BS) and biostimulation with bioaugmentation (BSBA). Good results were obtained in all treatments. O&G removal efficiencies of 59%, 77% and 80% were obtained for LF, BS and BSBA, respectively, indicating that nutrients and microorganism addition promoted higher improvements in the treatment process. It was also observed two patterns of O&G removal: a faster removal rate in the first 24 days (F24) and a slower rate in the last 105 days (L105). The kinetic parameters of zero order in F24, expressed in µg of O&G.g-1.day-1, were 0.87 for LF, 1.10 for BS and 1.21 for BSBA. The values in L105 were 97%; 96% and 98% lower than those obtained in F24, for LF, BS and BSBA, respectively. Total PAH removal efficiency of 87%, 89% and 87% at the end of the experimental period (129 days), for LF, BS and BSBA, respectively. Apparently, there were no significant differences between the used treatment techniques. However, the individual PAH analyses showed that in the BEBA treatment, the benzo[a]pyrene removal was 4% and 12% higher than those obtained in LF and BS, respectively. These results indicate again, that nutrient and microorganism addition is determinant in the removal efficiency.

2436 BENZO[A] PYRENE: BIODEGRADATION BY DIFFERENT *Trametes versicolor* MORPHOLOGY AND ENZYMATIC STUDIES

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Benzo[a]pyrene (BaP), a persistent organic pollutant included in the polycyclic aromatic hydrocarbon's group (PAH), is of environmental concern due to its known carcinogenicity and bioaccumulation potential. Because of its toxic properties, it is included in the European Community (EC) and United States Environmental Agency (EPA) priority pollutant list resulting in a much more strict regulation and complex tasks to be accomplished for remediation of PAH contaminated environments. Unlike other fungi, white-rot basidiomycetes can mineralize PAH.
The main objective of the research is to perform assays under different morphological cultures of the white-rot fungus *Trametes versicolor* in order to study the biodegradation of Benzo[a]pyrene in different bioreactors. Pellets biodegradation kinetics were carried out in 50 mL liquid medium. The results showed the ability of *Trametes versicolor* to degrade BaP (10, 15, 20 mg/L) obtaining yields between 54 to 27%. In order to study the BaP biodegradation under better oxygen conditions, experiments in an air-pulse fluidized bioreactor with the fungus in pellet form were carried out, reaching a 47% of biodegradation treating 30 mg/L BaP. In a bioslurry bioreactor were conducted with 40 mL of liquid medium, 5 g of sterile soil and with the fungus in mycelial form. Initial concentration of BaP was 30 mg/L and after 5 days biodegradation yield of 32% was obtained.
The role of the intracellular cytochrome P450 and the extracellular ligninolytic laccase enzymes in the BaP biodegradation were also studied. Experiments with ABT (inhibitor of the cytochrome P450 activity) shown that this enzyme may be involved in the BaP biodegradation mechanism by *Trametes versicolor*.

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2442 TPH REMOVAL BY A MICROFLORA APORTED BY ORGANIC MATERIALS AND HUMIC ACIDS AS SURFACTANT

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Nowadays, a great variety of hydrocarbonoclastic microorganisms with the capacity to remove hydrocarbons of low molecular weight (aliphitics and polycyclic aromatic hydrocarbons (PAHs) of simple structures) have been reported. Concerning to the PAHs of high molecular weight, one of the principal factors determining its degradation is their very low bioavailability which has been associated to adsorption phenomena and high hydrophobicity. In bioremediaton technologies TPH desorption has been carried out by addition of synthetic surfactants. However, those of natural origin, such as the humic acids are an interesting option due to its ability for incorporating aromatic organic structures to their structure. This last characteristic presents humic acids as an interesting material for PAHs immobilization through in situ biotechnologies.
The objective of this paper was the implementation of a bioremediation technology carried out on solid substrate fermentation and based on the interaction of a microflora supplied into organic materials: sugar cane bagasse (SCB), vermicompost (VC) and humic acids (HA), as well as the use of earthworms (EW) during the bioremediation of a clay soil contaminated with 83000 mg-kg^{-1} of a complex mixture of weathered total petroleum hydrocarbons (TPH) (Aliphatics (AH), 28.5 ± 2%; Polycyclic aromatics (PAH) 21.5 ± 3%; Saturates (SAT) 15 ± 2%; Asphaltenes (ASPH) 35 ± 3%). It was found that treatment composed by VC, SCB, EW and humic acids improved the removal of the different hydrocarbon fractions (AH, 85.5%; PAH, 78.4%; SAT 73.2%; ASPH, 35.8%) and TPH up to 60% in 94 days. These results show the good interaction among all the studied components. On one hand, the microorganisms provided by the VC and SCB, as well as EW which offers the possibility of removing the most recalcitrant and carcinogenic hydrocarbons. On the other hand the humic acid properties offer the possibility of reducing or, even more, eliminating lixiviation problems and consequently, pollution expansion of aromatic compounds in part due to immobilization processes.

2493 COMBINED USE OF MEDITERRANEAN LOCAL RESOURCES FOR RESTORING TCE-CONTAMINATED SOILS. THE BIOREMEDIATION EFFECT OF *Dittrichia viscosa* AND VERMICOMPOSTED OLIVE WASTES

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Trichloroethylene (TCE) and its metabolic intermediates are resistant to biodegradation in aerobic subsurface environments, which contributes to their persistence in soils and polluted groundwater. In this study, the combined action of *Dittrichia viscosa* (L.) and vermicomposted olive waste for the remediation of a TCE-contaminated soil was analyzed. For this, pots with 1 kg of TCE-contaminated soil were amended with vermicomposted olive waste and seeded with *Dittrichia viscosa*. Pots without vermicompost, without plants and without TCE were evaluated simultaneously.
The effect of the interaction plant x organic amendment on both soil pollution and quality was assayed by determining TCE levels, enzyme activities (dehydrogenase, β-glucosidase, phosphatase, urease, o-diphenoloxidase), as well as the bacterial community structure and biomass by PCR-DGGE and real-time quantitative PCR techniques, respectively.
We propose the combined use of Mediterranean local resources (*Dittrichia viscosa* and vermicomposted olive waste) for restoring chlorinated organic solvents contaminated areas.

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2586 APPLICATION OF SINGLE-DROP MICROEXTRACTION COMBINED WITH GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTOR FOR THE DETERMINATION OF γ-HEXACHLOROCYCLOHEXANE IN SOIL AND SEDIMENT SAMPLES FROM BIOREMEDIATION BIOREACTORS

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Single-Drop Microextraction is a novel sample preparation technique that integrates in a one step the extraction, enrichment and sample introduction. This miniaturized approach overcomes the main disadvantages of conventional extraction methods resulting in simple, low cost and faster procedures with little sample and solvent consumption. In SDME is performed by suspending a drop of a water-immiscible organic solvent in a stirred aqueous solution, after the prescribed time the drop is withdrawn from the aqueous solution and organic phase is analyzed. This technique is based on the passive distribution of analytes between microliter volumes of organic phase and the aqueous phase. In this work, the development of a Single-Drop Microextraction procedure combined with Gas Chromatography-Electron-Capture Detector for the determination of γ-hexachlorocyclohexane, an organochlrine pesticide, in soil and sediment samples from bioremediation bioreactors is presented. This compound has been identified as a priority pollutant by the US Environmental Protection Agency due to its toxic effects. In order to obtain the maximum extraction efficiency from the Single-Drop Microextraction procedure, several parameters, such as extraction solvent, solvent volume (drop size), stirring speed, extraction time and temperature were studied. Experiments were carried out using 10-mL screw-cap vial sealed with a PTFE-faced silicone septum and 6 mL of the samples. Extraction temperature was controlled using a water bath while stirring was achieved using magnetic bars. The extraction solvent was p-xilene. The selected conditions were: 1 µL of extraction solvent, a stirring rate of 400 rpm, and an extraction temperature of 40 ºC and an extraction time of 25 minutes. The proposed method was applied to the analysis of soil and sediment samples from a bioremediation process implemented in our lab and a relative recovery of 85.2 % with relative response deviations of 16.2 % was obtained.

2681 EFFECT OF LIQUID COW MANURE APPLICATION ON ATRAZINE DEGRADATION IN ANDISOL

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Application of animal manures on soil results in added organic matter and nutrients that contribute to improving plants growth, but these components could modify the behaviour of pesticides in the amended soils due to the content and quality of organic matter (OM) and pools microorganisms. For the corn crops liquid cow manure (LCM) is applied before sowing, during three months, however, the time between the last applications of LCM and herbicide application varies a lot. This could be affecting substantially the herbicide efficacy. In this work, the effect of the additions of LCM on the degradation of atrazine was evaluated under controlled conditions in an Andisol in the south of Chile. The LCM was added to soil at 0, 70, 140 and 200 mL kg^{-1}, corresponding to 0 (S-0), 100,000 (S-100); 200,000 (S-200) and 300,000 (S-300) L ha^{-1}. The soils were moistened to 70% of field capacity and incubation was conducted for 1, 10, 20 and 30 days in a dark room at 20°C. The kinetic study was carried out during 30 days for each sustrate. Atrazine was applied in equivalent doses to the field condition. The kinetic degradation of atrazina has indicated that degradation is strongly influenced by both, the incubation times of the amendment soils and LCM rate. Extractable atrazine found on amendment soils with a day of incubation was high compared to the unamended soil. For five days of kinetic study extractable atrazine was 70 and 30% respectively. This result can be explained because the microorganisms use available organic material and N from LCM, firstly. The kinetics study evidences, for amendment soils with 10 and 20 days of incubation, less percentage of extractable atrazine than the soil, indicating that atrazine would be being largely mineralized. At 30 days, there are no significant differences in relation with the soil, except for S-300, where extractable percentage of atrazine is smaller, specially at the beginning of the experiment. On the other hand, the relative percentage of atrazine degradation products in soil is so distinct compared to the amended soils. The soil is characterized by the formation of a big deisopropylatrazine (DIA), 80%, and values nearly 20 % for deethylatrazine (DEA) and hydroxyatrazine (HA). The relative composition of the degradation products for the amended soils is strongly influenced by LCM dose as by the incubation time. For a day of incubation, a similar percentage of DIA, DEA and HA was found, 30% for each one. This relative distribution varies for incubated amendment soils during 10 and 20 days, where HA is remarked with a high relative percentage (60%). Clearly, there are differences in relation with the effect of LCM dose which can become established for the amendment soils incubated during 30 days, especially in S- 300, where a bigger relative percentage is observed for HA.

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2692 2,4-DICHLOROPHENOXYACETIC ACID DEGRADATION BY A MIXED BACTERIAL COMMUNITY ISOLATED FROM PESTICIDE CONTAMINATED SOILS

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The increasing amounts of fertilizers and pesticides used in extensive agriculture are one of the main causes of contamination in aquatic environments. Phenoxy herbicides are chemical compounds widely used to induce rapid and uncontrolled growth of broad-leaf plants that eventually cause the plant death. One of the most used herbicides of this group is 2,4-dichlorophenoxyacetic acid (2,4-D). By their high solubility in water and their relative recalcitrance is an important contaminant that arrives by soil lixiviation to aquatic environments. In this work, it was evaluated the 2,4-D removal rate and removal efficiency, by microorganisms isolated from herbicide contaminated soils. The microbial community is constituted by five bacterial strains. By 16S rDNA amplification, sequencing and comparison with the GenBank data base, the following genera were identified: *Achromobacter* (AJ278451), *Burkholderia* (CP000150), *Leifsonia* (AY509237), *Klebsiella* (AY779526) y *Stenotrophomonas* (AY486381). Each bacterial strain was batch cultivated on 2,4-D (35 mg L^{-1}) added as the sole carbon and energy source. It was found that four of them were able to degrade partially 2,4-D, but *Burkholderia* was able to metabolize completely to herbicide. When binary bacterial combinations were cultivated in a batch system on 2,4-D at 50 mg L^{-1}, it was found that the highest removal efficiencies were superior to 80 % and obtained when *Burkholderia* was present. Finally was evaluated the 2,4-D removal efficiency in steady state continuous culture and the kinetic behavior of *Burkholderia* growing on 2,4-D. In the chemostat the flow rate was periodically measured and adjusted when necessary, to maintain the dilution rate D at 0.0065 h^{-1}, 0.0111 h^{-1} and 0.0168 h^{-1}. The herbicide concentration in the feeding line started at 50 mg 2,4-D L^{-1}. The samples obtained at each steady state were used to estimate 2,4-D concentration by HPLC and cell growth by dry weight. The highest removal efficiency in the chemost was obtained with the dilution rate at D = 0.0168 h^{-1} and growth yield was constant in steady state continuous culture.

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2870 DEGRADATION OF DICLORINATED DIBENZO-P-DIOXIN BY A CELL FREE EXTRACT FROM *Geobacillus midousuji* SH2B-J2

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Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) have been widespread environmental contaminants formed unintentionally as a by–products during the bleaching of pulp and paper, the manufacture of pesticides, and the incineration of halogen containing chemicals. PCDDs and PCDFs contamination has led to serious social problem because of their toxicity mutagenic and carcinogenic properties. Microbial degradation of model compounds of PCDDs and PCDFs such as non-chlorinated dibenzo-p-dioxin (DD), mono-chlorinated DD and di-chlorinated DD has been studied using some microorganisms. *Sphingomonas* sp. Strain RW1, which was isolated using non-chlorinated DD as a sole carbon source, has been studied extremely. The initial reaction of DD degradation occurred by dioxygenation at the angular position of two carbon atoms adjacent to the ether bond. But it could not degrade the highly chlorinated DD. For the past decades, the functions of various microorganisms have been analyzed for the bioremediation of chlorinated dioxins. However, none have been applied for bioremediation. Recently, we have isolated the bacterial strain *Geobacillus midousuji* SH2B-J2 having the ability to decrease PCDDs from compost under roadside trees in Osaka Japan. This bacterium has been Gram-positive, and thermophilic with an optimal growth temperature of 65 °C. *G. midousuji* SH2B-J2 have shown to reduce highly chlorinated dioxins in incineration fly ash. Since this bacterium has been capable of reducing highly chlorinated DD even the octa-chlorinated dioxins (OCDD), we have hypothesized that the initial reaction of PCDDs degradation occurred with intra-molecular ether bond cleavage which is common structure regardless of the positions of the chlorine substituents. The main goal of this study is to identify the intermediates of dioxin degradation with 2,7-DCDD as a model dioxin and indicates the degradation pathway of PCDDs by this bacterium and construction of bioreactor system for PCDDs contaminated soil.

1785 HEAVY METAL ACCUMULATION IN *Thlaspi caerulescens* ON Pb-Zn MINE TAILINGS FROM MIBLADEN AND ZAIDA (NORTH WEST OF MOROCCO)

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The Mibladen and Zaida Pb-Zn Mine in the North West of Morocco have been ceased in 1975 and 1986 respectively. Mine tailings are one of the main environmental problems in post-mining landscapes and their removal is often complicated due to their high heavy metal content and dimensions. In this sense, the use of plant species to stabilize or remove pollutants from soils, generally defined as phytoremediation, may be an interesting and low cost option.
This study tackles screen plants growing on contaminated soil to determine their potential for metal accumulation. The Seeds of *T. caerulescens* metallicolous have been collected in the vicinity of F.T. Laurent le Minier (North of Montpellier, South of France), but Seeds of *T. caerulescens* non-metallicolous were sampled on the Southern Border of the Larzac plateau (North of Montpellier, Southern France).
Soil substrates were collected from a soil mining of Mibladen and Zaida (North West, Morroco), normal Soil was sampled from Kenitra (Est, Morocco). Plant and surface soil samples were measured for cadmium (Cd), lead (Pb) and zinc (Zn) concentrations by inductively coupled plasma mass spectrometry (ICP-MS). A non-metallicolous (NM) ecotype of Thlaspi caerulescens and a metallicolous (M) ecotype are compared for growth and Pb, Cd and Zn accumulation in shoot and root in six metal contaminated soils and one normal soil. The growth of individuals from normal soil was greater than that of individuals from metal-contaminated soils. Both ecotypes had similar growth. The NM populations had markedly higher root: shoot ratio compared to M populations. The results thus indicate both ecotypes of *T. caerulescens* are highly tolerant of zinc and Cd. Ecotype NM has constitutively higher Zn uptake capacity than M ecotype and grew less well than ecotype M. *T. caerulescens* species accumulate higher amount of Zn and Cd in their tissues in polluted soil and both of the two ecotypes the root Pb concentrations were much greater than those of the shoot Pb contents. The results obtained indicate that *Thlaspi caerulescens* from both normal and metal-contaminated soils are interesting material for phytoremediation of zinc and cadmium.

2240 CHARACTERISATION OF SOIL AND PLANT-ASSOCIATED BACTERIA ON A METAL CONTAMINATED SITE

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Conventional methods for the remediation of heavy metal contaminated soils and ground water are very expensive and often damaging to the environment. Complementary to these traditional methods, especially for sites with a diffuse contamination in relatively low concentrations, phytoextraction is proposed as a promising technology for effective and inexpensive remediation. However, large scale application of phytoextraction presently faces a number of obstacles including the levels of metals tolerated by the plant, the bioavailable fraction of the contaminant, the physical availability of the metal to the plant's root system and inefficient translocation of the metal to the harvestable plant parts.
A possible solution for these problems can be the use of plant-associated bacteria. Both rhizosphere bacteria and endophytes may act as plant growth promotors by fixing atmospheric nitrogen, sequestering iron from the soil and/or synthesizing phytohormones and enzymes. Plant-associated bacteria, if equipped with the appropriate characteristics can influence the metal availability for the plant. They can also assist their host in tolerating higher concentrations of contaminants by f.i. the *in planta* sequestration of metals, making them less toxic for the plant; by consequence, they can promote metal accumulation in the harvestable plant parts.
A former agricultural soil, located nearby a zinc smelter, was characterized for its metal content and agricultural properties. Cd and Pb concentration in soil and crops exceeded the legal threshold values.
In order to test if any natural adaptation took place on bacterial level, we isolated and characterized the cultivable soil bacteria. Genotypic characterization of these bacteria was accomplished by Amplified R-DNA Restriction Analysis (ARDRA) and Box-PCR. The bacteria were also characterized phenotypically by testing them for their tolerance to Cd, Zn, Pb and Ni and for production of siderophores and organic acids.
In a second part, yellow lupine plants (*Lupinus luteus*) were grown on this contaminated soil. The cultivable bacterial population from rhizosphere, root and shoot samples was isolated and tested for the same characteristics as the population in the soil.
This characterization contributed to obtain bacteria equipped with interesting characteristics. In further experiments the bacteria with the most promising features will be re-introduced in plants. Effects of this re-inoculation on metal uptake in the plant and root to shoot translocation will be investigated.

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2243 METAL TOLERANT PLANT GROWTH-PROMOTING RHIZOBACTERIA AFFECTING GROWTH, SYMBIOSIS AND SEED YIELD OF LENTIL GROWN IN NICKEL AND LEAD

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Heavy metals, due to its effect on microbial diversity, soil fertility and the yields of various crops have attracted greater attention worldwide. Conventional methods for removing/detoxifying metals from polluted sites generate huge quantities of toxic products and are not economical. The use of microbial communities for remediation of heavy metals, from contaminated sites has provided an alternative to conventional techniques. In this context, plant growth promoting rhizobacteria play a significant role in the remediation of metal contaminated soils. In this study, the plant growth promoting activities and bioremediation potentials of nickel (300 µg/ml) and lead (350 µg/ml) tolerant Rhizobium sp. using lentil as a test crop was evaluated. The metal tolerant strain displayed the production of indole acetic acid, siderophores and hydrogen cyanide. The strain RL9 when used for coating lentil (lens esculenta Moench Medit.) seeds and sown in sandy clay loam soils along with 290 mg Ni/ kg and 195 mg Pb/kg soil, significantly ($P \leq 0.05$) increased growth, symbiosis, and seed yield of lentil. The metal tolerant strain increased the glutathione reductase activity by 43 and 22% even at 580 mg Ni/kg and 390 mg Pb/kg soil, respectively, compared to un-inoculated but metal treated soil. Metal tolerant strain reduced the concentration of nickel and lead in plant organs suggesting that this strain could be exploited for raising the productivity of lentil plants in nickel and lead polluted soils.

2369 RADIOCESIUM (^{137}CS) TRANSFER AND BIOACCUMULATION IN SOME VEGETABLE CROPS

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Radionuclides released from nuclear reactors are serious environmental pollutants. In recent years, radioecological studies have been concentrating on the restoration possibilities of contaminated ecosystems. Phytoremediation of radionuclides has been reported as one of the options. The potential of crop plants to remove radionuclides from soils can be successfully exploited for removal of radioactive contaminants in the terrestrial ecosystems. Among the fission products released during nuclear accidents, ^{137}Cs is of particular significance in view of its long half-life (30 y). We, therefore, focused on this radionuclide, determining its soil to crop transfer factor (TF) and bioaccumulation factor (BAF) in four vegetable crops, viz., *Allium cepa* L., *A. cepa var. aggregatum* L., *Amaranthus blitum* L. and *Coriandrum sativum* L. The crops were raised in pots after factitious contamination of a sandy clay loam soil with carrier-free ^{137}Cs as cesium nitrate in dilute nitric acid medium at 20 kBq and 40 kBq kg^{-1} of soil. The treatments were replicated four times in a completely randomized blocks design. The chosen individual crops were analyzed for ^{137}Cs activity in a NaI (Tl) crystal gamma ray spectrometer with optimal window settings. The highest values of TF and BAF were observed in *Allium cepa* L. among the species studied. The mean value of BAF for *Alliums cepa* L. ranges from 0.0543 to 0.1116, where maximum accumulation was seen in sheath. The results of our study suggest that *Allium cepa* L. could be used as a potential species for phytoremediation of ^{137}Cs.

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2373 THE ROLE OF PHYSIOLOGICAL ACTIVE SUBSTANCES IN PLANT ADAPTATION TO STRESS

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It is known, that brassinosteroids are capable in small quantities (10^{-12} - 10^{-7} M) to optimize physiology -biochemical processes in plants in stressful conditions. The aim of this study was to investigate the role of antistress and protective properties of phytohormone 24-epibrassinolide (24-epiBS), in view of its functional features and biological activity.
The invastigations were carried out in laboratory and greenhouse pot experiments with barley cultured in soddy-podzolic soil. By preliminary results of laboratory experiments the concentration of 24-epiBS - 10^{-9} M has been recommended as high stimulating. In long-term vegetative experiments it was established the influence of 24-epiBS, first of all, on a reproductive part of a plant. And this influence is connected to influence of 24-epiBS on passage by a plant of phases of ontogeny. Ontogeny of plants during vegetation is connected to levels of soil fertility. At optimal security of soil with nutritious elements (high doses of nitrogen, phosphorus and potassium) the biomass of a crop is maximal, but efficiency of 24-epiBS is less, than in extreme conditions. It emphasizes an ecological role 24-epiBS.
The ecological functions of 24-epiBS were showed in regulation of processes of absorption of cadmium (Cd) and In case of regulation of zinc absorption (Zn) by plant. And mechanism of this regulation is different, as the strategy of absorption of these substances by a plant is different. In this connection, the basic regulatory role 24-epiBS in diffuse penetration Cd into a plant. In a series of experiments it is proved, that regulation is connected to deduction of moisture in a plant and influence on photosynthesis. The regulatory role 24-epiBS is shown in amplification of efficiency of absorption Zn (it is proved in long-term researches on barley). The certain laws in view of which stability of growth of plants in the polluted regions raises are established and the opportunity to influence quality of agricultural production opens.

2374 USE OF URBAN COMPOSTS FOR THE REGENERATION OF A BURNT MEDITERRANEAN SOIL

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In Mediterranean region, forest fires are a major problem towards the desertification of the environment. Use of composts is considered as a solution for soil and vegetation rehabilitation. In this study, we determined the effects of three urban composts and their mode of application (laid at the soil surface or buried) on soil restoration after fire: municipal wastes compost (MWC), sewage sludge and green wastes compost (SSC) and, green wastes compost (GWC). Carbon and nitrogen mineralization, total microbial biomass, fungal biomass and soil characteristics were measured during 77 days incubations in microcosms. Impact of composts input on erosion risk limitation was estimated by measuring runoff, percolation and retention in rainfall simulator.
Input of composts increased organic matter and nutrients soil content and also carbon and nitrogen mineralization and total microbial biomass all along the incubations whereas it increased sporadically fungal biomass. For all these parameters, the MWC induced the highest increase while GWC input had no significant effect compared to the control.
Buried composts lowly limited erosion risk whereas composts laid at the soil surface significantly induced decrease of runoff and increase of percolation and retention particularly with the MWC.

2443 *Lycopersicum esculentum* ROOTS: A MODEL SYSTEM FOR ARSENIC PHYTOREMEDIATION STUDY

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Arsenic polluted soil is one of the most serious problems in Mexico, and a feasible option to this could be the phytoremediation with important advantages over many related clean up technologies, then is necessary to study the mechanisms –such as physicochemical and biochemical– involved in soil remediation. Some studies about arsenic caption had being made in some plant species –these techniques use hydroponic conditions and synthetic contaminated soils–. Our study involves the arsenic caption dynamic by an *in vitro* cultivated tomato roots technique. We made a caption model in order to study these mechanisms through a bifactorial experimental design; the factors are the time (levels 0, 1, 2, 3, 4, 5, 6 and 7 days) and the initial arsenic concentration in the culture medium (levels 0, 2 and 10 ppm), and the quantified responses are arsenic concentration in dry weight tissue (DWT), conductivity (mS/cm), pH, fresh root weight (FW) and dry root weight (DW). We found that tomato roots cultivated *in vitro* and contaminated with arsenic are capable to absorbe high concentrations quantities (˜1400 ppm). We found that arsenic uptake did not adjust to a linear model but instead adjust to a polynomial model for to 2 and 10 ppm concentrations into the medium. At 2 ppm arsenic concentration we found growth stimulation; in the other hand at 10 ppm arsenic concentration we found a growth inhibition. We can explain this behavior -in arsenic added mediums- by the plant stress response. It is necessary to study the phytochelatins and growth regulators roles in response to different arsenic exposures.

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2459 TRANSPORT AND LIXIVIATION IN SOILS OF THE HERBICIDES METHYL-IMAZAMETHABENZ AND CHLORSULFURON IN SOILS AT DIFFERENT SCALES

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The knowledge of the soil-water-solute systems is essential in order to assess the environmental fate of pesticides. The availability of a pesticide in the dissolved phase of a soil determines not only its efficacy, but also its potential for groundwater contamination. In this work, the behaviour of the herbicides Methyl-Imazamethabenz (IMZ) and Chlorsulfuron (CHL) is studied at different working scales ranging from laboratory controlled experiments to an undisturbed soil monolith.
Kinetics studies showed a negligible role of volatilisation and degradation under our experimental conditions. In batch equilibration sorption experiments, IMZ presented linear adsorption and remarkable desorption hysteresis, whereas CHL exhibited very low sorption.
Transport experiments in soil microcolumns (2 cm diameter, 10 cm length) under stationary water flow provided near symmetric breakthrough curves. The estimated retardation factors (1.39 for IMZ and 1.04 for CHL) confirmed the relatively higher adsorption of IMZ in soil. The values of the adsorption coefficient K_D, calculated from these experiments, were slightly lower than those obtained from batch experiments. No dependence of K_D with flow was found.
Transport experiments in a homogenous soil column (1 m length, 25 cm diameter) showed that a relatively high percentage of the applied herbicide was lixiviated (77% IMZ and 93% CHL). The curves of herbicide concentration in the lixiviate were modelled using the code STANMOD. The values of the retardation factors were close to those obtained in the microcolumn experiments.
An intermittent water regime, close to field conditions, was applied on an undisturbed soil monolith (1m length, 30 cm diameter). This experiment resulted in an increased residence time of the herbicides. A remarkable decrease in the lixiviate recuperation percentage (31.8% IMZ, 76.9% CHL) was attributed to the hysteresis in the desorption processes and degradation. The parameters obtained modelling previous experiments in homogenous soil columns, adequately described this new breakthrough curves.

2471 INFLUENCE OF LIMING ON THE TOXICITY OF LIXIVIATES FROM SOILS CONTAMINATED BY PYRITE TAILINGS

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This paper analyze the influence of $CaCO_3$ in the toxicity of lixiviates of soils affected by a pollutant solution (PS) coming from the oxidation of pyrite tailings. The aim is to determine the liming rates necessary to mitigate the toxicity of lixiviates of soils affected by this type of pollution.
The Ap horizon of a Typic Haploxerept was mixed with increasing amounts of $CaCO_3$ (2, 4, 6, 8, and 10 %). One dm^3 of a pollutant solution coming from the oxidation of pyrite tailing was added to one kg dry both to the original soil as well as to the five soil-$CaCO_3$ mixtures. The elements concentration in the pollutant solution (mg dm^{-3}) was: SO_4 15078, Fe 4360, Al 290, Zn 718, As 135, Ca 9.6, Mg 222, and Cd 2.6. This addition was repeated three times and lixiviates were collected, immediately passed through cellulose filters (0.45 Ìm pore), stored at < 4 ºC before analysis, and analysed after each addition. The concentration of Al, Fe, Ca, and Mg in the PS and lixiviates were measured by atomic absorption spectroscopy; Na and K by flame photometry; SO_4^{2-} by ion chromatography; and As, Zn and Cd by inductively couple plasma mass spectrometry (ICP-MS). The Al species present in lixiviates were processed using the computer program Solmineq (Kharaka, et al. 1989).The amount of each element precipitated was calculated by the difference between the concentrations in PS and lixiviates.
The results indicate that the $CaCO_3$ in soil was very effective in decreasing the concentration and activity of Al in drainage water, only lixiviates of soils without $CaCO_3$ showed toxic concentration. The effectiveness was lower in the case of As and after four additions of pollutant solution, only drainage water of soil with a concentration of 100 g $CaCO_3$ kg^{-1} dry soil didn't show toxic concentration of As.
In the case of Zn and Cd, the $CaCO_3$ added wasn't effective, and drainage water of all soils showed concentrations higher than toxic levels.

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2474 INNOVATIVE APPROACHES FOR THE REMEDIATION OF ARSENIC AND MERCURY POLLUTION VIA SOIL WASHING

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Soil washing techniques are *ex situ* remediation procedures to remove contaminants concentrating them into a minor volume through particle size separation, gravity separation, and attrition scrubbing; with or without the utilization of chemical additives. Systems incorporating these removal techniques offer possibilities for application to soils contaminated with a wide variety of heavy metal, radionuclides, and organic contaminants. Within them, Hg and As are outstanding pollutants of special interest, particularly in areas where the presence of ancient mining and metallurgy works has generated relevant environmental impacts.
In Asturias (northern Spain) there was an important Hg district with different deposits exploited until the end of the 1970s. One of the main points in this mining activity was the area of "La Soterraña" where, together with the mining activity, metallurgical procedures were carried out in order to obtain Hg. These works and the accumulation of both mining and smelting waste generated, through mechanical dispersion, oxidation and lixiviation of As-Hg rich materials, an important affection in a surrounding area of around 80.000 m^2. One of the possible remediation strategies for this site is soil washing, and the necessary technical-viability study is the objective of this work. In fact, before the design of proper real-scale schemes, a pilot-scale study used to be indispensable, and as a first step a detailed characterization of the textures, geochemical behaviour, and densities of the raw material is required.
In order to accomplish soil characterization three composite and representative samples of the soils were taken (more than 50 kg each one). After particle-size separation via wet sieving, ICP-MS analysis revealed very high concentrations of As (up to 30.000 ppm) and Hg (up to 1.600 ppm), and much more lower presence of other possible pollutants such Cd and Pb, which is consistent with the mineralization-type of the ores treated in La Soterraña Additionally, the concentration of Hg and As is privileged in the fine fractions (below 125 μm), but also quite high in coarse fractions. As consequence of that, soil washing strategies proposed consist of hydrocycloning for fine particles, multigravimetric separation (MGS) for fractions between 125 and 500 μm, and finally, vibrating tables and/or spirals for fractions between 500 μm and 4 mm.

2503 USE OF NATIVE SPECIES FROM CONTAMINATED SOILS IN PHYTOREMEDIATION

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Heavy metals are pollutants of much concern and have become a serious environmental problem due to their immutable nature. The use of plants capable of accumulating metals in their harvestable tissues to cleanup soils, a technique known as phytoextraction, is emerging as a new and profitable tool for in situ remediation, especially when native plants are employed. Because of this, we studied the potential phytoextraction capacity of three species growing on soils contaminated with heavy metals in Asturias (Spain).

Plants of *Dittrichia viscosa*, *Melilotus alba*, and *Betula celtiberica*, coming from seeds collected in polluted sites, were grown in vitro in the presence of Cd or Pb. For *D. viscosa* and *B. celtiberica* 10 mg Cd L^{-1}, and in the case of *M. alba*, 200 mg Pb L^{-1} was applied to the medium. After 40 days of culture organogenic data were recorded, and shoots and roots were separated, powdered, digested with HNO_3 and analysed for Cd or Pb by ICP-MS.

Except for root length in *B. celtiberica*, plant development was inhibited when Cd was added. There were differences in Cd content in the aboveground tissues among clones, reaching 1300 and 463 mg Cd Kg^{-1} dry wt. in *D. viscosa* and *B. celtiberica*, respectively. Thus, both species cultured in vitro reached and exceeded the hyperaccumulation values of 100 mg Cd Kg^{-1} dry wt. (Reeves y Baker, 2000)[1]. Plants of birch mycorrhized with a Cd-tolerant fungus, *Paxillus filamentosus*, and grown in the presence of Cd had a better development and accumulated more Cd in their shoots than the non-mycorrhized ones.

Pb reduced shoot and root length and number of leaves in *M. alba*. In terms of Pb content, it accumulated more than 1000 mg Pb Kg^{-1} dry wt. in shoots, so it can be classified as a hyperaccumulator species according to the value established (Brooks, 1998)[2]. These uptake abilities are being checked in the field to see if their Cd and Pb accumulation capacity is similar to the values we have observed in vitro, but in any case the use of these species seems very promising in phytoremediation programmes.

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2507 EFFECTS OF SHORT CHAIN ORGANIC ACIDS ON Zn AND Cu DESORPTION FROM MINING SOILS BY COLUMN LEACHING

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Phytoextraction is a cost-effective and environmental-friendly technology that uses metal hyperaccumulator plants to remove heavy metals from soils, transporting and concentrating them from the soil into the above-ground shoots, which can be harvested. These plants enhance metal uptake by releasing through root exudates chelating agents, such as Low Molecular Weight Organic Acids (LMWOA) which increase metal availability. The objective of this study is to investigate the release of Zn and Cu from two naturally contaminated soils by column leaching in response to the presence of different types and concentrations of organic acids. These soils were collected from the villages *Garganta de los Montes* and *El Cuadrón*, in the north of Madrid, where there was an intense mining activity more than 50 years ago. Soil samples were air-dried and passed through a 2mm sieve. They were analyzed for pH, Electric Conductivity (EC), Cation Exchange Capacity (CEC), Oxidizable Organic Carbon, Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC) and texture. Also a sequential extraction was used to fractionate Zn, Cu and Cd, following the procedure of Tessier et al. (1979). The column leaching experiment consisted of 36 glass columns with 300 g of soil in each one. Six treatments were applied to both soils (in triplicate) adding, every two days, 50 mL of a solution containing a background electrolyte with different treatments: without organic acid, 0.5 mM citric acid, 10 mM citric acid, 0.5 mM tartaric acid, 10 mM tartaric acid, and 5 mM citric + 5 mM tartaric. In the column lixiviates we measured pH and metal content (Zn and Cu) by Atomic Absorption Spectrometry (AAS). Zinc concentration in lixiviates was much larger in all treatments with *El Cuadrón* soil than with *Garganta* soil, due to a low pH and a higher initial adsorbed Zn content. The same tendency was found for Cu when we added low concentration of organic acids, but when using increasing concentrations of these, Cu desorption was higher in *Garganta* soil. Results indicate that adding increasing concentration of organic acids in both soils lead to a significant increase of Cu desorption, particularly with citrate. The desorption behavior of Zn with different treatments in each soil was not so evident.

2513 HEAVY METAL CONTENT EVALUATION OF SPECIES OF SPARTINA SCHREB. GENERA (*GRAMINAE*) FROM THE TINTO RIVER ESTUARY (HUELVA, SPAIN)

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Estuaries, as sinks of residues and sediments transported by river waters, are ecosystems especially sensitive to pollution. From this point of view, Tinto River estuary, located in Huelva province (Spain), is particularly interesting. Tinto River presents an average pH of 2.3 and elevated concentrations of metals as Fe, Cu, Zn, As and Pb in most of its length (nearly 100 km). When its waters rush into the estuary pH changes and metals precipitate. Furthermore, we must also take into account the effects of several residues produced by the local industrial activity: phosphate-based fertilizer, pyrite roasting and copper smelting plants and paper mills are present in this estuary among other industries. Perennial grass communities of species of *Spartina* from *Spartinetea maritimae* class are the permanent vegetation closest to water. The western Iberian communities of this class are: *Spartinetum maritimae* Corillion 1953 y Spartinetum densiflorae Rivas–Martínez, Costa, Castroviejo & E. Valdés 1980. *Spartinetum maritimae* is the pioneer coastal perennial grass community on intertidal mud and sand, mainly composed by *S. maritima*. This community, that grows forming wide strips close to water, is completely inundated by daily high tides of the Atlantic sea. Spartinetum densiflorae forms vast grasslands of the alochthonous *S. densiflorae*. This community occupies higher positions than *Spartinetum maritimae*, where is exposed to be partially inundated by either daily high tides or only by spring tides. The present work studies the metal content of the two species of *Spartina* that grow in the Tinto River estuary in comparison with their soils. The existence of both species in the Tinto River estuary suggests that both are extremely tolerant to high heavy metal contents in soils. The responses of accumulation / exclusion in their tissues are of special interest for the development of phytoremediation strategies for salt marshes and other soils and sediments affected by tides.

2521 RECLAMATION OF A BURNT FOREST SOIL WITH MUNICIPAL WASTE COMPOST. EFFECTS ON CO_2 EMISION AND SOIL CARBON SEQUESTRATION

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Soils play an important role in the study and prevention of the climatic change, because they can be source and sink of carbon. Forest fires that are becoming more frequent, affect physical, chemical and microbiological properties of soils. In recent years the necessity to protect burnt soils from degradation has allowed to find alternative practices to reclaim soil organic matter (SOM) content and functions. In this work we consider the application of municipal waste compost for a better and faster SOM recovery after fire, focusing the study in the dynamic of C mineralization.
The objectives of this work were: a) to quantify and to compare the values of soil carbon sequestration of a burnt zone with those of an unburnt adjacent one, b) to study the influence of municipal waste compost addition to a burnt and an unburnt soil on some properties related to CO_2 emission and C stabilization and sequestration.
A laboratory incubation experience was carried out with three municipal waste compost doses on a burnt soil and another unburnt adjacent soil, besides corresponding control samples. The soils were *eutric cambisols* developed under *Pinus pinaster* forest. 50 g of soil were thoroughly mixed with 0.5 g, 1.0 g or 2.0 g of municipal waste compost, wetted to 75 % of water holding capacity and incubated at 29 ºC for 92 days in closed chambers under laboratory-controlled conditions. The evolved CO_2-C was trapped in NaOH and titrated with HCl. Microbial biomass C and N were determined by chloroform fumigation extraction method.
With regard to the carbon sequestration we have found differences between the burnt and unburnt soils, especially with depth, related to the intensity of fire.
Regarding to its C recovery with the doses of compost, a different behavior has been detected in aspects as the metabolic quotient, or the more labile pool, in the burnt and unburnt soils.
As expected, the unburnt soil showed higher amount of mineralizable C than the burnt soil. The unburnt soil was sensitive and modified its behavior with the different doses of compost, but not the burnt soil, probably due to the different kind of SOM and microbial population, adapted at new conditions after fire. In both soils the compost amendment decreased or did not change soil respiration, without significant changes in microbial biomass.

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2522 EFFECT OF DIFFERENT CULTIVATION CONDITIONS FOR THE PRODUCTION OF LIGNINOLYTIC ENZYMES BY THE WHITE-ROOT FUNGI *Anthracophyllum discolor*

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At present, the study of the ligninolytic enzymes from white-rot fungi to degrade ligninolytic compounds has increased. Until now, most studies have been focused on the enzymatic system of *Phanerochaete chrysosporium* and *Trametes versicolor* due to its rapid growth, easy growing conditions and ligninolytic properties. Therefore, the enzymatic system for lignin degradation has been based on the enzyme system of these fungi, which is not always applicable to all white-rot fungi in general. There are many factors that influence the production of enzymes in ligninolytic white-rot fungi and not all respond equally to each of them. In the context, the aim of this study was to evaluate the production of ligninolytic enzymes, laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP), produced by the white-rot fungus *Anthracophyllum discolor* under different culture conditions in order to optimize the potential of this strain for bioremediation of contaminated sites.
The effect of temperature (20, 25, 30 35 and 40 °C) on the fungus biomass production in potato-dextrose agar was evaluated. Also, the effect of pH (3 to 7), C/N ratio, and incubation temperature (25 and 30 ºC) in liquid medium, containing glucose as carbon and ammonium tartrate as nitrogen sources, were evaluated. The flasks were incubated for 21 days and the production of ligninolytic enzymes in the extracellular fluid of *A. discolor* was evaluated periodically.
In solid medium, the fungus biomass increased with the increment on incubation temperature, obtaining the highest growth rates (0.94 Day^{-1}) at 30 °C. In liquid medium, it was determined that the optimal conditions for enzymatic production were: pH 5, with low nitrogen concentration and 25 °C. In these conditions, the maximum values obtained for Lac, MnP, were 288.5 UL^{-1} and 4278.8 UL^{-1}, respectively. LiP was not detected in any of the conditions evaluated.
These results show the potential use of the fungus *Anthracopyllum discolor* for the degradation of wastewaters containing lignin compounds, as well as contaminated soils with these types of compounds.

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2525 RESISTANCE TO HIGH LEVEL OF CU (COPPER) BY ARBUSCULAR MYCORRHIZAL, SAPROBE FUNGI AND *Eucalyptus globules*

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The effects of saprobe and arbuscular mycorrhizal (AM) fungi on growth, chlorophyll, root length colonization and succinate dehydrogenase (SDH) activity was measured in *Eucalyptus globulus Labill.*, plants growing in soil with high level of Cu were investigated. The application of Cu inhibited the development of mycelia of the saprobe fungi *Fusarium concolor* and *Trichoderma koningii* and the hyphal length of the arbuscular mycorrhizal fungi (AM) *Glomus mosseae* and *G. deserticola* in vitro. The application to soil of 100 mg kg^{-1} of Cu decreases the shoot and root dry weight and chlorophyll content of the non-inoculated plants with AM fungi. However, *G. mosseae*, *G. deserticola*, *G. intraradices* and *G. claroideum* increased the shoot and root dry weight, chlorophyll content, AM root length colonization and the succinate dehydrogenase activity of AM mycelia of *E. globulus* in presence of 100 mg kg^{-1} of Cu. The inoculation of Eucalyptus with *G. deserticola* and *G. intraradices* enhanced the amount of Cu uptake (953 and 921 mg kg^{-1} shoot plus root respectively), by Eucalyptus plants. We showed that it is important to select the most efficient AM and saprobe fungi to stimulate plant growth in heavy-metal-contaminated soil (particularly with Cu), and that the combination of both plays an important role in metal tolerance of Eucalyptus plants.

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Keywords: Phytoremediation, heavy metal, soil contamination, microorganism, symbiotic, synergistic.

2547 EFFECT OF Pb AND Zn ON *Brassica fruticulosa* ANTIOXIDANT SYSTEMS

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Vast areas of South-East Spain have been submitted to exhaustive mining exploitation for the last 3,000 years. These activities, specially those carried out in the last 50 years operating in big-open cast mines and applying differential flotation techniques, have resulted in the spread of tons of lead, zinc, copper, and other heavy metals and metalloids on the land surface. Phytoremediation is a promising methodology that could be applied in order to mitigate the detrimental effects of toxic elements on human health and on the environment. The success of plant-based remediation programs is mainly depending on the appropriate selection of the plant species to be used. Plants must be able to tolerate, and eventually accumulate, high metal concentrations without severe impairing of their metabolism. In this work, the effect of lead and zinc, the main pollutants in the Cartagena-La Unión Mining District, on both enzymatic and non-enzymatic antioxidant systems from *Brassica fruticulosa* is showed. *B. fruticulosa* is a species belonging to the *Brassicaceae* family, as *Thlaspi* or *Arabidopsis* species are, that spontaneously grows on heavy metal-polluted areas. Seed-derived plants were used for analyzing the evolution of H_2O_2-detoxifying enzymes (catalase, ascorbate peroxidase, guaiacol peroxidase) in response to lead treatments (3000 and 6000 ppm). In general, the activities of H_2O_2-consuming enzymes were higher in roots and shoots of treated plants than those determined in control plants, suggesting the involvement of these enzymes in the tolerance of *B. fruticulosa* to lead. In other set of experiments the effect of zinc treatment on several components of non-enzymatic antioxidant system from in vitro-propagated plants was studied. Levels of phenolics, thiol compounds, and antioxidative activity present in extracts obtained from *B. fruticulosa* shoots showed significant differences with respect to non-treated material. The final goal of these studies is to ascertain the involvement of antioxidant systems in the tolerance to high concentrations of heavy metals. Results obtained would help to improve plant performance in response to pollutants and, in turn, to design and to apply more effective phytoremediation processes.

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2548 SALICYLIC ACID-MEDIATED RESPONSES OF *Teucrium carthaginense* ANTIOXIDANT CAPACITY TO HEAVY METALS

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Three thousand years of mining exploitation have led to the deterioration of vast areas of the Cartagena-La Unión Mining District (South-East Spain). Natural local vegetation has been seriously affected by these activities, and some plant species are considered to be critically endangered. In many cases the local plant species have been replaced by other, well-adapted to high heavy metal concentrations, foreign plants. There is a major concern among Administrations about the necessity of restoring soils and preserving autochthonous fauna and flora. However, these goals can be hampered by the poor adaptation of native plants to the new conditions in the area. The high concentrations of heavy metals, among other environmental factors, could provoke a stress state in plants, leading to their death. Salicylic acid (SA) is a phenolic compound involved in plant cell signaling. It is thought that SA can stimulate plant defenses to successful face challenges of biotic or abiotic nature. In this work, the effect of SA on the antioxidant capacity of *in vitro*-grown *Teucrium carthaginense* shoots is described. *T. carthaginense* is an endemic and endangered species from South-East Spain that only appears in the heavily-polluted surrounding areas. Treatment of *T. carthaginense* shoots with increasing concentrations of SA showed a maximum of free radical-detoxifying activity for a SA concentration of 0.1 mM. Subsequent incubation of SA pre-treated shoots with Pb, Zn or Cu showed complex responses depending on the metal considered and the method used for antioxidant assessment. In general terms, it can be said that SA pre-treatment protects DNA, lipids, and proteins from oxidative damage provoked by heavy metal treatment, suggesting that this could constitute a valid strategy for improving *T. carthaginense* tolerance to high toxic metal concentrations. Although more studies are needed in order to further evaluate the protective effects provided by SA on Teucrium plants, the results here described could help to design future preservation programs with the aim to reintroduce the species in its original habitat.

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2874 COMPARISON BETWEEN PB PHYTOEXTRACTION CAPACITIES OF A HALOPHYTE (*S. portulacastrum*) AND A GLYCOPHYTE (*B. juncea*)

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The aim of this study was to compare the effects of different lead concentrations on growth and Pb^{2+} accumulation in two species, the first one is a glucophyte: Brassica juncea and the second is a halophyte *Sesuvium portulacastrum*. After multiplication, the seedlings were cultivated on nutrient solution supplemented with different Pb^{2+} (0, 200-400-800 µM) concentrations. After 1 month of treatment, plants were harvested and the dry weight, Pb^{2+}, Ca^{2+}, K^+ and Fe concentrations in tissues (roots and shoots) were determined.
Results showed that *S. portulacastrum* is significantly more tolerant to Pb^{2+} than Brassica Juncea. In fact, the growth of B. juncea was decreased progressively with increasing Pb^{2+} concentration in culture solution. However, *S. portulacastrum* plant-growth was not modified by the presence of Pb^{2+} in the medium, even at the highest concentration (800 µM).
With respect to plant nutrition's, lead decreased significantly Fe and K concentrations in the shoots of both species. However these effects were more pronounced in *B. juncea*. Pb^{2+} was more accumulated in the roots than the shoots in both *S. portulacastrum* and *B. juncea*. In the shoots, S. portulacastrum accumulated more Pb than *B. juncea*. Hence, the amounts of Pb^{2+} sequestered in shoots at 800 µM were 973 µg/gDW and 609 µg/gDW respectively in *Sesuvium* and *Brassica*. This result was related to the capability of the first species to absorb and transport Pb^{2+} from roots to the shoots concomitant to the conservation of its growth potentiality. These results suggest that *S. portulacastrum* (Halophyte species) is more efficient to extract Pb than *B. juncea* (glycophyte species).

2879 EVALUATION OF THE CAPACITY OF *Tagetes foetidissima* TO ACCUMULATE COPPER *IN VITRO* AND GREENHOUSE CONDITIONS

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The use of plants to clean contaminated sites is called phytoremediation and it is used in many countries to clean water and soils. In the Pacific of Costa-Rica a big area was contaminated with copper due to banana activities. Normal soils have a copper concentration between 2-100 mg/kg but the soils of the pacific have concentrations between 662-718 mg/kg. Plants show toxicity signals with only 20-30 mg/kg inside the tissues; it is evident that these soils are unproductive. It is very important to find a plant that can be used to clean this site. We evaluated the capacity of different plants to accumulate copper. We found that *Tagetes foetidissima* has the capacity to hyperaccumulate copper *in vitro*. We use the tissue culture to clone this species and diminish the genetic variation. *T. foetidissima* plants with adequate size were put in medium Murashige and Skook with increasing concentration of copper (20, 24, 27 and 30 mg/L). The solution of copper was changed every 48 hours to keep constant. We keep these plants in this medium for 15 days, after we washed the plants, extract the total water and determinate the quantity of copper inside the plants by atomic absorption. We found that *Tagetes foetidissima* hyperaccumulated copper between 3602-5600 mg/kg. Although this species can accumulate copper *in vitro* it is necessary to evaluate hyperaccumulation in conditions more similar to reality. We are evaluating the capacity of this species to accumulate copper in greenhouse conditions, we should acclimatize the species and plant it in contaminated soil (from the pacific site) when it is 2 months old. We will evaluate during the vegetative development that is approximate for 5 months. We will make an accumulation curve and determinate the location (roots, leaf or steam) and quantify the accumulation of copper in this species. If this species will result hyperaccumulator of copper in greenhouse conditions, it could be a good candidate to be used for clean an important area of our country.

2898 INFLUENCE OF ELECTROKINETIC PROCESS ON SOIL PROPERTIES IN A PILOT SCALE ASSAY

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Electrokinetic remediation is a developing technique that has a significant potential for in situ remediation of low permeability soils contaminated with heavy metal and/or organic compounds. The technique involves applying a low direct current or a low potential gradient to electrodes that are inserting into the ground. Due to electromigration and electro osmosis processes, the contaminants are transported to either the cathode or the anode and collected near the electrodes. Then they are removed by pumping the water in the electrodes zone

The aim of this work is to evaluate the effect of this technique application on physicochemical and biological properties of a soil contaminated six years ago, considering the importance of the soil parameters to achieve a soil restoration process after remediation preserving soil functionality.

The study was conducted in 1.5 x 1.1 x 0.9 m containers with a ton of a loam sandy soil from Central Spain. Soil was artificially contaminated with heavy metal soluble salts, $K_2Cr_2O_7$ and $Pb(NO_3)_2$ at doses of 1,500 ppm of chromium and 4,750 ppm of lead respectively. Direct current (100 volts) was applied to the soil during 30 days. At the end of the experiment, soil properties were analyzed. Besides, all the effluent extraction were analyzed to control the nutrient removal from the soil.

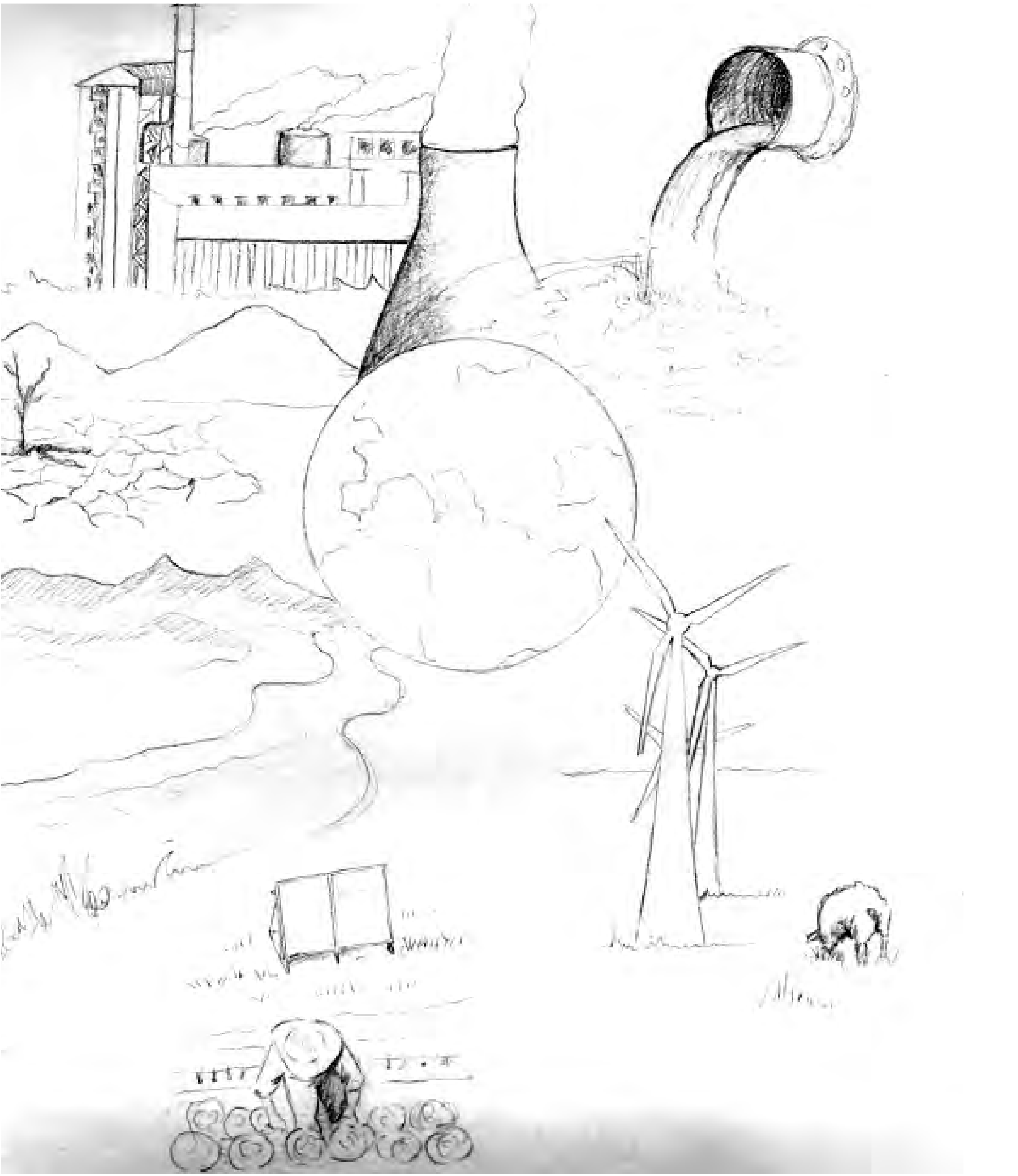
A good mobility of the contaminants was produced. However the mobility of chromium was lower than cadmium in the same conditions. Nevertheless, the total remove of soil pollutant were not achieved, even the removal yields were lower than those obtained when the technique was applied to recent contaminated soil, due to the effect of the time in the adsorption of the metals in the soil. In general, the effect of the technique produced a loss of mineral elements, and some changes in the soil structure due to the dragging in the pumping liquids of clay and slimes particles that go thorough the membranes.

No significant differences were observed when the soil biological activity was evaluated before and after remediation then application of the electric field doesn't seem to produce a measurable adverse impact in the assay conditions.

WEDNESDAY, 24 SEPTEMBER

POSTER COMMUNICATIONS

III.- Strategies for the protection and remediation of natural environments
IV.- Microbial Ecology
V.- Environmental education and legislative aspect



WEDNESDAY 24 SEPTEMBER 2008

III STRATEGIES FOR THE PROTECTION AND REMEDIATION OF NATURAL ENVIRONMENTS

Modelling and control of wastewater biological treatment systems

Techniques to prevent or reduce environment contamination

Sustainable agriculture

V ENVIRONMENTAL EDUCATION AND LEGISLATIVE ASPECTS

VI NEW PERSPECTIVES

Renewable energy and energy production from waste and wastewater

2194 ANALYZING THE BIOMASS FILTER BEHAVIOR IN AN ANAEROBIC WASTEWATER TREATMENT PLANT

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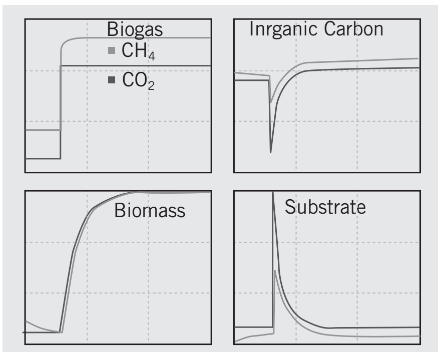
Nowadays, waste emissions in air, water and soils must be reduced in order to reach the more and more strict environmental rules. In the case of wastewater, there exists a big interest to improve treatment plants performances. The paper deals with the analysis, via the phase portraits method, of a biomass filter behavior in a completely stirred tank reactor for wastewater treatment. The main objective is to determine the effect of biomass filters on process properties e.g. stability, substrate degradation, biogas production, limits of operating conditions, with an automatic control and energy perspectives. A model experimentally validated of the anaerobic digestion process is implemented on specialized software. The biomass filter is integrated considering the biomass death rate in the model. Numerical simulations for several operating conditions are performed (Fig. 1), and then the process behavior is analyzed via phase portraits. Since this method is based on a nonlinear model, it allows to analyze the stability limits and the process behavior in face to changes on the operating conditions. The anaerobic process has two equilibria points: washout and functioning. Each one has an attraction domain which is separated by a socalled stability limit. Washout is characterized by the absence of biomass inside the reactor, the treatment is not possible, and then this point is to be avoided. Functioning point corresponds to adequate operating conditions for substrate degradation; this is the desired attraction domain for the process.

Fig.1. Anaerobic digestion variables



Results show that the anaerobic wastewater treatment process is improved using biomass filters. In fact, the stability limit is modified, reducing the attraction domain to the washout point and consequently enlarging the functioning area. The process is able to reject bigger disturbances on the influents and then it implies a bigger biogas production. Since the biomass filter improve the anaerobic wastewater treatment, it is necessary to find adequate mechanisms to implement this behavior in real processes. A current work is the materials characterization (natural zeolite, sand, biologic materials) with the perspective to be used as biomass filter.

2483 ALGORITHM FOR PARALLEL CONTROL FOR AN AEROBIC REACTOR

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Process control system typically utilize the same number of manipulated inputs and controlled outputs. A study biological system indicates that is possible to achieve superior performance by the judicious use of additional input variables. One can exploit the rectangular control structure to design redundant controllers working as a parallel control architecture which, under large load disturbance and control input saturations, can provide a smoother and safer process operation than the non-redundant control situation. In principle, a feedback control strategy designed within this parallel structure would lead to a closed-loop system with moderated control actions and improved performance.
The aim of this work is to propose a simple feedback control strategy for multiple-input single-output (MISO) linear plants. One obtains the control transfer function of each control input in a parallel control architecture. That is, every controller is driven by the same output regulation error. The parallel control scheme aimed to improve the disturbance rejection capabilities of the controlled process. A secondary variable is used in the above scheme mentioned to effectively reject disturbance to the biological system. In this way, the use of the recycle dilution rate as a secondary control input is in order to distribute the control effort. Simulation results are used to illustrate the control design methodology and the performance of the resulting controllers for an aerobic reactor.

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2219 INFLUENCE OF PINE OR OAK WOOD ON THE DEGRADATION OF ALACHLOR AND METALAXYL IN SOIL

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Sustainable technologies to retain or immobilize pesticides and, if possible, at the same time, able to facilitate their later degradation have been developed in the last years. They can contribute to reduce the point sources of water and soil pollution by pesticides and are decisive to avoid later difficult decontamination processes of soil and water. Wood can be good adsorbent of hydrophobic organic compounds due to the presence of lignin in its composition. The objective of the present work was to study the influence of pine or oak wood added to soil as amendment (5% w/w) on the degradation of two pesticides, alachlor and metalaxyl, with different hydrophobic character. The formation of pesticide metabolites and soil dehydrogenase activity of unamended and amended soil samples was monitored during an incubation period of 70 days for alachlor and 98 days for metalaxyl. Soil samples were spiked with alachlor or metalaxyl at 1 mg kg-1, and incubated at 20 ºC in the dark. Extracts at different sampling times were analyzed by HPLC-PDA-MS. Degradation of alachlor and metalaxyl in the soil samples studied followed first-order kinetics. Results indicated that degradation rate was slower for metalaxyl than for alachlor and followed the order pine amended soil < oak amended soil<unamended soil for both pesticides. Degradation rate was faster in unamended soil than in wood amended soil and this was attributed to the higher sorption of pesticides by wood amended soils compared to unamended soil. Furthermore, degradation rate was slower in pine amended soil than in oak amended soil due to the higher lignin content of pine wood responsible of the pesticide adsorption.The alachlor metabolite, alachlor ethane sulfonic acid (ESA), and two metalaxyl metabolites (2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid and N-(2,6-dimethylphenyl)-2-methoxy-acetamide) were detected at low concentrations during the incubation period. Soil dehydrogenase activity presented close values in unamended and amended soil for soil samples treated with alachlor. For soil samples treated with metalaxyl, dehydrogenase activity was higher in wood amended soil compared to unamended soil. Pine and oak wood must increase the immobilization of pesticides studied but they can also limit their bioavailability in soil decreasing their degradation rate in amended soil.

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2221 RETENTION OF PESTICIDES IN SANDY SOIL COLUMNS MODIFIED WITH A WOOD BARRIER

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Wood residues can be used as low-cost potential sorbents of hydrophobic pesticides in technologies aimed to prevent soil and water contamination and reduce the risk of environmental pollution produced by point pollution sources of these compounds. The objective of this work was to study the effect of a pine or oak sawdust barrier on the retention of linuron, alachlor and metalaxyl, with different hydrophobic character, in a sandy soil. Leaching of these pesticides in columns with a natural sandy soil and this soil modified with a wood sawdust barrier intercalated in the upper part of the column was carried out. The columns were washed by continuously applying 500 ml of water under a saturated flow regime. The breakthrough curves (BTCs) corresponding to the leaching of linuron, alachlor and metalaxyl and the distribution of these compounds in the soil column at the end of the experiment were determined by HPLC-PDA/MS. The modification of the sandy soil with a wood sawdust barrier gave rise to a delay in the maximum peak of BTCs for all pesticides studied compared to the natural soil. This delay represented 4 - 7 pore volume (PV) for linuron, 1 - 6 PV for alachlor and almost 1 PV for metalaxyl, approximately, compared to the leaching of these compounds in the column with natural soil. Moreover, the concentration of the maximum peak decreased, approximately, 6-3-fold for linuron, 4 - 2-fold for alachlor and 0 - 2-fold for metalaxyl. In general, the intercalation of a wood barrier of pine and oak sawdust in a sandy soil column gave rise to a decrease in the leaching of pesticides studied. However, the effect of the pine sawdust barrier was higher than that originated by the oak sawdust barrier. Furthermore, the efficacy was higher for the most hydrophobic pesticide, linuron, due to the higher sorption capacity of hydrophobic pesticides by woods. The BTCs obtained indicated an important modification in the leaching kinetics of these pesticides and a much slower washing process than in the natural soil. The degree of retention of the pesticide in the column depended on the wood type used for the barrier and the pesticide hydrophobic character.

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2295 EFFECT OF THE INCREASE OF 2,4,6-TRICHLOROPHENOL CONCENTRATION ON THE PERFORMANCE OF A FLUIDIZED BED BIOREACTOR

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The purpose of this work was to evaluate the effect of increasing the concentration of 2,4,6-trichlorophenol (TCP) on the performance of a fluidized bed bioreactor (FBBR). An initially partially-aerated methanogenic FBBR was operated for more than 360 days in three stages with increasing concentrations of a mixture of TCP and phenol (Phe) in the synthetic influent 120/30, 240/60 and 480/120 mg/L of TCP/mg/L of Phe as only carbon sources. The mesophilic, laboratory-scale FBBR was loaded with 1mm diameter granular activated carbon, colonized *ab initio* by an anaerobic undefined microbial. Before our experiments, the FBBR operated as a partially-aerated methanogenic system. The bioreactor was aerated with a 2 vvm airflow rate and operated a 1-day hydraulic retention time.
Average removal efficiencies of two first operational stages were high and similar with values of TCP removal efficiency $\eta_{TCP} \geq 99.7\%$, $\eta_{Phe} \geq 99.9\%$; $\eta_{COD} \sim 97\%$. However, in the third stage at the highest influent concentrations of TCP/Phe, removal efficiencies of TCP and COD decreased to averages 95.7% and 90%, respectively; in contrast, the η_{Phe} remained very high. Transient low concentrations of 2,4-dichlorophenol (0.05 mg/L) and 4-chlorophenol (0.07 - 0.26 mg/L) were observed at the beginning of the first stage. Afterwards, their concentrations decreased below the detection levels. Similarly during the first days of operation of the second and third stage, transient small peaks of 2,4,6-TCP (1.57 mg/L), 2,4-diclorophenol (0.056 mg/L) and 4-chlorophenol (0.056 – 0.18) and 2,4,6-TCP (3.12 mg/L), 2,4-diclorophenol (0.12 mg/L) and 4-chlorophenol (0.10 – 0.21) were found respectively. In the first and second stages, GC-MS analysis of effluent confirmed the absence of other degradation intermediates typical of the aerobic degradative pathway of chlorinated phenols, such as chlorinated aromatics and aliphatics typical of aerobic degradation pathways chloro-catechol, chloro-muchonic acid, etc. The patterns of TCP removal and concentration of intermediates suggest that there was only a slight and short term performance deterioration due to acclimation and adaptation to sudden jumps in xenobiotic concentrations at the beginning of the first and second stage. In the third stage, low concentrations of less chlorinated chlorophenols intermittently appeared and disappeared; this, along with the lower η_{TCP} suggested that the FBBR response to TCP loading would be hyperbolic, whereas further increases of TCP concentration would lead to its critical loading point.
Interestingly, methane in the biogas was not detected (below 0.2%) and the specific methanogenic activity of bioparticles from the reactor bed was below the detection level of the assay. In contrast, PCR-DGGE analysis revealed bands of methanogenic archaea in samples of the first stage that further attenuated or disappeared below the limit of detection of our PCR procedure. Dissolved oxygen in the effluent as well as aerobic specific respiration rate of bioparticles slightly increased with time, from 0.12 to 0.20 mmol O_2 /h gTKN. Net increase of chloride was high in effluents of first and second stages and near the theoretical value for 100% dechlorination of TCP which was consistent with the chromatographic analysis of xenobiotics, whereas its value in the third stage averaged approx. 85%. Long-term aeration of the FBBR seems to shift from partially-aerated methanogenic conditions to fully aerobic. Our FBBR holds promise for the effective removal of high concentrations of TCP and its chlorinated intermediates, particularly for groundwaters polluted with such substance and where the presence of easily biodegradable organic matter is negligible.

2390 PREPARATION OF NEW BIODEGRADABLE MATERIALS BY GRAFTING OF POLYCAPROLACTONE ONTO STARCH AND THEIR BIODEGRADABILITY STUDIES

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The starch is a natural polymer which has the advantage of being biodegradable, renewable and available in quantity unlimited at very accessible prices. However its poor mechanical properties, depending on its hydrophobic character, and also its absorption of water restrict its applicability considerably especially for packing.
Polymer blends and composites of starch (plasticizer or granular) and aliphatic, e.g, polycaprolactone, are of great interest as new biodegradable polymer materials. However, conventional melt-processing usually provides the starch-based composites with very poor mechanical properties, mainly due to thermal decomposition of starch, strong water absorption and poor interfacial adhesion. In order to overcome these drawbacks, physical (hydrophobic coating, cross-linking, addition of external plasticizers) and chemical (grafting reactions and substitution of the hydroxylic with functional group like esters, ethers, isocyanates, anhydride) modifications of the starch have been performed. In this work, we present the chemical modification of starch so as *in situ* ROP (Ring Openinig Polymerization) of ε-caprolactone monomer in melt phase using catalysis. It results a covalent grafting of hydrophobic polyester chains onto starch which reduce the strong absorption of water. We study also the biodegradability process of these new materials using ISO14855-1 and ISO14855-2 International standards.

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2456 THE ACTION OF SELECTED DISINFECTANTS ON CANINE ENDOPARASITE EGGS

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The goal of the work was to monitor occurrence of helmintologic disorders of dogs in the district of Košice-surroundings, to evaluate a potential devitalising effect of selected chemical substances and disinfective agents: NaOH (5%, 70 °C), Savo (10%), Saniten (10%), H1 (100%), J (100%) on eggs of *T. canis* under laboratory conditions. Using coprologic examination we realised that in dogs faeces most common are eggs or *T. canis* (41.4 %). Then follow eggs of *Trichuris* sp. (21.8%), *T. leonina* (11.5%), eggs from the group *Ancylostomatidae* (9.2%) and *Capillaria* sp. (2.3%).Under laboratory conditions the ovocide effectiveness of NaOH on nonembryonated eggs of *T. canis* by an exposition of 180 minutes was 23.98 ± 4.33%. Low effectiveness had also the disinfective agent SAVO, which by an exposition of 180 minutes devitalized 24.77 ± 5.33% of eggs of *T. canis*. Higher effectiveness had the disinfective agent Saniten. Using the disinfective agent Hviezda, 98.04 ± 1.77% eggs of *T. canis* were devitalized. Based on the testing of each agent under laboratory conditions we can recommend the usage of the disinfective agent Hviezda while it proved the best devitalizing effect on the eggs of *T. canis*.

2605 ASSESSING THE GRASS SCHIZACHYRIUM GRACILE FOR CAPACITY TO ECOLOGICALLY RESTORE THE POLLUTED SOILS OF ECOSYSTEMS IN A BAUXITE MINING AREA

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Abandoned bauxite mines in the tropical forests of the Dominican Republic's only biosphere reserve are leaving behind extensive areas and landfills, whose negative impacts need restoring because of the ecological interest of the sites they occupy. Given that any realistic recovery program should be based on knowledge of the ecological succession, in this report we present the results derived from the study of two populations of a pioneer species of these ecosystems. The species, *Schizachyrium gracile*, is a native grass of the family Poaceae and was collected both from its natural setting and when growing spontaneously in the secondary succession of the landfills that now occupy the site of an old tropical pine forest (*Pinus occidentales*), which was completely destroyed through bauxite mining activities.
The samples analysed were 3 populations of the grass taken from the unaltered ecosystem and a further 3 from the degraded ecosystem. Chemical analyses performed on the aboveground parts of plants revealed significant or highly significant differences between the two ecosystems in terms of the levels of macronutrients (N, Ca, Mg, K and Na) and micronutrients (Fe, Mn, Zn and Cu) in the grass. Significant or reliable differences were also detected for the heavy metals Cd, Ni and Cr, and for Al. We found no Pb or As in the plants. Higher mean contents of N, P, K and Mg were recorded in the samples from the unaltered ecosystem, while average Ca, Fe, Mn, Zn, Cu, Cd, Cr, Ni, and especially Al contents were higher in the populations from the degraded ecosystem. Differences between mean contents according to the ecosystem were particularly high for Fe (1803.1 versus 224.7 mg/Kg) and Al (3648.9 versus 269.4 mg/Kg). Differences were less marked in the roots although they reflected the trends observed in the aboveground portions.
Chemical analyses were complemented with cell studies based on electron microscopy observations (SEM in conditions of controlled pressure or vacuum). These observations revealed both the presence of further elements (Si, Ti, S and Cl) and greater proportions of Al and Fe in the cells of plants growing in the area of the abandoned bauxite mine. Our findings indicate that this species is particularly capable of accumulating Al in its aboveground mass (up to 9320 mg/Kg) and that it can also capture varying amounts of Cu, Zn, Cd, Cr and Ni.

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2899 DETERMINATION OF DORAMECTIN, AN EMERGENT VETERINARY CONTAMINANT, IN SOIL AND MANURE, USING HPLC WITH FLUORESCENCE DETECTION

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Doramectin is a macrolide endectocide belonging to avermectins group; it is widey used as antihelmintic because of their broad spectrum activity against ecto- and endo-parasities of cattle, pigs, sheep, goats, horses as well as cats and dogs. All avermectins are excreted mainly through faeces as non-metabolised drugs. Consequently, the application of residues from cattle to soil could represent a source of diffuse pollution, being the veterinarian treatments emergent contaminants towards soil and water.
The aim of our work was to develop an analytical method to determine doramectin in soil and manure samples in order to evaluate the potential diffuse pollution when these residues from cattle are applied to agricultural soils.
Doramectin was extracted from samples with acetonitrile, clean up using solid phase extraction and analysed by HPLC with fluorescence detection after derivatization with N-methylimidazole and trifluoroacetic anhydride. The method presents good recovery in the range 72-99% at different concentrations levels (2.5-42 ng/g soil) and a low detection limit (0.1 ng/g) defined as three times the signal-to-noise.
This method was applied to evaluate the doramectin levels in pig manures stored for one year before its use as organic amendment in an olive grove. Preliminary results showed concentrations of this emergent contaminant around 2 ng/g, so that studies about its environmental fate are necessary in order to control its behaviour in the soil-water system.

2900 EFFECT OF THE ADDITION OF SAND ON THE ENZYMATIC ACTIVITY OF CARBONATED SOILS CONTAMINATED WITH METALS

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Heavy metals can alter the composition, structure and metabolic activity of soil microbian communities. These alterations can be used as quick and sensible bioindicators of changes in the soil quality; therefore the study of enzymatic activity in the soil represents one of the prior objectives to determine its status.
The activity of deshidrogenase, β-galactosidase, alkaline phosphatase and urease, enzymes which are involved in the biogeochemical cycles of the main macronutrients, has been analyzed.
The work was carried out with carbonated soils coming from the experimental farm "El Encín", located in the river Henares corridor area. The soils, once collected, were contaminated with a mix of Pb, Cd, Zn, and Cu in two different concentrations (RD.86/278/CEE). Parts of the soils were amended with sea sand resulting on mix of 60/40 in weight of soil/sand. All measurements were duplicated.
The results obtained show that heavy metals significantly inhibit the activity of these four enzymes for the two contamination levels tested, corresponding the higher inhibiting effect to the higher metal concentration.
The decrease of enzymatic activity shows an elevated sensibility to the contamination with metals at concentration levels allowed by current legislation, in spite of soils being carbonated, thus having a high capacity to immobilize metals.
The addition of sand to these soils produces a significant increase of all valued enzymatic activities and a decrease, also significant, of the inhibiting effect of metal contamination on the analyzed activities.
These observations suggest that the addition of sea sand to carbonated soils contaminated with heavy metals could be used as a method to improve the soil activity.

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2905 ANAEROBIC AND AEROBIC SLURRY BIOREACTORS FOR REMEDIATION OF A HEAVY SOIL CONTAMINATED WITH LINDANE

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Slurry bioreactors can be used for bioremediation of polluted heavy soils such as those characterized by high contents of clay and organic matter, when the contaminatns are recalcitrant, toxic, and display hysteretic behavior, a and/or when bioremediation should be accomplished in short times under the pressure and monitoring of environmental agencies and regulators. Lindane or γ-hexachloro-cyclohexane (HCH) is a toxic and recalcitrant chlorinated insecticide widely used in developing countries, particularly in Mexico. The aim of this research k was to evaluate the effect of electron acceptor and supplementation of degradable organic co-substrate sucrose on lindane removal from a clayish agricultural soil with high levels of organic matter, using batch slurry bioreactors. A simple 3 x 2 factorial experiment was carried out with factor electron acceptor at three levels (aerobic, methanogenic and sulfate-reducing SB, *i.e.* A-SB, M-SB and SR-SB, respectively) and factor initial sucrose concentration at 0 a 1 g/L.
There was no general trends on effects of main factors; in fact, a strong interaction between factors was found. In this way removals of lindane in SBs without sucrose followed the order A-SB ~ SR-SB >> M-SB (86, 82, and 41%, respectively). On the other hand, the order of lindane removals in SBs with supplementation of sucrose was SR-SB > A-SB >> M-SB (88, 77, and 46%, respectively). Sucrose supplementation had a distinctive and positive effect on lindane removals *in only the anaerobic* SBs. Lindane-clastic bacterial counts were ca. 8 as log CFU for A-SB independently of sucrose or no sucrose addition, 6.5 in M-SB, and 7 in SR-SB. Biotic control A-SBs, and SR-SBs exhibited reductions of lindane in the order of 55% suggesting that the soil native microflora itself had a significant capacity for degrading or transforming lindane under the corresponding conditions. However, M-SB biotic controls could only remove 25% of lindane; this was consistent with lindane-clastic bacterial counts ca. 6.7, 5.7, and 5.0 in the aerobic, sulfate-reducing, and methanogenic biotic controls, respectively. Inoculation with acclimated biomass seemed to increase lindane removals by an absolute 25% (relative 35%) for A- and SR-SBs, whereas the positive effect on M-SB was outstandingly higher (absolute increase of 25% and relative increase of 90%). On the other hand, removals of lindane in abiotic control SBs were very low, with an average of 11%. The excellent performance of SR-SBs for lindane removal could point out to a promising alternative for the treatment of heavy soils rich in sulfate salts. On the other had, M-SBs poorer performance precludes its application as a main remediation option although it could be used as soil pre-treatment followed by one week aeration (conversion to A-SB); in this way substantial savings in aeration costs could be accomplished.

2912 ENHANCEMENT OF HEAVY METALS PHYTOEXTRACTION ON AN INDUSTRIAL CONTAMINATED SOIL USING EDTA AND NTA

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Large areas of agricultural soils from Romania are contaminated by heavy metals that mainly originate from industrial emissions.
To achieve high heavy metals removal rates, the concentrations of soluble heavy metals in the soils must be enhanced. Several chelating agents can effectively increase the solubility of metallic contaminants in soils and subsequently enhance their uptake by crops.
In the pot experiments, described in this study, we tested the direct and remanent effects of applying chelating agents (EDTA and NTA) on the accumulation of heavy metals by plants. Maize and *Phacelia tanacetifolia* have been used as test plants. Two experiments have been set up in the Green House in two time periods (Cycle I, Cycle II) using a soil material from an industrial contaminated area (Neferal – Acumulatorul, near Bucharest). Taking into account the heavy metals contents, the soil material can be considered high polluted with Cu (379 $mg.kg^{-1}$), high polluted with Zn (644 $mg.kg^{-1}$) and very high polluted with Pb (573 $mg.kg^{-1}$). The treatments with EDTA (0 - 2.7 $mmol.kg^{-1}$) and NTA (0 - 5.4 $mmol.kg^{-1}$) were applied before seeding, at the beginning of the experiment (Cycle I).
Statistical data revealed that there are significant differences as concern to the biomass and the Pb, Cu and Zn accumulation in the shoots related to the treatments applied. Also, the residual treatments influenced the weight and height of plants and the heavy metals accumulation.
The Pb, Cu and Zn concentrations in the shoots of maize and *phacelia tanacetifolia* were directly proportional to the amount of EDTA and NTA added to the soil. Some other results demonstrated significant decreases for soil reaction both Cycle I and Cycle II experiments.

2913 STRATEGIES TO REDUCE THE ENVIRONMENTAL IMPACT CAUSED BY THE POTENTIAL LOSSES OF N IN SOIL AMENDED WITH ORGANIC RESIDUES

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For many years, nitrogen mineral fertilization has been regarded as a most highly productive and profitable farming practice. The downside, however, is represented by the negative environmental repercussions of its use. A potential source of N is found in organic residue, which has increased dramatically due to human activity. For instance, organic debris generated in urban areas and resulting from intensive livestock breeding. This field study analyzes N transformation using different sources of nitrogen (organic and mineral) in an almond tree plantation. It aims at evaluating three types of organic residue as sources of N as well as their short-term impact on soil quality in an almond tree plantation. The following composts were used: A mix of manure and plant residue from a green (organic) farm (E); sewage-sludge-and-pruning-waste compost (S) and a mixture of composted sewage sludge and swine slurry and manure (X).Two compost doses determined according to N content were compared. A high dose (H), which corresponds with the 210-Kg-N/ha input. A low dose equivalent to 105 Kg N/ha (L). Composted soils (E, S, X) treated with the low dose were compared with other plots also treated with an L dose of compost and a mineral N supplement, namely an NPK fertilizer 5:15:15, which was applied at a dose of 0.45 t/ha (O+M). Chemical variations in the soil were analyzed two months after applying both the organic and mineral fertilizers. Both N-NO_3^- and respirometry were used to analyze the transformation of the N sources under study. Generally speaking, C, N and P levels increased in all the treatments with respect to the control treatment (unamended). There were, however, no significant differences among treatments and doses. Supplementary mineral N did not affect the chemical characteristics of the organically amended soils studied, such as pH, electrical conductivity, organic matter content, as well as N and P levels. Electrical conductivity, however, increased significantly in soils treated with mineral fertilizer and compost E. Nitrate content increased between twofold and sevenfold (E to L) with respect to the control treatment. Respiration increased in all those soils treated with compost. The mineral N supplement along with the organic amendments caused a decrease in soil activity, which resulted in lower CO_2 production, with lower N and O.M. contents. Interestingly, these soils also showed lower nitrate concentration when compared to soils that did not receive a N supplement. Nitrate concentration went from 68.75 to 25.49 mg/Kg in soils supplemented with mineral nitrogen. The mineral nitrogen supplement in organically amended soils seems to have led to a decrease in biological activity with respect to the same soils when only treated organically. This may be due to an environmental unbalance caused by a competition over N-substrate. The short-term nature of the study limited the possibility of determining the exact balance between soil quality and the environmental impact of nitrogen transformation caused by mixed fertilization (organic-mineral).

2916 METALS AND METALLOIDS ACCUMULATION BY WILD PLANTS FROM A MINING ZONE OF MEXICO

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In extreme environments, as mineralized soils, there are adapted organisms to these abnormal conditions. Plants that inhabit these kinds of soils are called metallophytes, and their capacity to tolerate and/or accumulate metals is important for the cleanup of metal polluted ecosystems. The objective of this study was therefore to identify the species of metallophytes present in the mining zone of Zimapan, Hidalgo. For it, plants and rhizospheric soil samples were collected, and analyzed in order to quantify As and Pb concentrations. In addition to this, an As speciation was carried out to determine the form in which As is been assimilated by the plants. At the moment we have identified two Pb accumulator plants; *Notholaena sulphurea* and *Argyrochosma formosa* which accumulated into their roots 1 102.84 mg kg^{-1} and 2 385.95 mg kg^{-1} respectively. Both plants showed high translocation factors (bigger than 3), but small bioaccumulation indexes (less than 1 %). For Pb, we have found three accumulator species; *Viguera dentate* (716.67 mg kg^{-1} in leaves and stem), *Eschscholzia californica* (1 324.02 mg kg^{-1} in leaves and stem) and *Bricrellia veronicifolia* (626.22 mg kg^{-1} in roots). High translocation factors (bigger than 2) and high bioaccumulation indexes (bigger than 29 %) were observed in all of this plants The information obtained from As speciation indicated that As V is the predominant specie like $HAsO_4^{2-}$ and $H_2AsO_4^-$, which are absorbed by the plant. The results suggest that five plants identified have potential for application in remediation of metal in contaminated soils.

2918 COMPARISON OF ALTERNATIVE METHODS FOR THE CONTROL OF POTATOES NEMATODES (*Globodera rostochiensis* AND *Globodera pallida*) IN SOILS O SA POBLA. MAJORCA, SPAIN

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Among phytoparisitic nematodes, *Globodera rostochiensis* and *Globodera pallida* represent one the higest problem of potato crop and as so need to be controlled years after years. Traditionnaly, this control was performed through soil chemical disinfection before starting the crop. Nowadays most of the products used for this disinfection are eliminated from the market due to the UE directive 91/444. For this reason since some years new control methods such as solarization and biofumigation are evaluated. Solarization consists in watering the soil until maximum capacity and covering it with a perfectly sealed plastic film for 6 to 8 weeks during the summer. In this way the sun heats the soil producing a disinfection effect. From its side, biofumigation consists in incorporating to the soil organic plant residues or agroindustrial waste. In this case, the disinfecting effect is obtained through the gases and other products liberated during the degradation of this organic matter. Wastes of crucifers are often used for this purpose since they contain sufur compounds with known nematicide properties. During the present study both techniques were tested in a field of SA Pobla (Majorca) which is usually used for potato crop. A previously non cultivated plot was subdivided into 24 sub-plots to which were applied 6 different treatments: No treatment (control plot), treatment by solarization, treatment by biofumigation using (1) organic animal wastes or (2) cabbage waste or (3) turnip waste or (4) composted mushroom waste. The same treatment was applied in 4 different plots. The nematodes present in the plots before and after treatment were determined through kist extraction according to Fenwick method. Viable nematodes were counted under microscope. The treatments were applied during 8 (control and solarization) and 7 weeks (biofumigation). Following the treatments, the levels of *Globodera* spp. decreased by: 22 % for the control plots, 55 % for the plots treated by solarization and 38 % for the plots biofumigated with animal organic waste. Contradictory results were obtained for the plots fumigated with plant organic wastes. Indeed while the number of nematodes decreased in some of the replicate plots they increased in others giving very high standard deviations on average values. A preliminary analysis of these results suggests that solarization and biofumigation with animal manure are effective methods for the control of potato nematodes. Biofumigation tests with plant organic waste must be pursued in order to adjust application dose and period of application since good results using this method have been reported by others. Particularly it would be interesting to test plant waste abundant at the vecinity of the fields to be treated. Moreover it is important to study not only the impact of biofumagation on the amount of a phytoparasitic nematode such as *Globodera* spp, but also how biofumigation affect the soil environment.

1791 DROUGHT EFFECT ON GROWTH, GAS EXCHANGE AND YIELD IN TWO LOCAL BARLEY LOCALITIES UNDER WATER DEFICIT CONDITIONS IN THE SOUTH OF TUNISIA

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Drought is a worldwide problem, constraining global crop production seriously (Wang et al., 2003). It is an important abiotic factor limiting yield, and barley seems to be relatively well adapted to water deficit (Ceccarelli, 1987). In Tunisia, barley is reserved for the semiarid and arid zones in the middle and the south. The remarkable resistance of the photosynthetic apparatus to dehydration (Quick et al., 1992) suggests that plant survival under drought results partly from the maintenance of photosynthetic capacity by the leaves, thus allowing rapid recovery of the plant after re-hydration. Plant strategies to cope with drought normally involve a mixture of tolerance and stress avoidance mechanisms. In order to screen drought tolerance in barley, gas exchange, growth and yield were investigated in two barley (*Hordeum vulgare* L.) localities (Switir and Tlalit) from the south of Tunisia (respectively from Medenine and Tataouine). A glasshouse experiment was conducted in the south of Tunisia and local barley (cv. Ardhaoui) was grown on sandy soil. Treatments consisted of deficit irrigation (50% of field capacity (FC)) and full irrigation (100 of (FC)) from tillering to maturity using meteorological data of the experimental site. Our results suggest that the rainfed treatment recorded the lowest net photosynthetic rate (A). Nevertheless, Tlalit kept the highest A at tillering and proved to be the least affected. At tillering, we observed a reduction of leaf area (LA) in Switir. Values recorded in irrigated and stressed regimes for Tlalit are almost the same. Re-watering increased leaf appearance rate (LAR) which was about 0.26 leaf day^{-1} for stressed plants. Switir and Tlalit are strongly affected and their grain yield hardly exceeds 1.54 g plant^{-1}. Thus, Tlalit recorded the highest grain yield. Analyses of variance showed that the effect of locality was highly significant on grain yield ($P < 0.001$). The developmental stage had a highly significant effect ($P < 0.01$) on LAR and LA. In summary, the present results clearly show that Tlalit tolerates water stress more than Switir. So it would be preferable to integrate Tlalit's seeds in semi arid regions of the south of Tunisia in order to enhance yield.

1816 ANTINUTRITIONAL EVALUATION OF THE FLOUR OF THE NIPPLES OF HOLM OAK (*Quercus ilex*) AND OAK CORK (*Quercus suber*) RAW AND PRESSURE-SEALED

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The present study contributes to the development of the food potential of the nipples of oak like new resource likely to be exploited on an industrial scale for their use in animal feeds. Our work relates to two species S of nipples of oak, edible, the holm oak and the other fodder one, the oak cork known S for their spontaneousness and their very significant geographical distribution. The oak offers among its multiple products, of tannins which are extracted in great quantity in the barks and also on the level from the fruit but with variable quantities. Certain studies showed that the nipples of Algerian oaks contain a rather significant content tannin which varies between 0.2 and 0.9%. The analysis of the phenolic compounds, enabled us to highlight two types of tannins, digest and hydrolysable. The determination of the tannin rates reveals their variation according to the vegetable species considered, but also of the state of maturity of the fruit. Condensed tannins are present in high at the fodder species compared to the edible species. There is a correlation between condensed tannins and toxicity. The contents tannins hydrolysables vary relatively with the species. A low content characterizes the edible species whereas the fodder species are richly equipped with it.
The presence of tannins or other toxic substances not yet identified in the believed nipples are probably at the origin of their bad nutritional effectiveness. Consequently, the presence of such compounds in a diet not controlled could have annoying consequences during a voluntary and abusive consumption of massive amounts of the nipples of fodder species by the cattle. The objective would be to decrease this high content in tannins by heat treatments for an improvement and of a durable consumption or to carry out the extraction of the various toxic tannic fractions before nourishing the animals. The hydrothermal treatment (pressure-sealing with 100 °C Pd 30 mn) appears adapted best to detoxify the nipples of the alleochimic substances, without involving serious losses in nutritive principles. It thus allows a reduction of the anti-nutritional effect which tannins of the nipples believed in the level of the digestive tract exert.

1817 BIOCHEMICAL AND NUTRITIONAL STUDY OF THE NIPPLES OF ALGERIAN OAKS FOR THEIR USE IN THE ANIMAL FEEDS.

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The fodder intensification constitutes a major concern for the farmers, this comes owing to the fact that currently Algeria knows a deficit in the fodder production and out of food of cattle more particularly. It devotes enormous efforts to increase the production and much resources to face this deficit by the importation in the capacity as raw materials intended for the industry of food of cattle is 209,646 $, in particular the various types of oil cakes of but, soja sunflower. In order to highlight the importance of the Algerian flora from the economic point of view and with an aim of discovering new food resources as well animal as vegetable, which can contribute in a direct way to the revival of the agricultural sector, current biochemical research tasks convergent towards a valorization of the natural substances likely to provide glucids, lipids and proteins. Of this rebirth of interest with regard to the floristic resources in general and forest products in particular, it appeared interesting to us to conceive a new approach of the nipple of oak in order to emphasize the possibilities of valorization of this fruit. The idea of development of the nipple appeared in the majority of the country having the oak with an aim of benefitting from the food potential of its pulp on a scale laboratory and from industry, in order to study the economic viability of the transformation of the nipple by its conservation into flour like food of cattle. Our study relates to the determination of the chemical composition of three species of oak, edible, the holm oak and two fodder, the oak cork and the kermes oak. In the light of the results obtained, it arises that the fractions on the one hand glucidic, represented particularly by the starch (51.63% of the matter dry) and on the other hand lipidic (7.64% of the matter dry) constitute the major part of the reserves of the believed nipples of holm oak, cork and kermes. The contents mineral matter, proteins and crude fibre are relatively weak for the three species of oak, and account for 2.23% respectively; 7.16% and 2.66% of the matter dry.

2195 ENZYME ACTIVITIES AND MICROBIAL INDICES OF MEXICAN VOLCANIC SOILS UNDER DIFFERENT MANAGEMENTS

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Soils at the Mexican Trans-volcanic Belt are extremely important because the lack of agricultural land in overpopulated areas in Mexico. In addition, contents of soil organic matter (SOM) have been declining since the Mexican fields have been cultivated intensively. The aim of this work was to study how different agricultural management practices affect the SOM quality, using biochemical and microbiological parameters as indices.
Selected experimental plots, located in the Mexico's Central Volcanic Belt (Atécuaro catchment; State of Michoacán), were managed according to 4 different managements: Traditional (Tt); improved (Ti); organic (To); and fallow system (Tf). An uncultivated soil under grass cover (Sg) was used as reference.
Soils of the area are *Acrisols*, acid and rich in clays and sesquioxides. Composed soil samples were collected at 0 - 10 cm depth and chemically analyzed. Soil organic C, total N, water-soluble C, humic C, microbial biomass, soil respiration, net N mineralization, dehydrogenase activity, arginine ammonification, and extra-cellular enzymatic activity were determined.
The biological activity had a favourable response to the addition of composted and farm manures (To treatment), and to mulching and fertilizer inputs adjusted to the crop demand (Ti treatment). The increase of values of biochemical and biological parameters with the two latter treatments was related to an increase of the SOC content; hence, both managements contributed to the regeneration of the degraded *Acrisol* studied. In addition, results indicated that managements that minimize the input of organic residues toward the soil (Tt and Tf) decreased, in general, the enzymatic activities, affecting the soil capacity to recycle and release available nutrients to be absorbed by plants.
Results also demonstrated that the biochemical indices studied could provide good indications about the SOM quality attained under different managements applied to these volcanic soils.

2224 BARLEY SEEDS COATING WITH HUMATES-PHOSPHATASE COMPLEXES IN ORDER TO IMPROVE P UPTAKEAND PLANT GROWTH

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Although plants may uptake some forms of organic phosphorus compounds, most of them must be first mineralized to inorganic forms to become available to plants. This hydrolysis is catalyzed by extracellular phosphatases produced by plant roots and microorganisms when plant P availability is limiting. P fertilizers added to soil rapidly become unavailable to plants by forming insoluble P compounds. Furthermore, continuous and massive application of these chemical fertilizers is one of the major causes of eutrophication. In order to find natural fertilizers more convenient to sustainable agriculture, we intend use seed coating with immobilized phosphatases as a tool to enhance P bioavailability and minimize pollution. Barley seeds were film coating with *E. coli* alkaline phosphatase free and immobilized in soil humates [1], using methylcellulose (MC) as a binder. Additionally, seeds were encapsulated in calcium alginate hollow beads with both free and immobilized humates-phosphatases. Germination tests, phosphatase activity in the rhizosphere and the effect of seed coating on phosphorus mobilization in soil and plant growth were assessed. After assaying five different MC concentrations and enzyme/MC ratios, the best results of seed film coating with immobilized and free enzymes (45% and 30% phosphatase activity retention, respectively) were obtained with 4% MC. In these experiences, yield differences depended on binder concentration and free or immobilized form of the applied enzyme. Seeds encapsulated in hollow beads with free enzyme showed coating yields around 21% in all enzyme/CMC ratios. Nevertheless, encapsulation with humates-phosphatase complexes decreased from 25 to 11% enzyme activity retention when enzyme/CMC ratio was enlarged from 0.02 to 0.20 g/ml. On the other hand, the percentage of germination using free phosphatase remained almost unchanging (97 - 99%), confirming the utility of these methods. Fim coating and hollow beads encapsulation with phosphatase-humates complexes showed 83.8 and 84.5 - 44.3% germination rate, respectively. To study the effect of seed coating on rhizosphere phosphatase activity, a qualitative technique was used [2]. Finally, we found that the soil samples cultivated with the coated seeds increased their inorganic P content (38%) and that the seed coating improved the P uptake (29%) and plant growth (increased 35% of the shoot dry weight).

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2289 SOIL PROTECTION THROUGH ALMOND TREE CULTIVATION

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Most threat to soil are particularly severe in areas with step slopes and suffering dry periods followed by heavy rain such as the Mediterranean regions. Severity is aggravated by lacking or inappropriate farming systems. Therefore the objective of this work was to demonstrate that land management based on cultivation of new varieties of local crops (almond trees) suited to these conditions may result in a sustainable system to prevent soil degradation. Three experimental sites with different were selected after a previous study of several zones in Murcia region (SE Spain) and Matera (SE Italy) attending to soil characteristics and climatic conditions. Each selected Site was divided into two sub-plot in order to perform the almond cultivation under two different farming systems (traditional and organic). Soils for organic cultivation were amended with ecological compost at a rate of 20 t/ha. Two different rootstocks, franco and GF677, grafted with different almond tree varieties were selected for this study, three trees of each almond variety/rootstock combination being planted in each sub-plot. The orchard fields were set up in late May 2006 and soils were sampled immediately after planting and each four months. Both mineral and organic amendment improved soil nutrient status with respect to the control sites, which lead to the stimulation of the activities of the enzymes involved in C (β-glucosidase), P (phosphatase) and N (urease) cycles. In turn, soils under organic farming showed higher organic carbon content and higher microbial population size and activity than those under conventional farming. Differences in soil properties due to the rootstock type or almond tree variety were not observed neither at the second sampling nor at the third sampling (nine months after planting). However, a certain influence of tree cultivation on some of the monitored parameters was observed from 9 months after planting (sampling of February and June 2007), cultivation contributing to maintain soil organic carbon content through root exudates and root debris, and to activate soil microbial growth and activity. One year after planting differences in soil properties were observed between the conventional and organic farming system, the effect of the organic amendment being still notorious.

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2290 ECOLOGICAL FARMING SYSTEMS IMPROVE SOIL MICROBIAL QUALITY AND SUSTAIN PRUNE TREE PRODUCTIVITY

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The present concern for protecting a natural resource as important as the soil has promoted changing from conventional agricultural farming systems to more sustainable production systems, such as ecological production, tending to the conservation of soil quality and productivity. Although Ecological Agriculture is increasingly gaining adepts, there are many unknown aspects with regards this production system. Therefore, this work was aimed to evaluate the incidence on yield and soil and prune crop quality of different ecological types of fertilization, as well as to compare the results obtained with these organic production systems with those obtained with a conventional one. To this, different plots were established in an orchard devoted to prune cultivation, sited in the North of the Murcia Region, and submitted to the following treatments: a) a conventional farming with use of inorganic fertilizers and pesticides; b) annual addition of ecological compost (32 kg/tree); c) biannual addition of ecological compost; d) annual green amendment (sowing dose 120 kg/ha); e) biannual green amendment; and f) annual addition of a commercial biofertilizer (*Azospirilium brasilense* + *Pantoea dispersa*). All treatments were performed in triplicate. Soils were sampled for analysis three times at year during two years: immediately after treatment addition, at the middle of the crop, and after harvesting. Parameters such as total organic carbon, soil respiration, ATP, dehydrogenase activity, and hydrolase activities, related to soil quality were analysed in soil samples. Biometric parameters on trees and fruits were also determined as well as yield. Yields obtained under the ecological farming systems were higher than the average yields obtained under conventional farming. The best results were obtained with the compost and biofertilizer treatments, although differences not always were statistically significant. Plots submitted to green amendment showed lower yields than the rest of the ecological treatments, probably due to the fact that the organic matter incorporated to the soil with this practice is very young promoting a great stimulation of soil microbial populations, which will compete with the plant for nutrients. Conversely, this kind of organic fertilization followed by compost addition was the best option to encourage soil microbial activity. All microbiological and biochemical parameters determined in soils showed higher values under ecological farming system than under conventional agricultural farming highlighting the suitability of such ecological production for soil protection.

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2319 CONSERVATION TILLAGE INFLUENCE ON SOIL CARBON AND AGGREGATION IN SEMIARID RAINFED AGRICULTURE (SW SPAIN)

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Soil aggregation has a strong influence on soil physical properties. The aim of this study was to determine the influence of two tillage systems, traditional tillage and conservation tillage, on soil aggregate size distribution and water aggregate stability. Two experiments were established: a long-term experiment (15 years), in which traditional tillage (TT_{15}) and reduced tillage (RT15) were compared, and a short-term experiment (3 years) in which TT_3 and no-tillage (NT_3) were compared. A wheat (*Triticum aestivum*, L.)-sunflower (*Helianthus annuus*, L.) crop rotation was established in all treatments. In 2005 a fodder pea crop (*Pisum arvense*, L.) was included in the rotation. Dry aggregate size distribution (ASD), mean weight diameter (MWD) and aggregation index (AI) values were slightly greater in both conservation tillage treatments than in the corresponding TT treatments. However, water stability (WAS) values for 1-2 mm size aggregates were greater in TT_{15} than in $RT_{15,}$ despite the soil organic carbon (SOC) and $CaCO_3$ contents in these aggregates were greater in RT_{15}. On the contrary, this variable was slightly greater in NT_3 than in TT_3. Aggregation is a temporal variable parameter, product of interactions of the soil biota, mineral and organic components, that is affected by soil use and management, especially under no-tillage.

2397 ALMOND TREE AND LAND MANAGEMENT PRACTICES FOR SOIL EROSION PROTECTION IN MEDITERRANEAN AREAS

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The soils of many European regions are frequently exposed to erosion and desertification processes. These are particularly severe in areas with steep slopes and suffering dry periods followed by heavy rain such as the Mediterranean regions. This study is focused on demonstrating that the cultivation of almond trees suited to these conditions and a proper land management, may result in a sustainable system to prevent soil erosion. The selected experimental sites, at three different slopes (0.2 and 6%), were located in Basilicata (Southern Italy) and in Murcia regions (Southern Spain). Each selected site was divided into two sub-plots in order to perform the almond cultivation under two different farming systems (mineral and organic) and the soils were sampled every three months for one year.
Biological, chemico-physical, and structural parameters were considered in order to asses the soil vulnerability and effects of management practices in contrasting soil erosion.
Dehydrogenase enzyme activity, which is considered an index of soil microbial metabolism, resulted higher in 0 and 2% slopes with respect to 6% slope. This activity was found correlated with water soluble carbon, an easily mineralized substrate, suggesting an interaction between the available energy-rich compounds and biological processes. Soil permeability (which is influenced by soil physico-structural properties) increased in 0% and 2% slopes only in organic farming system, suggesting that the addition of organic matter can improve soil physical structure, thus preventing soil erosion. In 6% slope not significant differences were observed in chemico-physical parameters between organic and mineral system, probably because at this slope the effects of the management practices will be more evident during the time of planned experiments. In addition, different treatments showed different fine clay-mineral dispersion in water extract, meaning different capacity of soil to form stable aggregates.

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2893 INFLUENCE OF GREEN TOP COVER FOR SOIL PROTECTION IN SPANISH OLIVES TREES. ROOT TENSILE STRENGTH

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Natural development of certain top living covers is the best way for soil protection under arid climate conditions. A number of certain gramineas was experimental field trial for 5 years looking for soil cover protection and adaptability under the olives tree rows.
Olives trees are a fully expanded crop in Spain occupying 2.5 million of hectares. Is very interesting crop because one ha of pre-treated olive tree pruned residues will produce 0.3 t of ethanol (Cara, C. et al. 2007)? We are beginning a research to divide the tree in two zones according with the N-W cardinal point orientation. This is because cultural practices must be in relation with the physiological facts in which the trees spend efficiency the water by evaporation and get energy by sun to perform the photosynthesis. Cultural practices, like irrigation and pruning trees, must include parts of the tree because they not react equally to the cardinal point orientation mainly in agricultural precision practices. We include as new cultural practices the presence / not presence of top green cover under the trees.
The cultivated olive tree (*Olea europaea* L.) is known to be sclerophyllous plant and effective at tolerating drought (Moreno, F. et al. 1996).
Nine years old young trees were used of two varieties numerated at random inside the row, of three rows field experience. On a farm call "La Malena" Experimental field was layout 4 km from Linares town, (Jaen) Spain. On a farm call "La Malena" UTM coordinates X448300Y4219715.
The two varieties used were: Picual and Arbequina, including two collecting time data: winter/spring, two cardinal point orientation: N-W and with Picual variety we design the drought induced stress plot by no irrigation.
We have found differences in N-W oriented leaves both in photosynthesis measured by florescens rise OJIP test and stomatal conductance. And no influence of the green top cover on the size of the olive tree fruits.

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2917 EQUILIBRIUM BETWEEN ENERGY SAVING AND IMPROVEMENT O ENVIRONMENTAL QUALITY VERSUS AGRICULTURE PRODUCTIVITY

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During decades, an inadequate use of agricultural techniques has lead to negative consequences for the environment. One of these consequences corresponds to the loss of organic matter resulting in soil erosion. Other type of problems corresponds to the saturation of the soil capacity to accept contaminants. When thinking about environmental quality as one of the major problems to be solved by European politics, very often the important role of soil is forgotten, particularly that of agriculture for the ecological equilibrium. The systems of production for sustainable agriculture, especially in the case of cereals, are directed to a reduction of soil mechanical preparation. This type of agriculture, known as "conservation agriculture" consists on leaving in the fields the organic residues resulting from the crop. Such a production system allows reducing energy consumption thanks to a decreased number of tractor rotations and so diesel consumption. This is extremely important in this century where the crisis resulting from oil resource exhaustion start to be seen. The decrease of reactor rotation number results also in less soil compacting and emission of green house gases and so on less damage caused to the environment. The main objective of this work was to compare the "conventional" and "conservation" agricultures in terms of energy savings measured as diesel consumption and soil quality determined by the measurement of chemical characteristics, particularly the changes in nutrient content such as C, N and P, but also the biological activity evaluated through the microbial biomass content. The study was performed with barley (Hordeum vulgare) crop, from sowing to harvest, corresponding to a period of 6 months. The sowing was done with a tractor by the conventional way and in parallel by direct sowing on a field where the residues of the previous crop had been abandoned on the soil after harvest. Analysis of barley productivity showed a non significant decrease between the conventional and direct sowing from 1944 to 1742 kg/ha. This decrease was not important taking into account the high reduction of diesel consumption (calculated for all the performed activities) achieved with direct sowing. From a soil quality point of you, the characteristics which were evaluated during the 3 crop periods (sowing, germination and harvest) showed a slight improvement of the organic, nitrogen and phosphorus content when a direct sowing and an abandonment of stubble had been applied. Concerning the biological activity no significant differences in terms of microbial biomass were found between the two crop management modes. The slight differences observed for the soil characteristics in the two cases were more related to the abandonment of the residues left after the previous harvest than to direct sowing. Since this study is starting and so do not have sufficient background it needs to be pursued for a longer period in order to determine if the "conservation" management has really a positive effect on soil quality which could compensate the apparent loss of productivity, a loss already compensated by a lower energy consumption.

2919 MANAGEMENT OF ORGANIC RESIDUES AS POOLS OF NITROGEN IN TOMATO CROP ("RAMILLETE"), A VARIETY COMMONLY GROWN IN MAJORCA.

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Growing "ramallet" tomatoes (bunch tomato) is very traditional in Majorca. This variety is characterized by its resistance to drought and its long post-harvest conservation period. Some studies conducted on this type of tomato aim at finding alternative management practices to improve both production and quality. Since it is also a major crop on the island of Majorca, it is intended that tomato quality and production go hand in hand with minimum environmental impact. Normally, slow-release nitrogen mineral fertilization is used in tomato fields of this sort. There are, however, other types of potential organic fertilizers on the island, such as sewage sludge, swine slurry, and manure. Therefore, this study focuses on comparing the effect of a slow-release nitrogen fertilizer, and two different types of compost on bunch tomato (ramallet) farming. The first compost type was solely made up of sewage sludge whereas the second one consisted of a mixture of sewage sludge, swine slurry and manure. The aim was to assess the best farming practices involving the aforementioned compost types and their effect on production, quality, and fruit preservation, as well as on chemical characteristics of the soil. Three different doses of the two compost types were used.Namely, 3,500, 7,000, and 10,000 Kg/ha. These were compared to a slow-release NPK fertilizer (14-17-17) plus potassium sulphate. Soil sampling took place at different stages of development. Samples were collected 40 and 100 days after the application of all the treatments (organic and mineral), as well as at the time of harvest. Soil chemical parameters associated with fertility were measured at each stage, along with ammonium and nitrate levels. Three developmental stages were studied, where the following fruit parameters were analyzed: fruit weight, fruit number per surface area, hardness, pH, and acidity. Generally speaking, no significant differences in soil characteristics were found between soils treated with the two different kinds of compost. The 40-day measurement showed a significant increase in electrical conductivity at the highest dose of both types of compost. However, the most significant and worth-highlightingn increase was that of electrical conductivity in soils treated with slow-release mineral fertilizer, which went up to 2.24 dS/m. On the whole, production saw an increase as the dose of fertilizer also went up. Production went from 64% at the lowest dose to 76% at the highest dose, when it came to mineral fertilization. Consequently, based on the data collected, it could be said that the production – soil preservation balance would make organic fertilization more environmentally profitable than mineral fertilization.

2206 DETERMINATION OF ENZYMATIC PROFILE OF THE LIGNINOLYTIC FUNGI ISOLATED FROM MEXICO

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The State of Hidalgo (Mexico) has a large extent of forest, that is the Huasteca Hidalguense where there is a big amount of microorganisms, these represent an important resource from the ecologic and biotechnological point of views due to them can be used in a broad variety of industrial processes with the climatic conditions of this place the fungi must be thermopile or at least thermotolerant, since the temperatures can be higher to 50 °C in summer and this temperature in declines to 20 °C in winter. The applications of ligninolytic fungi are based on their capacity to produce enzymes of industrial interest, this topic has been a continuous investigation for investigators and industries. Among the most important enzymes we can find the protease which are widely used due to its biotechnology applications with a higher economic impact. Other enzymes, Laccases, Peroxidases and Lipases can be used in industries of Hidalgo State, especially in the Textile Industry specifically in the effluent processing.
156 fungi were collected in the Huasteca Hidalguense of which 100 were isolated in Potato Dextrose Agar and maintained in inclined tubes. After that, the enzymatic activity was determined, laccase, protease and Lipase in plate. The purpose is to select the fungi with a higher potential for their biotechnological applications. The fungi generally grow to 30 °C and these one growth to 37 °C and presented enzymatic activity. The results were present like relationship between enzymatic activity and rate growth. 51 fungi presented lipase activity, 3 protease activity and 63 laccase actitivity and in most of the case the enzymatic activity was higher than rate growth indicating that the fungal isolated have a great biotechnology potential.

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2273 POTENTIAL OF THERMOPHILIC MICROORGANISMS FOR THE DEGRADATION OF SYNTHETIC POLYMERS

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Nowadays, synthetic polymers are used in all areas of human activity, mainly due to their high stability against environmental conditions and microbial attack. However, these properties are also a problem from an environmental point of view, and thus it is necessary to find biodegradable synthetic polymers that can be easily removed in nature after disposal, and decomposed into biomass, CO_2 and water.
Polyester-based materials are a primary example of synthetic biodegradable plastics. The first step in their biodegradation is the cleavage of ester bonds, after which the solid polymer is progressively broken down to short chain soluble intermediates and monomers. The ability of specific microorganisms or enzymes to degrade polyesters is often assessed by evaluating their capacity to hydrolyze ester bonds in model molecules.
In this work, whole cells of lipolytic enzymes-producing thermophilic microorganisms from the genus Thermus have been used for catalyzing the hydrolysis of dibutyl adipate, a small diester that could be used as model substrate for aliphatic polyesters biodegradation. Diester hydrolysis was determined by automatic titration, at pH 8.0, in a temperature-controlled and magnetically stirred system. First of all, the kinetics of dibutil adipate hydrolysis with respect to substrate concentration was investigated, both with and without addition of a surfactant to the reaction medium. When no surfactant was present, the reaction rate appeared to be mostly independent of dibutyl adipate concentration, probably due to limited interfacial area for activity. Also, when the influence of the biocatalyst concentration on the reaction rate was assessed, a linear relationship was found, up to a certain enzyme concentration. The influence of temperature on hydrolysis rate was investigated, as well as the possibility of biocatalyst reutilization. Repeated batches allowed discovering the deleterious effect of surfactants (Triton X-100, Genapol X-80, and Tween 80) on reuse of the catalyst. Surfactants might remove hydrolytic enzymes from the cell membrane, thus reducing activity in successive batches.

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1815 TREATING SWINE WASTE WITH ANAEROBIC SEQUENCING BATCH REACTORS AT HIGH AMMONIA LEVELS: PERFORMANCE AND MICROBIAL COMMUNITIES

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The capability of anaerobic systems to produce large quantities of biogas from animal wastes has been demonstrated, however, a lack of knowledge on the bacterial composition within these systems prevails. Four 5-liter anaerobic sequencing batch reactors (ASBRs) were operated for over two years treating swine waste (with low solids levels – 20 g volatile solids [VS]/l) at 25 °C. During the first year (0-378 days), all four ASBRs were operated at similar conditions. In particular, total ammonium concentration was kept at ~1200 mgNH_4^+-N/l. During the second year (379-730), total ammonium concentration was increased in two out of the four reactors to ~4000 mg-N/l, while keeping the other ASBR pair at relatively low levels (~1200 mg-N/l). The reactors performance was assessed with conventional techniques and additional tests were performed on the biomass of the ASBRs. For instance, methane yield was not statistically different among the four reactors in the first year, and was 0.32 ± 0.020 l CH_4/g VS (volatile solids fed). For the second year, methane yields were ~0.30 ± 0.018 and ~0.17 ± 0.007 l CH_4/g VS for low and high ammonia reactors, respectively. Therefore, the performance of the anaerobic digesters was indeed affected with increasing total-N concentration (subsequently increase of free ammonia level as well). The bacterial community structure was assessed through 16S ribosomal RNA gene sequencing surveys for inoculums, swine waste, and the ASBR biomass samples at low and high ammonia levels. Even though the reactors performance was affected by total ammonium addition, bacterial community analyses did not show any significant difference between low and high ammonia biomass samples neither at phyla levels nor at species levels, with *Firmicutes* (~55%) being the most predominant phylum, followed by the phyla *Bacteroidetes*.

1831 PNT EFFECT ON DIVERSITY OF RHIZOBIA ASSOCIATED WITH SESBANIA SESBAN

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Tilemsi rock phosphate (TRP) of Mali is one of the best rock phosphates in West Africa. But it is less used because of it's insoluble form.

The main objective of this study is to investigate it's effect on rhizobia living under a multipurpose leguminous tree was been investigated. The substrate was Senegalean soil which was less poor in phosphorus and not sterilized. The experience included treatments with TRP and treatments without TRP. The study was conducted during 105 days and nodules was been taking of every 21 days. At the end of experience, 116 nodules were been collected and analysed by molecular tools (PCR/RFLP).

In the results, 16 strains of rhizobia have been detected from 116 nodules. The presence of rhizobia strains varied in presence or absence of the insoluble phosphate.

It seem that TRP of Mali has an effect on rhizobia diversity contrarily to an soluble phosphate (TSP) which haven't no effect on rhizobia diversity under the same leguminous (SACKO, poster AABNF 2004, Dakar, Sénégal).

1877 PETROLEUM-INFLUENCED BEACH SEDIMENTS OF THE CAMPECHE BANK, MEXICO: DIVERSITY AND BACTERIAL COMMUNITY STRUCTURE ASSESSMENT

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In Mexican, either spilled or seeped out petroleum impacts nearly 300 km of the beach between Dos Bocas (Tabasco State) to Champoton town (Campeche State), where between 9 to exceptionally 300 tonnes of oil as tar balls have been measured. This study was focused to explore, for the first time, the bacterial diversity and community structure (α-diversity) -in a kilometric scale- on petroleum influenced sediments of ~100 km the sandy beach.
Molecular tools were based on DNA extraction, from both sediments and sediment bacterial strains; 16S-23S Ribosomal Intergenic Spacer Analysis (PCR-RISA) and rDNA libraries construction. Banding (risotype) patterns were obtained and rarefaction analysis performed. By using RISA bands as operative taxonomic units, Shannon (H) and Simpson (D) diversity indices were calculated. Fourteen plasmid inserts from rDNA libraries were sequenced (GenBank EF191388 to EF191401), and the taxonomic placement of these sequences was done with the Classifier tool RDB II.
Risotype patterns revealed different ·-diversity of bacterial communities along the shoreline. Based on ecological indices, diversity varied inversely to the degree of petroleum hydrocarbon influence: the highest petroleum concentration (range 8,379.0 mg kg dry sediment^{-1} – 47,645.0 mg kg dry sediment^{-1}), the lowest the alpha-bacterial diversity (H=0.80; D=0.45). For low petroleum influenced sediments (range 97.0 mg kg dry sediment^{-1} – 547.0 mg kg dry sediment^{-1}), diversity was the highest (H = 2.14; D = 0.88). Rarefaction showed that sediments with a medium oil-influence (range 1,223.0 mg kg dry sediment^{-1} – 7,490.0 mg kg dry sediment^{-1}) harbored more number of risotypes that the remaining oil-influenced sediments. In addition, 50% sequences were associated with Unclassified Bacteria, while the remaining 50% was ϒ-Proteobacteria (Eubacteria).
Petroleum affected both the bacterial diversity and community structure in the beach studied, where ϒ-Proteobacteria might be the foremost component. Further studies should be addressed on the inventorying of the unidentified bacterial component, as well as the genetic capabilities of beach bacteria. Genes associated with the transformation of petroleum hydrocarbons and production of bioactive compounds could be of potential biotechnological application in beach clean up activities upon oil spillage.

1884 QUANTITATIVE SHIFTS IN METHANOGENIC COMMUNITY STRUCTURES IN ANAEROBIC DIGESTERS TREATING VARIOUS WASTEWATERS

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Anaerobic digestion has been widely used in wastewater treatment due to the low formation of sludge and production of methane, a valuable energy source. Although several previous studies have focused on the microbial behaviour in this useful bioprocess, little quantitative information about how methanogenic communities evolve and grow in anaerobic processes is at hand. Therefore, this study aimed to quantitatively investigate and compare changes in methanogenic community structures in relation with process data. For the purposes, three lab-scale batch digesters treating different wastewaters (i.e., synthetic glucose wastewater, whey permeate, and liquefied sewage sludge) were operated. All digesters were seeded with anaerobic sludge obtained from a local municipal wastewater treatment plant. Dynamics of methanogenic populations were assessed at the taxonomic levels of order and/or family using real-time PCR targeting 16S rRNA genes. The monitoring results demonstrated that, in each digester, quantitative structure of methanogenic community varied continuously over operating time and such variations were generally well related to process performance data. Biphasic production of methane associated with successive increases in aceticlastic (mainly *Methanosarcinaceae*) and hydrogenotrophic (mainly *Methanomicrobiales*) methanogens was clearly observed in all digesters. This corresponded to the diauxic utilization of acetate and longer chain volatile fatty acids (C3-C6), mainly propionate. The non-metric multidimensional scaling (NMDS) analysis based on the real-time PCR quantification results showed that the methanogenic communities in three digesters shifted in quite different ways. Considering the operating conditions in all digesters were identical except for substrates, these differences were probably due to the effect of different substrate compositions. This suggested that the wastewater treated could directly affect the shaping of methanogenic community structure in an anaerobic digestion process. This conclusion is contrary to the conventional wisdom that methanogenic communities change little in terms of diversity (i.e., qualitatively), suggesting the need to pay attention to quantitative as well as qualitative approaches.

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1886 MOLECULAR TRACKING OF UBIQUITOUS ACIDOGENS IN ANAEROBIC PROCESSES TREATING VARIOUS WASTEWATERS: QUALITATIVE AND QUANTITATIVE STUDY

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Acidogens play a primary and critical role in producing substrates for methanogens, but an imbalanced acidogenic community can cause the accumulation of unfavourable acidogenic intermediates (i.e. sour reactor). Such a phenomenon results in inhibition of methanogenesis leading to poor performance and even to process failure. This is why we often see digester upset during the start-up period. Nonetheless, as yet, relatively little attention has been paid to the behavior of acidogens during this critical phase which is probably most decisive for the imbalanced acidogenesis. On this, we aimed to identify and quantify prominent and actively growing bacterial populations in the start-up period, which are potentially responsible for the initiation of overall process and the process imbalance. For the purposes, three anaerobic batch reactors treating different wastewaters (i.e., synthetic glucose wastewater, whey permeate, and liquefied sewage sludge) were operated. Bacterial population dynamics in the start-up of each reactor was assessed by using a combination of denaturing gradient gel electrophoresis (DGGE) and real-time PCR techniques. The DGGE profiles suggested that the bacterial populations assigned to *Aeromonas* and *C. sticklandii* were suggested to be common and functionally important in all trials. Two distinct primer and probe sets targeting Aeromonas and C. sticklandii were newly developed and applied to quantitatively assess the dynamics of target populations in relation to process data. The real-time PCR results clearly confirmed that *Aeromonas* and *C. sticklandii* were commonly prominent and actively growing in our systems. *Aeromonas* and *C. sticklandii* populations accounted for up to 86.6 to 95.3% and 8.5 to 55.2% of total bacterial 16S rRNA genes during the start-up phase. Furthermore, considering their putative roles and process performance profiles, our results suggested that these two populations would be functionally important as well as ubiquitous in all reactors studied. This hinted that they may play critical roles in anaerobic digesters regardless of the substrate composition. To the best of our knowledge, this is the first report on comparative quantitative monitoring of ubiquitous acidogenic populations in distinct anaerobic digesters. This study also discusses the development of a new tool for quantification of key acidogenic populations commonly found in all trials.

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2148 EVALUATION OF THE NATURAL ATTENUATION POTENTIAL OF *S*-TRIAZINE-CONTAMINATED SOIL-WATER SYSTEMS USING THE GENE ATZB AS A FUNCTIONAL MARKER

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s-Triazine compounds form a common group of herbicides used for the control of a wide variety of broadleaf weeds in agricultural soils. For this reason, the presence of these herbicides in the soil-water system is considered an environmental hazard. Functional gene-targeted fluorescence *in situ* hybridization (FISH) is an advantageous technique as compared to traditional approaches restricted to culturable microorganisms. Since most strains that mineralize s-triazine harbour atzB gene, nucleotide sequences of specific regions of this gene could be powerful probes to detect bacterial strains capable of removing these herbicides from soil. We propose a new approach to evaluate the natural attenuation capacity of the soil-water system microbial population based on fluorescence *in situ* hybridization (FISH). A specific oligonucleotide probe AtzB1 was designed based on the sequence data of the atzB gene involved in the hydrolytic deamination of s-triazines. This gene, located in a multiple copy plasmid was detected by the optimized FISH protocol in pure cultures of simazine-degradative bacteria such as *Pseudomonas* ADP. The FISH protocol was applied on two agricultural soils (Lodi and Henares), and two aquifers with a different history of s-triazines contamination. AtzB1 probe-target cells were found in the Lodi soil in a greater percentage, compared to the Henares one. Moreover, the greatest percentage of AtzB1 probe-target cells in Lodi was accompanied by a greater mineralization rate, compared to the Henares soil. AtzB positive cells were also found in the herbicide-contaminated aquifer, whereas a non-specific fluorescence signal was observed in the non-contaminated one. The presence of this gene in the autochthonous microbial populations suggests not only the effective applicability of this molecular technique in monitoring active bacteria, but also the presence in the studied environmental samples of a specific metabolic capacity involved in the potential herbicide degradation. The FISH method used in this study was suitable for the detection of s-triazine-degrading bacteria and could be a useful indicator of the potential of the soil-water system bioremediation.

2159 COMPARISON OF CLASSICAL AND MOLECULAR TECHNIQUES FOR THE DETERMINATION OF MICROBIAL BIODIVERSITY IN MEDITERRANEAN CROP SOILS

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Soil microbe ecology has lately become increasingly important in the study of soil microbe populations. Direct extraction of soil bacteria DNA allows PCR and DGGE analyses for the microorganism identification of these complex samples, avoiding the need for time-consuming culture-dependent techniques. The aim of the present study was to compare the two techniques (culture-dependent and molecular culture-independent) in four different plots of maize (*Zea mays* L.) crop under irrigation in south-western Spain. The soils, classified as District Luvisol, were studied under three management regimes (conventional tillage, direct seeding, and direct seeding with cover). Soil samples were collected every two months, and the microbial populations were analyzed by identification of the species using seven culture media and DNA-PCR-DGGE techniques. Some limitations and special features of each technique are discussed, including how different microorganism groups are detected in each. The microbial biodiversity determined by culture-independent techniques was lower than by culture-dependent techniques. Complementary data extracted with the two techniques showed, however, that each had its advantages and disadvantages.

2161 CONSIDERATIONS ABOUT THE FREEZE STORAGE OF SOIL SAMPLES FOR FUTURE MICROBIAL ANALYSIS

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Soil microbial populations are preferably analysed from fresh soil samples, but this may not always be feasible in field conditions. We studied the effect of freeze storage (at -20 °C, up to 44 months) on soil culturable microorganism viability. Most culturable soil bacteria were not affected by the prolonged freeze storage and freeze-thaw stress, but the viability of the fungus and sporulating bacillus populations significantly decreased after 8-12 months frozen. These changes did not, however, significantly affect the total culturable microorganism count, biodiversity index, or differences in biodiversity between soils. Therefore, freeze storage for up to 8 months may be allowed for analyses of culturable microbial population biodiversity, and longer times may be reasonably acceptable.

2197 RATE GROWTH COMPARISON OF BASIDIOMYCETOUS FUNGI ISOLATED IN MEXICO

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Huejutla de Reyes is a place with a warm-humid climate and counts on an annual average temperature of 30° C. We collected fungi that growth in wood or trees with the purpose of isolation this ligninolytic fungi in two seasons (one in spring, before raining station and another one in autumn, during raining station). The experimental work was done in the laboratory of the university, where the fungi were isolated in Petri dishes with culture media to Potato Dextrose Agar (PDA), the culture means were supplemented with Ampicillin and Chloranfenycol to avoid the growth of bacteria, and also add Benomile to avoid the growth of ascomicetos fungi. This means of culture was prepared with the purpose of isolating basidiomycete fungi solely. In total we collected 156 fungi and isolated 99 fungi (61 Fungi of the first collection and 38 fungi of the second collection). We determinated the radial rate growth of the fungi in three different temperatures: 32, 37 and 42 °C. The rate growth of the fungi was determined by measuring the diameter of the fungus as it was growing in the Petri dishes. The data were treated with the SPSS statistical package for one-way ANOVA and the statistical level of significance for all treatments was 5%.
The results indicate that during raining station the fungal variability was higher than spring season. 59% of fungi analyzed was a higher radial rate growth at 32 °C; 37% at 37 °C and 4% at 42 °C indicating that this last fungi are thermophilic. 48% of fungi are thermotolerant. The rate growth is different between 0.05 to 2.2 mm/h in the first collection and 0.4 to 3.9 mm/h.

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2198 IDENTIFICATION OF LIGNIONOLYTIC FUNGI ISOLATED IN MEXICO USING ITS LIKE MOLECULAR MARKER

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Huejutla de Reyes is a place with a warm-humid climate and counts on an annual average temperature of 30 °C. We collected fungi that growth in wood or trees with the purpose of isolation this ligninolytic fungi in two seasons (one in spring, before raining station and another one in autumn, during raining station). The experimental work was done in the laboratory of the university, where the fungi were isolated in Petri dishes with culture media to Potato Dextrose Agar (PDA), the culture means were supplemented with Ampicillin and Chloranfenycol to avoid the growth of bacteria, and also add Benomile to avoid the growth of ascomicetos fungi. This means of culture was prepared with the purpose of isolating basidiomycete fungi solely. In total we collected 156 fungi and isolated 99 fungi (61 Fungi of the first collection and 38 fungi of the second collection). To identification of isolated fungal we use Internal Transcribed Spacer like molecular marker, so we extract de DNA of each fungi and amplified this region by Polimerase Chain Reaction using universal primer for this region. Later, the DNA obtained was sequenced and compared with sequence previously reported in GeneBank data base. We isolated fungi species belonging to species like *Trametes, Fomes, Pycnoporus* and *Ganoderma*.

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2200 DETERMINATION OF ENZYMATIC PROFILE OF THE LIGNINOLYTIC FUNGUS ISOLATED FROM MEXICO

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The State of Hidalgo (Mexico) has a large extent of forest, that is the Huasteca Hidalguense where there is a big of microorganisms, these represent an important amount from the ecologic and biotechnological point of views due to them can be used in a broad variety of industrial processes with the climatic conditions of this place the fungus must be thermopile or at least thermotolerant, since the temperatures can be higher to 50 °C in summer and this temperature in declines to 20 °C in winter. The applications of ligninolytic fungus are based on their capacity to produce enzymes of industrial interest, this topic has been a continuous investigation for investigators and industries. Amongst the most important enzymes we can find the protease which are widely used due to its biotechnology applications with a higher economic impact. Other enzymes, Laccases, Peroxidases and Lipases can been used in industries of Hidalgo State, especially in the Textile Industry specifically in the effluent processing. 156 fungi were collected in the Huasteca Hidalguense of which 100 were isolates in Potato Dextrose Agra and maintained en inclined tubes. After that, the enzymatic Activity was determined, laccase, protease and Lipase in plate. The purpose is to select the fungi with a higher potential for their biotechnological applications. The fungi generally grow to 30 °C and these one growth to 37 °C and presented enzymatic activity. The results were present like relationship between enzymatic activity and rate growth. 51 fungi presented lipase activity, 3 protease activity and 63 laccase actitivity and in most of the case the enzymatic activity was higher than rate growth indicating that the fungal isolated have a great biotechnology potential.

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2202 ABILITY OF LEGUMES TO REGENERATE MICROBIOLOGICAL PROCESSES IN SOIL OF TUNISIAN ARID REGIONS

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Nitrogen fixing legumes and their microsymbionts are a fundamental contributor to soil fertility and prevent their degradation in arid and semi arid ecosystems. In Tunisia, few data are available on the contribution of these legumes in microbial activity in the arid soil. In this objective, a study was undertaken on five leguminous species from different arid regions to evaluate their ability to regenerate microbiological processes of the soil: *Genista saharea, Genista microcephala, Acacia tortilis ssp raddiana, Retama raetam* and *Prosopis stephaniana*. Soil was sampled from the rhizosphere of plants and soil's microbiological state was studied through biological parameters (microbial biomass, basal respiration and metabolic quotient of soil) and by biochemical parameters (enzyme activities). All analysed soils showed that legumes enhance soil fertility and microbial activity as judged by increase of all parameters. So, we concluded the role of legumes in improving soil quality in arid regions.

2256 A REVIEW ON DEGRADATION OF HEXACHLOROCYCLOHEXANE AND INVOLVED ENZYMES

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Lindane or γ-hexachloro-cyclohexane (γ-HCH) is a chlorinated insecticide widely used in developing countries, particularly in Mexico, in spite of its banning in first world countries. Negative impacts of lindane on the environment and human health have been reported worldwide. The main goal of this work is to present a critical review on the degradation of γ-HCH in the context of pathways and the associated enzymatic and molecular mechanisms.
The scope of this paper encompasses the following subjects: (i) aerobic and anaerobic degradation pathways of γ-HCH; (ii) the instrumental methods for identifying and quantifying intermediate metabolites, such as GC-MS and other techniques; (iii) important genes and enzymes involved in the metabolic pathways of γ-HCH degradation; (iv) advances on identification and characterization of strains and consortia able to transform and degrade γ-HCH.
It can be concluded that typical anaerobic and aerobic pathways of γ-HCH are well known for a few selected microbial strains, although less is known for anaerobic consortia where the possibility of synergism, antagonism, and mutualism can lead to more particular routes and more effective degradation of γ-HCH. Chromatography coupled to mass spectrometric detector, especially GC-MS, is the most used technique for resolving for γ-HCH metabolites, although there is an increased participation of HPLC-MS methods. Scintillation methods are very useful to assess final degradation of γ-HCH. Enzyme and genetic characterization of the molecular mechanisms involved are in their early infance; more work is needed to elucídate them in the future. Advances have been made on the enzymes of *Sphingomonas paucimobilis* where the gene LinB codifies for the enzyme haloalkane dehalogenase that acts on 1,3,4,6-tetrachloro 1,4 cyclo hexadiene, thus debottlenecking the pathway. Studies on microbial degradation of γ-HCH indicates that a few pure strains exhibits the traits of overall degradation or mineralization of this compound. The application of microbial consortia in bioremediation of soils and sediments seems to be a promising alternative, although challenges for characterization and dynamics monitoring of consortia are more complex than those for pure strains. In this regard, molecular biology tools are called to play a fundamental role in the near future. Exciting applications of bioaugmentation and process control dependo on the advances on knolwedge on γ-HCH- degrading microbial community.

2260 CHANGES IN THE STRUCTURE AND FUNCTIONING OF A BACTERIAL COMMUNITY OF A SOIL AMENDED WITH OAK AND PINE RESIDUES AND TREATED WITH LINURON HERBICIDE

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The effects of wood amendments on soil microbial community functioning, structure and on the potential of this community for linuron degradation have been studied on an agricultural soil. For this purpose the soil dehydrogenase activity, the number of live bacteria and the phylogenetic structure of the bacterial community by Fluorescence *In Situ* Hybridization (FISH) were assessed in soil treated with linuron and either amended with pine or oak wood or un-amended (sterilized and non-sterilized). Moreover, the ability of specific microbial groups to degrade the herbicide linuron was evaluated in terms of half-lives ($t_{1/2}$) in the un-amended and amended soils for an incubation period of 66 days. Monitoring of linuron metabolites in different soils was also carried out. The overall results show that the bacterial community had a significant role in linuron degradation. The linuron half-life values indicated a slower degradation rate in pine and oak amended soils than in un-amended soils. This is attributed to the higher sorption of linuron by these soils compared to the un-amended ones. N-(3,4-dichlorophenyl)-N´-methoxyurea was the only metabolite found in the non-sterilized soils. Furthermore, linuron did not affect the microbial community, presumably because the latter had adapted to its presence. However, the specific activity of the herbicide degrading populations was negatively affected by the amendment owing to a lower bioavailability of the herbicide for microbial degradation.

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2271 POTENTIAL BIOTECHNOLOGICAL APPLICATION OF MYCORRHIZAS AND YEASTS ASSOCIATED WITH *Nothofagus nervosa* (RAULÍ)

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Nothofagus nervosa is an ecologically and economically important species of South American temperate forests. In Argentina, it has a reduced natural distribution area due to overexploitation, overgrazing and forest fires. This critical situation led to the implementation of conservation and domestication programs. Among the different factors that should be considered in these programs and subsequently in reforestation activities are the mycorrhizas and yeasts associated with this species. These microorganisms function as growth promoting and phytodefense agents, so they are of great importance for seedlings growth and for their implantation in the field. The general aim of this work is to analyze the abundance and diversity of mycorrhizas and yeasts in *N. nervosa*, to compare them between native and nursery cultivated individuals and finally to select some mycorrhizas and yeasts strains which would have significant biotechnological applications in domestication processes and therefore in forest management. The first step was to analyze the yeasts associated with *N. nervosa* seeds, they influence the microbial succession that occurs when seeds germinate and consequently they have an effect on plant fitness and development, hence it is crucial to study and describe them. Yeasts were isolated from 30 seeds. Each seed was put into an eppendorf containing 1mL of sterile 0.9% NaCl and sand, and was vortex-mixed for 2 minutes. Aliquots of 100ul were spread on MYP agar plates supplemented with 0.01% chloramphenicol. Plates were incubated at 20 °C for 72hs. One-hundred-forty-nine strains were isolated. Hitherto, they have not been completely identified, because their morphological and biochemical characterizations are still being carried out and molecular identification has to be done. According to these preliminary results, *N. nervosa* seeds have a diverse community of yeasts, including some strains that develop pseudomicelia and some black-yeasts. This work contributes to the description of yeasts biodiversity in natural environments of Patagonia and constitutes the initial step in the study of the influence of these microorganisms in plant growth. It also provides a base for further investigations, such as yeasts-mycorrhizas interactions and possible biotechnological applications of both types of microorganisms in the improvement of plant fitness and the enhancement of domestication programs.

2279 STUDY OF THE NATURAL ATTENUATION OF LINDANE CONTAMINATION IN SOIL MICROCOSMS

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The organochlorine pesticide lindane has been largely used as a powerful insecticide for crop, animal and human health protection. Lindane is the gamma-isomer of hexachlorocyclohexane (HCH), which is obtained along with α-, β-and δ-HCH isomers by photochemical chlorination of benzene. All of them are potential carcinogens, and are listed as priority pollutants by many developed countries. Due to their persistence, moderate volatility and high potential for bioaccumulation along the food chain, trace amounts of HCH isomers are worldwide distributed and are still considered a serious environmental hazard. Although several microorganisms capable of degrading HCH isomers have been isolated from soils in agricultural areas and at the pesticide production sites, little is known about in situ biodegradation. The potential of natural attenuation of a soil artificially contaminated with α-, β-, γ-, δ-isomers has been studied at microcosms scale (500 g soil). One hundred-day incubation under controlled conditions of temperature (25 ºC), air humidity (65%), soil moisture (18% air-dried weight) and, the daily thoroughly mixing for soil aeration permitted the removal of 90%, 70% and 50% of the α-, γ- and δ-isomers, respectively. In contrast, up to 80% of β-HCH remained in soil. The analyses of soil extracts were performed by gas-chromatography, using an electron capture detector and pure purchased compounds as standards for calibration. The analysis of bulk DNA from soil microcosms by means of 16S rDNA PCR amplification and the subsequent electrophoresis of amplicons in a denaturant gradient gel (DGGE) showed that the composition of bacterial populations changed along the incubation period. New bands in the profile pointed at the increase of certain strains under the selective pressure that the presence of the contaminants exerts.

2284 AMMONIA TOLERANT SYNTROPHIC ACETATE-OXIDIZING BACTERIA INVOLVED IN THE METHANOGENIC DEGRADATION OF PROTEIN-RICH MATERIAL

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Inhibition of aceticlastic methanogens by ammonia during anaerobic degradation of protein-rich materials, e.g. fish industry waste water, slaughter house waste or distillers waste causes development of an alternative mechanism of methane formation from acetate. This pathway, offering a way to run stable biogas processes at high levels of ammonia, involves two organisms operating in a syntrophic relationship. Here, acetate is converted to hydrogen and carbon dioxide by one bacterium, followed by the subsequent reduction of carbon dioxide to methane by a hydrogen utilizing methanogen. Up to date only limited information of syntrophic acetate-oxidizing bacteria is available and only three organisms have been isolated so far. The aim of this project was to isolate and identify new mesophilic acetate-oxidizing bacteria.

Two mesophilic methanogenic digesters, operating at high level of ammonia, were used as material source for the isolation. Materials from the digesters were repeatedly diluted in agar shakes with 7 different substrates. Colonies appearing were transferred to liquid medium and later checked for acetate oxidizing ability by co-cultivation with hydrogen consuming methanogens of the genera Methanoculleus. The 16S rDNA sequence of the isolates was determined and phylogenetically analysed.

Two organisms, strain Sp3 and Re2, isolated from agar shakes with syringate as substrate showed acetate oxidizing ability in co-cultivation with a Methanoculleus sp. These isolates originated from the two different digesters. Nearly full length (>1400 bp) of the 16S rDNA sequences were determined and showed that both strains belongs to the Firmicutes-Clostridia subphylum. The most closely related specie of strain Sp3 is *Thermoacetogenium phaeum* with a 16S rRNA gene sequence similarity of 92%. For strain Re2 the closes related bacteria are *Tepidanaerobacter syntrophicus* (96% similarity). Reciprocally the strains Sp3 and Re2 had a 16S rDNA sequence similarity of 85%.

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2291 CLONING AND EXPRESSION OF LCC1 LACCASE GENE PROMOTER IN *Aspergillus niger*

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The white rot fungus *Trametes* sp. I-62 is a strain with laccase activity and a great potencial for biotechnological applications given its ability to detoxify distillery effluents. The *lcc1*, *lcc2* and *lcc3* laccase genes of this basidiomycete have been cloned and sequenced. The promoter region of *lcc1* laccase gene contains a putative site for xenobiotics (XRE). We are interested in using the promoter lcc1 laccase gene, induced by aromatic compounds, for heterologous expression in Aspergillus niger. In the present work, it is described the cloning and expression of the promoter region of the laccase gene *lcc1* from *Trametes* sp. I-62 in *A. niger* using EGFP as a reporter gene. The *lcc1* gene secuence was used to design the primers. Genomic DNA was isolated from *Trametes* sp. I-62 according to the method of Raeder and Broda, and it was used as a template for amplified promotor sequence by PCR. The PCR reaction was carried out at 95 °C for 5 minutes, followed by 30 cycles of 45 seconds for denaturation (95 °C), 45 seconds for annealing (55 °C), 45 seconds for polymerization (72 °C) and final extensión at 72 °C for 10 minutes. The 618 base pair DNA fragment was obtained by PCR, cloned into pGEM® - T-Easy Vector (Promega, Madison WI, USA) and sequenced. We used technologies molecular biology for construction of a vector that it contains an EGFP reporter gene. We have adapted the electroporation method of Sanchez and Aguirre to transform germinated conidia of *A. niger*. For electroporation, 2 µg of DNA were added to 50 µl of the ice cold conidial suspension. Following electroporation, transformants were spread on malt extract agar media containing 100 µl/ml spectinomycine and incubated at 30 °C, the transformants were evident after 48 hours.

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2294 TWO PSEUDOMONAS STRAINS ISOLATED FROM A FLUIDIZED BED BIOREACTOR ARE ABLE TO DEGRADE 2,4,6-TRICHLOROPHENOL AS THE ONLY SOURCE OF CARBON

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Toxic chlorophenols are ubiquitous organic pollutants in wasterwater, aquatic ecosystems and groundwater all they had to anthropogenic sources. Previous recent works of our research group has shown the excellent performance of a fluidized bed bioreactor (FBBR) using engineered aerobic–anaerobic systems to improve the removal of a mixture of 480 mg/L of 2,4,6-triclorofenol (TCP) and 120 mg/L phenol at major pollutant. Despite the fact that microbial degradation of chlorophenols has been investigated for many years, there is still considerable interest in the metabolic capacity of bacteria able to degrade chlorophenols within indigenous microbial consortia in various ecosystems. The aim of the present work was to investigate 2,4,6-TCP-degrading bacteria isolated from an FBBR fed with 2,4,6-TCP as major pollutant.
Two pure cultures of 2,4,6-TCP-degrading bacteria were isolated by cycles of replating on Mineral Medium (MM) and 2,4,6-TCP agar plates with 1% vitamin solution. For degradation studies, each culture was inoculated into each sterile shake-bottle containing MM, 120 mg/L of 2,4,6-TCP as only source of carbon and incubated under aerobic conditions for 192 h at 180 rpm. In addition, abiotic controls were run to asses abiotic removal of the xenobiotic. It was found that the cultures were Gram-negatives with dimensions 1.8 and 1.9 – 4.0 μm. Comparative sequence analysis of the 16S rDNA (1545 bp) in the GenBank database revealed that the bacteria were most closely related one to Pseudomonas aureoginosa and another to *Pseudomonas sp.* (94% and 95% of similarity respectively). Bacteria genus was corroborated by biochemical tests as well as colonial and bacterial morphology test. In a final of experiment for 2,4,6-TCP removal with 120 mg/L initial concentration of the xenobiotic as only carbon source, the culture similar to *P. aureoginosa* exhibited higher degradation potential (70 ± 2%) than the culture similar to *Pseudomonas sp.* (60 ± 1%) under aerobic conditions. There was a net growth of biomass in both cases. Interestingly, the net increase of chloride in the medium was similar for both cultures, *i.e.*, 32 mg Cl^-/L. HPLC chromatograms did not show peaks corresponding to TCP transformation products, such as phenol, o-methylated phenol, and monochlorophenols; this suggested, along with biomass growth and the net increase of chloride, a cleavage of the aromatic ring, significant dechlorination, and TCP uptake by the cultures.
In conclusion, our results indicate that the bacterial community in the FBBR contains strains such as *P. aureoginosa* and *Pseudomonas sp.*, which exhibit a high potential for effective degradation of 2,4,6-TCP under aerobic conditions. The contribution of such species could be associated to the long time operation of the FBBR under partial aeration conditions, that could allow for the adaptation and enrichment of these strains. Finally, it is worth highlighting that (i) development and presence of aerobic TCP-degrading bacteria points out to the usefulness of the FBBR approach for bioremediation of groundwaters polluted with TCP, (ii) to the best of our knowledge, this is the first report on aerobic bacteria isolated from FBBR that are able to degrade TCP as only carbon source.

2302 TESTING THE TOLERANCE AND GROWTH OF ELEVEN TRICHODERMA STRAINS TO CRUDE OIL, NAPHTHALENE AND PHENANTHRENE

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Petroleum hydrocarbons (PH) are major organic contaminants in soils, and are subjected to degradation process mediated by either rhizospheric or soil microorganisms. Filamentous fungi such as *Cunninghamella elegans* and *Phanerochaete chrysosporium* have a significant role on degradation of organic contaminants in soils. However, little is known about the tolerance and the ability of *Trichoderma* species to oxidize and to degrade PH. This research evaluated the tolerance and the growth of eleven *Trichoderma* strains to crude oil (CO), naphthalene (NAPH), and phenanthrene (PHE) using an in vitro system. Petri dishes containing potato dextrose agar (PDA, Baker®) were separately contaminated with CO, and with seven concentrations of either NAPH or PHE (250, 500, 750, 1000, 2000, and 3000 mg L^{-1}) on the agar surface. Non-contaminated plates were used as controls. The *Trichoderma* strains were exposed to all the PH concentrations by triplicate, and incubated for 10 days at 28 ºC. The growth of each fungal colony for each contaminant was daily recorded. No significant differences were observed among the *Trichoderma* species when exposed to CO, indicating that all the strains tolerate the presence of this contaminant; the maximum fungal growth was reached at 96 h. NAPH resulted in significant differences ($P \leq 0.05$) on delaying or inhibiting the fungal growth. The concentration of 3,000 mg NAPH L^{-1} significantly inhibited the fungal growth of five fungal strains. Although *Trichoderma* strains showed growth on PHE-contaminated media at all concentrations, PHE-concentrations significantly reduced ($P \leq 0.05$) the fungal growth. NAPH showed greater toxic effects on growth of *Trichoderma* than PHE. This study demonstrates that *Trichoderma* strains may be considered in bioremediation of PH-contaminated systems due to their tolerance to these contaminants. The potential role of *Trichoderma* strains to degrade PH is in progress.

2305 EFFECT OF THREE POLYCYCLIC AROMATIC HYDROCARBONS ON NODULATION OF *Rhizobium tropici CIAT* 899 ON *PHASEOLUS VULGARI*

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Polycyclic aromatic hydrocarbons (PAH) are ubiquitous organic pollutants that are considered toxic and carcinogenic compounds to living organisms. There is scarce information about the effect of PAH on symbiotic systems such as *Azolla-Anabaena*, arbuscular mycorrhizal fungi-plants, or legume-rhizobia. Thus, the objective of this study was to evaluate the effect of three PAH on the symbiosis between *Phaseolus vulgaris-Rhizobium tropici.* Common bean seeds were surface disinfected and germinated on water-agar 1%. Then, seven-days old seedlings were transplanted to a 3-split growth pouch system containing an artificially-contaminated Jensen's nutrient solution with PAH: naphthalene (NAPH), phenanthrene (PHE), or benzo[a]pyrene (BAP). Each PAH was dissolved in acetone and applied to the respective nutrient solution at the following concentrations: 20, 40, 60, 80, and 100 μg mL^{-1}. Uncontaminated plants with or without acetone were included as controls (T). Seven days after treatments were established, plants were inoculated with 1.0 mL of a *Rhizobium tropici* strain CIAT 899 (87.2 UFC x 1010 mL^{-1}). Plants were grown under growth chamber conditions: 70% HR, 23 °C, 12 h, during 21 days. Root nodules were counted and recorded from day 5 to day 21. Plants were harvested to evaluate the nodule dry mass at the end of the experiment. The three PAH significantly reduced the number of nodules in plants. All the NAPH and BAP treatments resulted in significantly lower number of nodules than controls. Also concentrations of PHE higher than 40 μg mL^{-1} resulted in significantly lower number of nodules; however, at 20 μg mL^{-1} this PAH stimulated the nodulation. Similar response was observed for nodule dry weight where the highest concentration of any PAH resulted in the lowest dry mass of nodules. This study shows the negative effects of PAH on either formation or dry mass of nodules, in the *Phaseolus vulgaris-Rhizobium tropici* symbiosis.

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2306 MICROANALYSIS OF CASUARINA, EUCALYPTUS, PINE TISSUE AND MYCORRHIZAL MYCELIUM COLLECTED FROM MINE SOILS

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Potentially toxic elements (PTE) deposition in soils due to mine exploitation has been recorded since XVI century for Mexico. Plants established in such environments have developed tolerance and adaption to PTE. The objective of this study was to determine the accumulation sites and spatial distribution of PTE in plant tissues and mycorrhizal mycelium. Tissues of *Casuarina equisetifolia* L., *Eucalyptus camaldulensis* Dehnhardt y *Pinus greggii* Englem, and mycorrhizal mycelium (MM) were collected from mine sites of Zacatecas, Mexico. Transversal cuts of stem, leaves and roots were analyzed with Scanning Electron Microscopy with X-ray microanalysis (SEM-EDS), and Particle-Induced X-ray Emission (PIXE). Roots and stems were the main plant tissue for PTE-accumulation in the three tree species, as well as the MM. Root cortical cells mainly accumulated Pb, Br, Sr, Mn, and Fe, whereas the MM and the stem cortex contributed on the accumulation of Pb and Ti. PTE accumulation among the plant species was as follows: *Casuarina>Eucalyptus>Pinus*. SEM-EDS allowed the determination of trace amounts of Pb and Ti, which are not usually detected via atomic absorption analysis. This research shows the potential of utilizing SEM-EDS and PIXE for estimating the spatial distribution of PTE in biological samples.

2326 ANALISYS OF BACTERIAL COMMUNITY IN TWO WASTEWATER TREATMENT PLANTS BY DENATURING GRADIENT GEL ELECTROPHORESIS AND FLUORESCENT IN SITU HYBRIDIZATION

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A combination of denaturing gradient gel electrophoresis (DGGE) and oligonucleotide probes was used to characterize and follow the dynamic of bacterial community in two wastewater treatment plants with an A_2O (anaerobic, anoxic, oxic) system. DGGE was used to know how the bacterial community changes, and to stablish differences between bacterial community from different compartiments in the A_2O system of each plant. To complete this technique, we used Fluorescent *in situ* Hibridization (FISH) to quantify the main groups in activated sludge samples. In the same way, the two molecular techniques allowed us to know the differences between the bacterial community structure of the two wastewater treatment plants.
Activated sludge samples were amplified with bacterial general PCR primers using the extracted genomic DNA before the PCR products were loaded on DGGE gels for bacterial community analysis. For FISH we used ten different oligonucleotide probes to assure us the detecction of the majority bacterial community.
In order to find out if there are differences between different compartiments of the two plants, DGGE analysis was performanced using samples from two different compartiments of each plant. The DGGE pattern showed that the bacterial community structures among compartiments in each system were very similar.The DGGE pattern showed too that, although the two plants had similar bacterial community structures in activated sludge, the bacterial populations presented some differences. Some bands are characteristic of each plant, so the next excision of these bands and their sequencing will allow us to know what organisms are more important in each plant.
Analysis of the bacterial community by FISH demonstrated that the most important group in activated sludge was β-Proteobacteria inside of Bacteria Domain. This group contain an important number of phosphorus accumulating organisms (PAO,s). Another important fact observed by FISH was that the metabolic activity of the bacterial community of each plant was different, it was higher in a plant than in the other, so we will study the causes of this fact. Finally, the group of nitrifing and denitrifying bacteria were not very important in this two plants, so we are considering to employ different oligonucleotide probes for these groups.

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2328 MOLECULAR CHARACTERIZATION OF TEX (TOLUENE, ETHYLBENZENE, AND XYLENE) DEGRADING MICROBIAL COMMUNITIES FROM AIR BIOFILTERS

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The biodegradation of alkylbenzene hydrocarbons has been studied in three lab-scale air biofilters operated simultaneously. These biofilters were inoculated with liquid microbial enrichments on toluene, ethylbenzene, and xylene (TEX compounds), respectively, and subsequently fed with air streams that contained the same substrates. A pelletized organic material was used as biofilter packing. Despite poor watering of the filter bed, high elimination capacities were obtained immediately, and maintained throughout the biofiltration experiments. It has previously been hypothesized that good biofilter performance is linked to the action of hydrocarbon-degrading fungi. Here, samples from the bed were taken after more than 2000 hours of operation in order to characterize the fungal-bacterial interactions. Microbial populations were studied by extracting the total DNA and by amplifying the ribosomal gene by PCR, using specific primers for eubacteria (16S rRNA) and fungi (ITS1 rRNA). The obtained amplicons were characterized by DGGE profiling (denaturating grading gel electrophoresis). Preliminary results have shown that the bacterial and fungal population profiles were very specific to the substrate, and clearly distinct from the endogenous populations already present in the packing. This indicates that both types of organisms might play a direct role on the biodegradation of the contaminant. Current efforts are directed towards the sequencing and identification of the predominant genotypes.

2338 DNA-BASED DIAGNOSTIC TESTS FOR SALMONELLA STRAINS TARGETING *hilA, agfA, spvC* AND *sef* GENES

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Salmonellae are invasive enteropathogens of humans and animals. During the past decade, a dramatic increase in the occurrence of *Salmonella spp* infections was principally responsible for the rise of food-borne salmonellosis. The goal of this study was to evaluate the suitability of the, *hilA, agfA, spvC, sef*, gene amplification by PCR as a specific method for detection of Salmonella strains. Twenty six isolates of *Salmonella spp* including 8 different serotypes were analyzed in this study. The bacteria were isolated between 2005-2007 and serotyped at the Clinical Hospital of Infectious Disease, Cluj-Napoca. We used a direct PCR technique, DNA extraction had been skipped and the bacterial cell wall denaturated in the first step of the reaction. PCR amplicons were electrophoresed (at 6 V/cm for 90 min) in a 2% agarose gel. After staining with ethidium bromide, the amplified fragments in the gel were visualized. PCR amplicons were sequenced to provide a characterized DNA probe. This rapid detection method has potential for use in the food, agricultural, public health and environmental control sectors.

2342 *Sedimentibacter sp*. WITH CORROSIVE CAPABILITY, FERRIC-REDUCING, ISOLATED FROM AN OIL SEPARATION TANK

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Biocorrosion is a common problem in oil and gas industry facilities. Characterization of the microbial populations responsible for biocorrosion and the interactions between different microorganisms with metallic surfaces is required in order to implement efficient monitoring and control strategies. Microbial anaerobic communities present mainly at oil and gas producing, transporting and storage facilities, have been poorly characterized. Some studies reported the characterization of oil field microbial communities both by culture-dependent and cultureindependent methods, revealing the presence of several physiological types of bacteria as sulfate-reducers, sulfidogens, fermentative bacteria, metal reducers, methanogens and acetogens, depending on the physicochemical conditions of the reservoirs. Other species belonging to the order Clostridiales have so far been isolated from oil wells, they include *Dethiosulfovibrio peptidovorans*, family *Syntrophomonadaceae*, these bacteria produce the highest rate corrosion in a pipelines, about 4 mmy and produce sulfides from thiosulphate. The goal of this study was to characterize a fermentative bacterium isolated from oil environment and to determine its participation on the carbon steel corrosion. The Isolate IMP6C3 was identified by 16SrDNA gene sequence as *Sedimentibacter sp*. With the closest homology with *Sedimentibacter saalensis*, for that, it suggests that this is a new species. In this study, *Sedimentibacter sp.*, was determined his important corrosive capability. This bacterium has adhesion metal capability and utilizes iron as final acceptor electron with biomass increase in presence of this element.

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2366 CHANGES IN MICROBIAL COMMUNITIES IN GREEN WASTE AND SEWAGE SLUDGE COMPOSTS FOLLOWING MATURITY: COMPETITION AND CO-METABOLISM

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Composting is an interesting way to valorize various biowastes and is becoming an increasingly used soil amendment. Compost is a product obtained after a humification process. However, compost utilization as amendment needs to know precisely its stability and maturity. Since composting is mainly a microbial process, knowledge of the various microbial groups and their role in the process of bio-oxidation is essential. Compost maturity can be potentially estimated by changes in microbial communities during the composting process. So, the main objective of the work was to study the relationship between the microbial functional diversity and the compost maturity. Community level physiological profiles (CLPPs) with Biolog Eco and FF microplates and enumeration of microorganisms were used to access changes in functional diversity of the microbial communities in a sewage sludge and green waste co-composting over time. Each stage of composting was characterized by different microbial groups. Even if eubacterial numbers stayed high from beginning to end of composting, actinomycetes densities only increased after the bio-oxidation phase i.e. after 40 days. Numbers of fungi were higher during the last stage of decomposition. Cluster analyses of metabolic profiles gave the same information with a clearly separation between two groups of composts: before and after 67 days for bacteria and before and after 40 days for fungi. Principal Component Analysis (PCA) on bacterial and fungal CLPP data showed the same clearly chronological distribution of composts. These results show that the structure of microbial communities changes with composting time. Another interesting aspect is the evolution of the metabolic diversity within the communities. Indeed, standard deviations of barycentres on PCA were higher for mature composts than young composts. This result could indicate the increase of metabolic diversity within the communities. To study particular utilizations of carbon sources, PCA was applied to bacterial and fungal CLPP data and the influent substrates were selected. The results show that microbial communities adapt their metabolic capacities to the compost. Maturation phase appeared as multiple metabolisms, and not only centred on easily degradable substrates. Bio-oxidation phase produced a competition between microbial communities whereas maturation phase involving a co-metabolism.

2428 LONG-TERM FIRE EFFECT ON SOME CHEMICAL PARAMETERS AND MICROBIAL DIVERSITY IN A CONIFER FOREST SOIL

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Soil microbiota are one of the soil components most affected by wildfires. The data from the present study were obtained from a conifer forest soil at Sierra de Gredos (Ávila, central Spain) twenty years after fire of low-to-moderate intensity. A set of soil characteristics indicated the extent to which the spontaneous recovery of the soil is produced as a result of vegetation regrowth. Ten months after fire a strong increase in soil pH, organic C and N, and exchangeable Ca and K, with respect the control soil. Twenty years after this fire it was observed a decrease of soil organic C and N, whereas other variables such as pH, exchangeable Ca and K were slightly increased with respect to control soil.
The recolonisation after twenty years of natural fire by bacteria was different, because after incubation with *Bacillus* and *Clostridium*, inoculated bacteria have increased bacterial CFU in burnt soils with respect to control soils. The highest value was in the contaminated soil with *Clostridium*. Recently after fire with an enrichment of available nutrients, we observed a stimulation of bacteria probably because of development of new strategies of recolonisation.

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2432 INFLUENCE OF CULTIVATION REGIME OF AN ARBUSCULAR MYCORRHIZAL FUNGAL ISOLATE ON ITS SYMBIOTIC EFFICACY IN PHYTORESTORATION OF DISTURBED ECOSYSTEMS

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Arbuscular mycorrhizal fungi (AMF), from the Phylum Glomeromycota, are a group of soil organisms that forms symbiotic associations with plant roots and can contribute to increase plant biomass and promote phytorestoration of disturbed ecosystems. The influence of cultivation regime of a *Glomus geosporum* isolate, obtained from a highly alkaline anthropogenic sediment, on its symbiotic efficacy was investigated. Two cultivation lineages of *G. geosporum* (BEG199 and BEG211) were created by sub-culturing with or without the stress of the alkaline sediment. Symbiotic efficacy of the cultivation lineages was assessed after inoculation onto Conyza bilbaoana, a potential target plant species to be used for phytorestoration of disturbed ecosystems. *Glomus geosporum* BEG199 produced a larger number of spores, a more extensive extraradical mycelium and was more effective than *G. geosporum* BEG211 in increasing the biomass and leaf phosphorus concentration of *C. bilbaoana.* The stress tolerance and symbiotic efficacy of *G. geosporum* was, therefore, shown to decrease when cultivated without the stress of the sediment.
The results indicate that environmental conditions under which AMF are cultivated can influence their symbiotic efficacy and that AMF might quickly lose gained-tolerance to environmental stresses when maintained without the selective pressure of those stresses.
Production of AMF inoculum and maintenance of fungal cultures should, therefore, be conducted using substrates containing the original edaphic stresses. This practice should be adopted for the native AMF to be used as inoculants in phytorestoration of disturbed ecosystems.

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2435 SOIL ENZYME ACTIVITIES, BACTERIAL BIOMASS AND BACTERIAL COMMUNITY STRUCTURE. CHANGES THROUGH SOIL PROFILE DUE TO DIFFERENT MANAGEMENTS

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Dry climate regions are particularly susceptible to impacts from soil degradation. In these areas, deteriorating soil quality is mainly related to inappropriate farming techniques, but the use of environmental friendly agriculture practices has proven to be effective in restoring or improving soil quality.
In this work, the long-term effect of four different managements (tillage, non-tillage, cover crop + herbicides, and cover crop + mower) on some biological indicators of soil quality was assayed in an olive orchard under rainfed conditions. For this, the activity of five enzymes (dehydrogenase, β-glucosidase, phosphatase, urease, o-diphenoloxidase) were determined, and PCR-DGGE and real-time quantitative PCR were used to detect changes in bacterial community structure and biomass, respectively, through the 0–20 cm in depth.
The results indicate that overall biochemical activity, as well as bacterial biomass and diversity, are helpful approaches to evaluate the impact of different managements on soil quality, and to decide how we can manage agricultural systems sustainably.

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2451 CHARACTERIZATION OF CELL-FREE EXTRACTS OF *Pseudomonas aeruginosa* MGP-1 THAT OXIDIZES *n*-ALKANES

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The alkanes are a kind of the principal compounds of petroleum. When the n-alkanes are solid, also called paraffins, are spilled into the field, they form a dense layer in the surface that hinders the oxygen and nutrients transport, affecting the quality and functionality of the soil. A few bacteria have been reported to be able to degrade paraffins, therefore it is interesting that the *Pseudomonas aeruginosa* MGP-1, a strain isolated from petroleum contaminated soil in a previous study, can degrade kerosene, diesel, pure alkanes between 11 and 40 carbons atoms, mix of paraffin and other compounds with high content of alkanes. In the present study enzymatic activity of alkane oxidation was demonstrated in cell-free extracts obtained from culture supplied with hexadecane (C16) or tetracosane (C24) as sole carbon source. From each extract obtained the enzymatic activity of oxidation of alkanes was detected and quantified by UV spectrophotometer and gas chromatography. The products alkane oxidations by the cellular-free enzymatic system were identified by chromatography of gas-masses and infrared spectrometry. *Pseudomonas aeruginosa* MGP-1 has only one system of alkane oxidation. The alkanes are hydroxylated by monoterminal oxidation and NADH+H^+ or NADPH +H^+ are used as reducing agent. This system is sensible to changes of pH, and the optimal pH is neutral, as well as to the concentration of reducing agent. It is little sensible to extreme high temperatures and to the concentration of the substrates. The oxidation products are alcohols, aldehydes and carboxylic acids corresponding to the alkanes that oxidized. The system is partiality specific, although it displays greater affinity to eicosane. The rapidity to degrade and to oxidize these compounds diminishes as the hydrocarbon chain increases or diminishes around this alkane. *Pseudomonas aeruginosa* MGP-1, could be a candidate to be used in the bioremediation of soils contaminated with paraffins and the produced of long chain alcohols and linear acids can be of industrial interest.

2470 CELLULAR POLYMORPHISM AND NITROGENASE ACTIVITY OF *Azotobacter nigricans* DURING KEROSENE REMOVAL

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It has been reported that certain plants grow in soils contaminated with hydrocarbons developing nitrogen-fixing bacteria. For example in the rhizosphere of beans plants grown in soil contaminated with kerosene it was found the presence of *Azotobacter* which removed kerosene and produced polymorphic cells when they grew in nitrogen free cultures. However, it is important to differentiate the morphological shape of this strain during kerosene removal to know whether the cysts of *Azotobacter* are involved in kerosene degradation. In this work, the assays were in liquid cultures with kerosene as a sole carbon source, incubated at 28 °C and 150 rpm for 192 h. The concentration of kerosene nitrogenase activity, cell ultraestructure and biomass were monitored by gas chromatography, the acetylene reduction method, transmission electron microscopy and dry weight respectively.
Both, the growth and the nitrogenase activity of A*. nigricans* were severely affected by the kerosene compared with cultures grown with sucrose as a sole carbon source. On the other hand, the nitrogenase activity kinetic was similar with both carbon sources sucrose and kerosene, up to the end of the exponential phase of growth, but in cultures with kerosene, when the nitrogenase activity decreased the biomass increased slowly removing 75.9% of kerosene. Thus, the cultures with kerosene seem to fix nitrogen not to increase biomass, but rather to lead the metabolic pathway to cellular maintenance and biosynthesis of carbon storage. During the exponential phase of growth in culture with sucrose, cell population showed vegetative cells without PHB granules, but these cells had some electro-transparent peripheral bodies. The vegetative cells turned into encapsulated ovoid shape which showed abundant peripheral bodies on the cytoplasm peripheral, close to the cell membrane and the removal of kerosene was of 63.8% at the end of the exponential phase. It is important to mention that peripherals bodies were particularly abundant in the exponential phase of growth, where the nitrogenase activity was maximum (11.9 μmoles C_2H_4 h^{-1}culture^{-1}) and the presence of PHB granules in the cells were rare. The nitrogenase activity was decrease in the culture with kerosene after 24 h, but the size of PHB granules was increasing. Though, the presence of cysts was found at 192 h of growth when the remaining kerosene was 25%. These results suggest that the cysts were not involved neither in removal kerosene nor atmospheric nitrogen fixation. However, kerosene or metabolic intermediates compounds induce the encystment process.

2488 METHANOGENESIS IN AN ACID AND HIGHT CONTENT OF HEAVY METALS ENVIRONMENT

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Methane is, after water vapour and carbon dioxide, the most important gas that contributes to greenhouse effect. Methanogenic archaea are ubiquitous in many anaerobic natural and engineering ecosystems, but they are generally found in habitats with reduced redox potentials (under -200 mV) and circumneutral pH. The Tinto river (Huelva, SW Spain) is well known by its reddish (*tinto*=red wine) waters. The color is due to the very high content in dissolved iron (2.3 g/l), although other heavy metals are also present (0.23 g/l Zn, 0.1 g/l Cu, etc). Metabolism of the pyrite by chemolithotrophic microorganisms associated to iron- and sulfur cycles (*Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans*) gives its unusual physic-chemical characteristics: low pH (2.3), high redox potential (> 400 mV). Ferric iron plays a key role in its maintenance according with the following equation:

$$Fe_3+ + 3\ H_2O \rightleftarrows Fe(OH)_3 + 3\ H+$$

The extreme acidic and oxidative conditions of the river are in direct contrast with the methanogens requirements. But contrary to what was expected, field and laboratory observations suggested that methanogenic activity is widely distributed in the Tinto river basin. Microcosms inoculated with sediments and water from the river produced methane. When the microcosms were biostimulated by addition of different substrates methane production was increased. In all cases the production of methane was associated with a decrease in redox potentials (from + 410 to -110 mV) and with an increase of pH (from 3.5 to 6). Something similar was observed *in situ*. 40 cm deep cores, extracted from a dam in the river, showed well-defined black bands in the middle of otherwise brown-red sediments. The black bands were associated with reduced redox potentials and higher pH values compared to the high redox potentials and acidic pH of the adjacent sediment and water. Sequences corresponding to *Methanosaeta concilii* could be retrieved from the black bands sediment. The development of microniches with different pH and redox potential values that those in bulk environment could explain the presence of methanogens in the Tinto river. Consequently, the natural sources of which greenhouse gases come from must be revisited.

2502 BIOLOGICAL ACTIVITY IN LONG-TERM TREATED WASTEWATER IRRIGATED SOILS OF MALLORCA

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Soil properties and soil biological activity were studied in different arable soils irrigated for more than 20 years with treated wastewater in the island of Mallorca. Soil properties measured were: Cation exchange capacity (CEC), pH, $CaCO_3$ content, organic matter content (SOM), nitrogen content (N), Olsen phosphorous content (OP), and water-soluble organic carbon content (SOC). Biological activity was determined by parameters related to microbial presence and activity, such as microbial biomass (MB), soil basal respiration (BR), dehydrogenase (DH), β-glucosidase (GL) and alkaline phosphatase (AP). Results showed that the parameters analysed to define biological activity were highly positively correlated between them. Biological activity was also highly positively correlated to cation exchange capacity, organic matter content and nitrogen content. By contrast, $CaCO_3$ content was negatively correlated to soil basal respiration, and β-glucosidase and dehydrogenase activities. There was no relationship between Olsen phosphorous and water-soluble organic carbon contents with any of the parameters related to biological activity. Values of microbial biomass and enzymatic activities were in the same range of similar soils reported in the literature, so any negative effects of long-term treated wastewater irrigation was considered.

2509 DIVERSITY OF CHROMATE RESISTANT BACTERIA PRESENT IN THE TANNERY OF FEZ IN MOROCCO

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One of the main sources of income of Morocco is the leather goods made by craftsmen with tanned and tinted animal skins. One of the more important tanneries of Morocco is that located at the Medina of Fez, which constitutes one of the sources for heavy metals contamination of the Sebou River, since wastewater treatments are either inexistent or inefficient and obsolete because the high costs required for their improvement.
Most of the chromium used in these tanneries is trashed to this river arriving to the Atlantic Ocean where fisheries for human consumption are performed. Since this kind of contaminant accumulates in the fish and environment, it is finally ingested by humans, originating public health problems. The use of clean technologies is quite limited in Morocco because their high costs of adaptation and the lack of a consciousness of people and authorities to achieve a good management that might warranty a clean and healthy environment. For a better knowledge of the pollution levels, as well as to determine the microbial populations in the Sebou River, we have analysed the physico-chemical parameters of its waters and detected the microorganisms present in this River. The microbial populations have been identified by classic Microbiology and Molecular Biology techniques (DGGE, FISH) and electronic microscopy. These studies have allowed us to determine the level of heavy metals contamination and the microorganisms able to grow in their presence. The use of these microorganisms as possible decontamination agents is been studied.

2517 STUDY OF THE POPULATION DYNAMICS OF A MIXED BACTERIAL CULTURE ABLE TO DEGRADE CYANURIC ACID IN A PACKED BED REACTOR, USING RAPD (RANDOM AMPLIFICATION OF POLYMORPHIC DNA) TECHNIQUE

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Cyanuric acid is a biodegradation byproduct of triazinic compounds. Because of its low carbon to nitrogen ratio, a complementary carbon source is usually needed for its complete biodegradation. In this work, glucose was used as extra carbon source.
Cyanuric hydrolase is the first enzyme in cyanuric acid (CA) catabolism, and is produced by a wide number of microorganisms. Although axenic cultures could use cyanuric acid, it could be degraded more efficiently by mixed cultures. The stability of a microbial community depends of the environmental conditions prevailing in the microcosm represented by the packed bed reactor; thus, the study the microcosm's species-richness is of relevance to understand CA biodegradation kinetics.
For cyanuric acid degradation, a two-stage packed bed column was used. A bacterial community composed of Acinetobacter sp. and *Agrobacterium tumefaciens* was immobilized in the porous material support (volcanic stone). The column was concurrently operated with a hydraulic retention time of 33.4 hours. Air flow was maintained at 1.2 vvm. Effluent wasperiodically sampled for cyanuric acid and total soluble nitrogen determinations. In aseptic conditions, samples of porous support were withdrawn for DNA extraction, purification and random amplification RAPD. The primers used for RAPD were previously probed for their DNA amplification performance using both strains separately. The initiators used in the screening were: rapd3, rapd4 , rapd5, rapd6, rapd7, rapd8, rapd9, rapd10, rapd 11, 14306, 10514, 1283, 1290.
Throughout the biodegradation process, the CA removal efficiency rounded 99%. Using the primer 1283, it was witnessed the predominance of the *Agrobacterium tumefaciens* strain in the attached biofilm. Two primers; rapd 10 and rapd 11, amplified specifically for DNA of *Agrobacterium tumefaciens* and *Acinetobacter sp.* respectively, but when combined and with DNA from both strains, interference were found.

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2523 GLOMALIN IN A MEDITERRANEAN ECOSYSTEM AFFECTED BY MINING ACTIVITIES AND ITS CONTRIBUTION TO HEAVY METALS SEQUESTRATION

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Glomalin-related soil protein (GRSP), a glycoprotein produced by arbuscular mycorrhizal fungi (AMF) and usually presents in high amounts in soil, may stabilize heavy metals such as Cu and Zn in soils affected by mining activities, as large areas of central Chile. The amount of GRSP, its contribution to the sequestering of Cu and Zn in the soil, and the micro-spatial variation of other soil traits (pH, soil organic carbon-[SOC], Cu and Zn contents) was studied in a natural semi-arid Mediterranean ecosystem near a copper smelter in central Chile affected by deposit of metal-rich particles since 1964. Rhizospheric (R) and non-rhizospheric (NR) soil of four representative plants (*Argemone subfusiformis, Baccharis linearis, Oenothera affinis* and *Polypogon viridis*) was analyzed. The results showed a strong variability in GRSP (6.6-36.8 mg g^{-1}), Cu content (62-831 mg kg^{-1} for the total Cu and 5.8-326 mg kg^{-1} for the available Cu) and pH (4.2-5.5) in the different plant and rhizospheric zones analyzed. A strong correlation between the contents of GRSP with contents of total soil Cu and Zn was found (r=0.89 and 0.76 for Cu and Zn respectively, $P < 0.001$). The Cu content in GRSP ranged from 3.8 to 89 mg g^{-1} soil and represents 1.4-28% of the total Cu content in soil. Moreover, the contribution of GRSP to total soil organic C reached 89% in P. viridis R, which represents the most extreme conditions of soil degradation within the ecosystem, with the highest content of heavy metals (both total and available fractions) and lower pH values (pH_w=4,2). This study gives new evidences about the role of the GRSP in Cu and Zn sequestration and suggests a highly efficient mechanism to mitigate this environmental stress.

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2543 DIVERSITY AND COMMUNITY COMPOSITION OF TRIBUTYLTIN-RESISTANT BACTERIA UNDER DIFFERENT CONDITIONS

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Tributyltin (TBT) is an organometallic compound used as antifouling agent in marine paints. This compound is toxic not only for eukaryotes, but also for bacteria. Based on the literature review, a few researchers have reported evidence for the presence of TBT-resistant bacteria in natural seawater and marine sediment.
The objective of this study was to assess the diversity and community composition of TBT-resistant bacteria under different conditions (TBT, monobutyltin (MBT) known as microbial degradation product of TBT, and dextrin/peptone mixture as an external carbon source). Resistant to TBT was examined in bacterial populations enriched using TBT-contaminated marine sediment (Ulsan Bay, South Korea) sample as inoculum, at the initial TBT concentration of 10 and 100 ppm which was much higher level than the observed TBT level in the contaminated sediment.
Phylogenetic trees based on the 16S rDNA sequence revealed that *Marinobacter* sp. and alpha proteobacterium sp. were identified for 100 ppm TBT-amended culture samples regardless of the presence or absence of carbon source and 100 ppm MBT. Those bacteria to date have never been reported from the TBT-contaminated marine sediments. Quantitative fluorescence in situ hybridization (FISH) analysis confirmed that the Alphaproteobacteria were the most dominant group in all 100 ppm TBT-amended cultures. For 10 ppm TBT-amended cultures, along with *Marinobacter* sp. and alpha proteobacterium sp., Halomonas sp. were identified in the absence of carbon source, and in the presence of carbon source, *Marinobacterium sp., Desulfovibrio sp., Thiomicrospira sp., Soehngenia sp., Acholeplasma sp*. were additionally identified. However, in the presence of 10 ppm MBT, phylogenetic analysis revealed that only *Marinobacter* sp. and alpha proteobacterium sp. were identified for all 10 ppm TBT-amended cultures. Similar to the results obtained in 100 ppm TBT-amended cultures, quantitative FISH analysis resulted that the Alphaproteobacteria were the most dominant group in all 10 ppm TBT-amended cultures. Both quantitative FISH and restriction fragment length polymorphism (RFLP) analyses resulted that the community composition of TBT-resistant bacteria changed significantly when the external carbon source and/or MBT were amended.

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2562 CHARACTERIZATION OF *Pseudomonas nitroreducens* STRAIN MHP41 AND ITS APPLICATION IN BIOREMEDIATION

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s-Triazines are herbicides widely used in agriculture for the control of broadleaf weeds and for grass control in non-crop lands. The potential toxicity of these compounds causes environmental concern. Soil quality is strongly influenced by microbe-mediated processes and changes in the microbial community structure could be an indication of pollutant biodegradation in soil. Our research has focus in the biological characterization of *Pseudomonas nitroreducens* MHP41, a native strain isolated from Chilean agricultural soil, and its biocatalyst potential for simazine bioremediation. *Pseudomonas nitroreducens* MHP41 was grown in a minimum medium with simazine as nitrogen source. Growth rate was determined by measuring turbidity at 600 nm and by CFU counts. To evaluate the capability of native soil microbiota and bioaugmentation strategies on simazine removal, microcosm experiments with agricultural soil were used. High Pressure Liquid Chromatography (HPLC) was used to estimate simazine degradation. Soil samples were collected for microbiological and chemical analysis. Total (DAPI stain) and cultivable heterotrophic bacteria were counted. Soil simazine catabolic activities were determined by most probable number method. Fluorescent *In Situ* Hybridization (FISH) was performed to analyze changes in soil microbial community structures due to simazine amendment and bioaugmentation strategies. The strain was able to grow using simazine as nitrogen source reaching a high biomass of 2.10^8 CFU/ml. Resting cells of strain MHP41 degrade more than 80% of simazine within 60 min. In soil microcosm, the addition of strain MHP41 increases simazine catabolic activities and enhances the herbicide attenuation. FISH analysis showed that bioaugmentation modified the structure of soil microbial community, and α-Proteobacteria, *Planctomycetes* and *Acidobacteria* were the most affected phylogenetic groups. *Pseudomonas nitroreducens* MHP41 is an interesting biocatalyst for bioremediation of s-triazine herbicides.

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2587 RELATIONSHIP BETWEEN MICROBIAL DIVERSITY AND XENOBIOTIC POLLUTION IN SOILS FROM A MUNICIPAL SOLID WASTE LANDFILL

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In the last few years the interest to know the soil health has remarkably increased because this approach could contribute to predict the ability of such soil to be recovered from disturbances, such as drought, climate change, human exploitation and pollution. The concept of soil health refers to different attributes of the soil related to functionality aspects such as mineralogy, chemical-physical characteristics, and organic material, including the microorganisms, which play an important role in the capacity of soils to function as vital living systems.

The main aim of this work is to study the relationship between microbial diversity and different pollutants, such as polyaromatics hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in soils from a municipal solid waste (MSW) landfill placed in Torrejón de Ardoz (Madrid, Spain).

Soil samples were collected from four selected areas (T2, T2B, T8 and T9) in which the amount of PAHs and PCBs were determined. Soil microbiota has been estimated by viable counts technique and direct DAPI counts in an epifluorescence microscope. The physiological profiles of soil microorganisms have been characterized by measuring the following enzymatic activities: alkaline- and acid phosphatases, β-glucosidase, invertase, cellulase, urease, β-N-acetylglucosaminidase and dehydrogenase. In addition, analysis of the microbial biodiversity of soils was carried out by using molecular techniques, including DGGE (denaturing gradient gel electrophoresis) and 16S rDNA gene cloning from *Bacteria* and *Archaea* domains.

High concentrations of total hydrocarbons (aliphatic- and aromatic hydrocarbons) were detected in T2 and T9 samples (165.60 and 189.91 ppm, respectively) being remarkable the presence of PCBs (4.14 ppm) and benzopyrene (2.29 ppm) in T9 sample. Results corresponding to microbial estimation and microbiological enzyme activities showed that highest values were determined in T2B and T8 areas. It is noticeable that in such areas the lowest concentration of the pollutants PAHs and PCBs were detected. Preliminary results from the analysis of microbial biodiversity were also obtained. The different pattern of bands obtained though DGGE analysis reflect the presence of different microbial communities in every analysed area. In order to provide phylogenetic information about the microorganisms in such areas, Bacteria and Archaea 16S rDNA gene sequencing are in progress.

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2682 CHANGES ON SOIL MICROBIAL COMMUNITY BY DAIRY COW MANURE AND ATRAZINE APPLICATION IN AN AGRICULTURAL SOIL CULTIVATED WITH MAIZE

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Several researches have shown that pesticides can present negative effect on soil microbial community. However, this trend has been modified in organic amended soil. The effects of herbicides are conventionally studied by techniques based on cultivation of the soil microorganisms or by measuring their metabolic activities. Recently, various molecular techniques, such as denaturing gradient gel electrophoresis (DGGE) of 16S rRNA genes, have been developed to assess temporal and spatial changes of the soil community. In this study, the aim was to determine whether the applications of dairy cow manure and atrazine on soil affect the soil microbial community. Different doses of animal manure and atrazine were applied on soil. The effects on soil microorganisms were assessed through microbial respiration, fluorescein diacetate activity (FDA) and β-glusosidase activity, microbial numeration and molecular techniques (PCR-DGGE). The results showed that the application of dairy cow manure on soil increased the microbial respiration, FDA activity, and number of bacteria after 15 days of applying the amendment. However, after this period the activity and microbial number began to recover the activity presented in the unamended soil. Finally, the amendments modified the microbial community with the application of bigger cow manure doses. The application of atrazine, carried out in a stabilized amended soil (30 days after application of amendment), did not present adverse effects on soil microorganisms. On the contrary, microbial respiration and enzymatic activity were increased with the application of atrazine during 80 days after their application on soil and amended soil, while that the microbial numbers were augmented. The study of the bacterial community by PCR–DGGE reflected different banding patterns under different atrazine concentrations after 10 days of atrazine application. However, the community was recuperated rapidly after this period of time. With the obtained results, it is possible to indicate that atrazine application stimulates the soil microbial community which can be explained based on the apparition of bands that they would be representing a specific group of soil microorganisms with the capacity of atrazine degradation. A variety of bacteria with capacity for atrazine degradation has been isolated from agricultural soils that have had some contact with this agrochemical, including several *Pseudomonas, Acinetobacter, Agrobacterium y Nocardioides* sp. Probably this response could be causing a minor efficiency of atrazine due to the adaptation and accelerated degradation in soil amended organically and cultivated with maize.

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2687 ISOLATION AND IDENTIFICATION OF ACTINOMYCETES FROM A COMPOST-AMENDED SOIL AS BIOCONTROL AGENTS

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Compost capability to suppress soil-borne plant pathogens has become an interesting subject as a strategy for reducing the adverse effects of massive fungicides' application in the environment. In this context, actinomycetes have received considerable attention as biocontrol agents, particularly *Streptomyces* species. Thus, about 60% of the products with antifungal activity developed for agriculture have been isolated from *Streptomyces* spp. The aim of this investigation was to isolate actinomycetes from a soil, 2 two-phase olive mill waste ('alperujo') composts, and a compost-amended soil to perform an in vitro antagonism assay as well as to identify the actinomycete strains with the highest inhibitory activity. Actinomycete strains were isolated from selective media and co-cultured with 5 phytopathogenic reference fungi –*Fusarium oxysporum, Sclerotinia sclerotiorum, Phytophthora cinnamomi, Phytium debaryanum* and *Thanatephorus cucumeris*– by placing plugs of active growing fungi and actinomycete strains on potato dextrose agar and yeast-malt extract agar. After 7 days of incubation at 28 ºC, antagonism was determined by measuring the distance between the growing edges of actinomycete and fungi. Total DNA was extracted and subjected to PCR amplification using primers 27f and 1525r; the PCR product was purified with the same sets of primers. The 16S rRNA gene sequences were assembled by the PHYDIT software package, obtaining an almost complete sequence of each 16S rRNA gene. From these sequences, the identification was determined using the Basic Local Alignment Search Tool. In addition, different phenotypic characteristics were examined using standard procedures: chitinase activity, diaminopimelic acid isomers, aerial spore-mass colour, substrate mycelial pigmentation, and diffusible pigments. A total amount of 49 actinomycete strains were isolated, 12 of them showing a high antagonistic activity towards the phytopathogenic fungi tested. Eleven of these 12 strains were presumptively assigned to the genus *Streptomyces* due to their distinctive colonies and pigmentation characteristics. Identification revealed that the antagonist strains belonged to *Streptomyces variegatus, Streptomyces griseoruber, Streptomyces lincolnensis, Streptomyces aureoverticillatus, Streptomyces lusitanus, Streptomyces roseogriseus, Streptomyces coeruleorubidus* and *Lechevalieria xinjiangensis*. Right results were obtained from both phenotypic characterization and molecular techniques hence providing a reliable strains' identification. Further research should focus on in vivo antagonism tests to study the potential use of antagonist strains in commercial production.

2915 PLANT BIOMASS INCREASE LINKED TO BIOLOGICAL ACTIVITY IN AMENDED SOILS

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Sewage sludge compost application to almond tree plantations presents a potential management alternative to combat soil mismanagement in Mediterranean areas where almonds are grown. This practice could also be used to restore vegetable biomass to soils which are not fertile enough to support other crops, as well as to fight climatic change. The main objective of this study is to estimate aerial vegetable biomass increase from organic matter fluctuations and nutrient dynamics associated with soil microbial activity. To this end, the relationship between chemical - biological changes in compost-amended soils and vegetable biomass production was analyzed in a long-term study (2 years) conducted in almond tree plantations. An experiment consisting of almond trees planted in composted soil contained in dustbins was carried out. Four types of Majorca-sewage-sludge compost were compared, in their ability to increase soil N content to 1%, at two different doses. The control treatment contained unamended soil. Soil and tree sampling took place two years into the growth process. For the latter, the trunks, branches and leaves of those almond trees which grew in amended soils. Soil chemical characteristics and biological activity were measured. Microbial biomass carbon analysis and enzyme activity analysis (β-glucosidase, alkaline phosphatase, protease and urease) were conducted to gauge biological activity in the soil. C, N and P levels increased significantly in amended soils with respect to the unamended soil. Microbial biomass carbon in amended soils was not statistically different at any dose. However, there was an increase of microbial biomass carbon content in all the amended soils when compared to the control treatment. Branch biomass weight doubled its initial weight in soils treated with high doses of urban sewage sludge as compared to unamended soil. Significantly highest biomass weight increase (trunk, branches and leaves) with respect to organic carbon content in the soil originating from microbial biomass carbon was found in soils treated with the most stable or mature compost, the highest C/N ratio. Both phosphatase and protease activity, analyzed per gram of microbial biomass carbon, were significantly higher in amended soils. However when levels of enzymatic activity per gram of dry soil were analyzed, it was found that the levels of alkaline phosphatase were the highest in all the soils treated with compost.

2387 THE DEVELOPMENT OF TRANSFORMATION FUNCTIONS FOR THE QUANTITATIVE EVALUATION OF ENVIRONMENTAL IMPACTS IN THE AGRO-FOOD INDUSTRY

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In recent years, numerous environmental problems have appeared, so there is a need to change the focus to deal with today's environmental problems, abandoning ideas like unlimited development and accepting the concept of sustainable development, to avoid compromising future generations' needs.
Therefore, preventative measures are increasingly adopted which are effective in environmental protection, to minimise or eliminate many environmental impacts associated to humankind's activities.
The evaluation of Environmental Impact, as a preventative tool for the control of environmental projects, appeared at community level in 1985, with EEC Directive 85/337 for the evaluation of environmental impacts of public and private works. In 1986, with Spain's adhesion to the European Economic Community, this tool became part of our procedures for introducing environmental criteria into projects.
Since then, the legislation has received several modifications, finally reaching state level on 11 January 2008 (Law 1/2008). Of the changes introduced, we note the considerable increase in the number of projects now subject to this evaluation procedure. Some experts in this field attribute a series of deficiencies to this procedure. We can highlight the following: absence of a valuation methodology which permits unifying criteria; tuning environmental indicators; absence of transformation functions; excessive qualitative valuations; etc.The present work therefore seeks to study the choice of standard environmental indicators to evaluate quantitatively certain impacts associated to the agro-food industry, and the construction of the corresponding transformation functions associated to these indicators. This enables us to obtain homogeneous values of environmental quality, compare alternatives, study the environmental viability of a specific project, study the efficacy of a correction measure, etc. The construction of the transformation functions is undertaken using data of representative current practice activities of the agro-food sector, to thus be able to establish the situation with project. To determine what the minimum conditions required should be, we have used the values established in current legislation, depending on the indicators.Therefore, we consider that this work can contribute to the development of methodologies that tackle quantitative valuation of environmental impact, and to tune the standard indicators depending on specific problems of the sector, current legislation, etc.

2486 MINOR AND TRACE METALS LEVELS IN HUMAN MILK IN NORTH WESTERN CITIES OF LIBYA

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Levels of twelve minor and trace metals were determined by using [AAS,ES and ICP/MS) in breast milk obtained from 60 women living in north western cities of Libya.
Samples were collected at one week up to two years after delivery. Women with age > 21 years old to an age of < 43 years old were investigated. The samples were digested in 86% w/v nitric acid and few drops of perchloric acid. The treated samples were measured with flame atomic and emission spectroscopy for their minor and trace metals contents. The same samples were also treated with 25% w/v tetramethylammonium hydroxide and then measured directly with ICP/MS for their metals content. The results obtained for human milk showed that minor metals levels were Ca (0.02-0.7% w/v), Mg (0.001-0.2% w/v), while Na and K had maximum values of 2.0% w/v and 1.4% w/v respectively. Trace metals levels in human milk were (0.32-1.88) ppmZn, (0.15-0.74)ppmFe, (10-196) ppb Pb, (< 0.4-14) ppb Cd, (< 0.4-109) ppb Cr, (2-30) ppb Li, (5.1-296) ppbAl, and (2.7-10.4) ppb Mn. These values were compared to previous studies in different parts of the world.

2520 HOW DOES A WASTEWATER TREATMENT PLANT WORK?. DIDACTIC SCHEME ON WASTEWATER TREATMENT IN THE FRAMEWORK OF A SPREADING SCIENTIFIC'S FAIR

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Every spring takes place the fair *"Feria de Madrid por la ciencia"* devote to the science spreading. It is a very popular event with 147,500 visitors last year. The fair is focused on general public, including a broad range of ages and education. The main purpose is to put in contact citizens with researchers and to spread science in a understandable way. The event is managed by scientists underlaying their important role in the popularization of science. The present edition will take place on April 24th to 27th and it will be focus on water, including several environmental aspects and problems. In this context, we planed to carried out a didactic experience with the goal that people become aware about the pollution of waters, meanly domestic ones, showing how a wastewater treatment plant operates and its microbiological basis. In order to do this, a model of Wastewater Treatment Plant - including all the facilities and phases (pre-treatment, primary, secondary and tertiary treatment) - will be displayed. A short explanation on the associated (physical, chemical or microbiological) processes will be given. The experience will be evaluated by question forms in order to know the effectiveness of this sort of events.

2584 COMBINED ACTION OF HEAVY METALS IN SOILS SUSTAINING CORN AND SORGHUM: ASSESSMENT METHODS, PHYTOREMEDIATION AS AN OPTION AND EDUCATION STRATEGIES

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Heavy metals generated through geochemical processes in some tropical ecosystems, besides affecting the productivity of the systems, could also affect human health. Based on this hypothesis, we set out to assess the bioavailability of heavy metals to these crops used as a main food source or component of fodder.
Four real scenarios in the Dominican Republic were examined whose soils contained Al and more than one heavy metal (Cd, Cu, Zn, Cr or Ni). Soil samples from the different sites were used to conduct bioassays in microcosms under controlled conditions planted with local varieties of corn and sorghum.
Three replicates were prepared per site and control. The techniques used to evaluate bioavailability were plasma emission spectroscopy, scanning electron microscopy (SEM), low temperature SEM (LTSEM) and transmission electron microscopy (TEM). These microanalytical methods allowed us to determine the plant organs and tissues in which heavy metals build up and to observe any damage produced.
Statistical tests revealed significant differences in the metal contents of the above-ground mass versus the roots of the plants in the soils. Levels of Al, Cu, Cd and Mn were high in the above-ground parts of the plants and Cu, Cd, Cr, Ni and Mn in roots. The accumulation by these crops of toxic metals was assessed as a possible method of phytoremediation but also in terms of its impact on the health of both the crop systems and humans. We also discuss possible effects on the livestock consuming these crops. Both issues are considered to substantially compromise the chances of tropical countries unaware of their soil pollution problems to escape poverty. Also proposed are strategies for education in this issue and for sustainable development in regions faced with this problem.

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2590 LOCAL HABITATS RECREATION IN GARDENING AS AN ENVIRONMENTAL EDUCATION TOOL

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A garden has been implanted at IMIDA facilities in La Alberca (Murcia) which recreates different habitats of Murcia Region, with two main objectives: 1) to be used as a tool for environmental education, encouraging social awareness in habitats and flora species protection, and 2) to obtain relevant information for the use of regional wild flora in gardening, both for the ornamental interest of not extensively spread species, and its low water irrigation needs.
The design phase included zoning by habitats (Coastline"ramblas"; Zone for J. phoenicea ssp. turbinata; Zone interior garrigue; Zone for P. halepensis grove; forest "rambla" area; Zone sclerophyllous scrublands with *Juniperus*; Zone consolidated dunes; Zone fossilized dunes; etc.): It was considered of great importance the inclusion / exclusion of the surrounding landscape (including views of the Sierra de La Pila, 40 km to North), or the use of local materials (sandstone floors, rocks...). As an added value it is expected to include some species subjected to different figures of protection. The implementation of the main works was carried out between November 2007 and March 2008. Then the plantations started to be conducted, and it will take place gradually, depending on the difficulty to obtain plant material.

2897 PRESENCE OF BERYLLIUM (Be) IN URBAN SOILS: HUMAN HEALTH RISK

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Beryllium (Be) is, together with As, Cd. Hg, Pb and Ti, one of the trace elements more toxic for human being (Vaessen and Szteke, 2000; Yaman and Avci, 2006), but in spite of the exponential increment of its applications during the last decades, surprisingly there isn't hardly information about its presence and environmental distribution.
The aim of this work is to evaluate the presence of beryllium in urban soils in Alcalá de Henares, (Madrid, Spain). Considering the obtained results, the carcinogenic risk for human health (ER) due to the ingestion and inhalation of these soils has been evaluated, using the criteria proposed by the US EPA (2004).
Soil samples were selected from public gardens located in different areas in the city. Beryllium content was determined by ICP-MS (Perkin Elmer Elan 6000) after an acid treatment. Results show the presence of beryllium in most of the samples in values around 0.35 ± 0.17 mg/g. These values are higher than those founded in other European soils (Yaman and Avci, 2006, Bath et al. 1997) However, higher values have been found in soils in the vicinity of municipal waste incinerator in values around 0.63 μg/g (Nadal et al. 2005, Meneses et al, 1999). Beryllium presence in Alcalá de Henares soils could be associated with the brick and ceramic manufacturing industries located near the city and the presence of a highway that split up the city into two parts letting cars to expel a big amount of fumes for years in the middle of the city. On the whole, it has been observed that it doesn't exist a significant risk for human health due to the presence of Beryllium in the Alcalá de Henares soils although it is necessary to develop basic and risk evaluation studies to monitor the element, especially in our country. Besides, it would be very important to carry out more studies focused on the knowledge of this trace element, at the moment much unknown.

1790 ACHIEVING SUSTAINABLE BIOMASS CONVERSION TO ENERGY AND BIOPRODUCTS

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The present effort in to maximize biomass conversion-to-energy and bioproducts is examined in terms of sustainability practices. New goals, standards in practice, measurements and certification are needed for the sustainable biomass industry.

Sustainable practices produce biomass energy and products in a manner that is secure, renewable, accessible locally, and pollution free. To achieve sustainable conversion, some new goals are proposed. The goal for energy crops should be to grow a renewable resource for biomass-to-energy conversion, in a sustainable manner, and to utilize the energy or molecular constituents of the waste stream, to the fullest extent possible before placing the materials in the ground. The goal for biomass waste management should be to utilize the energy or molecular constituents, in a sustainable manner, to the fullest extent possible before placing the materials in the ground. The activities in energy crop production, biomass-to-energy and bioproducts conversion, conducting biomass waste management, as well as construction of a biomass-to-energy and bio-product conversion facilities, should be held to standards-in-practice to assure sustainable biomass systems. Standards-in-practices for biomass systems are proposed based on principles of greenhouse gas balance, carbon sinks, existing food supplies, biodiversity, water availability, air quality, local economic development, social well-being of employees, and transparency to the public.

A method of measurement is proposed to hold developers and operators accountable to the standards-in-practice. This method must be instituted in order to achieve the maximum level of biomass conversion at sustainable levels.

It is anticipated that both voluntary and compliance-based markets for energy or products from biomass conversion will drive the need for a firm to become certified. As a level of certification is reached, compliance is required in all areas of bio energy and product production. Ultimately, the market for energy or products derived from sustainable biomass conversion will drive actual amounts of production.

2293 ENERGY PRODUCTION BY ANAEROBIC TREATMENT OF CHEESE WHEY

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Anaerobic treatment and methane generation potential of cheese whey, diluted with mud, were determined in the digester of an existing wastewater treatment plant in Switzerland. Lactose, main sugar in cheese whey, can be a useful indicator to evaluate serum anaerobic treatment.

Conventional parameters of anaerobic digestion (Volatile Matter, Dry Matter, Fatty Volatile Acids, Total Alkalimetric Title) were measured after the introduction of different whey/sludge ratio demonstrating that, despite an overcharge of whey digester, its stability is never compromised. Additionally, Capillary Zone Electrophoresis was performed in order to detect lactose, main sugar of cheese whey, in the incoming and outcoming flows of the digester. Results revealed fast lactose removal, with efficiency higher than 90%, since the sugar in sludge output appeared only four hours after its introduction. Finally we measured variation of methane concentration in biogas for different whey/sludge ratio: results revealed a light diminution since a maximum decrease rate of 14%, measured in correspondence of an incoming flux with a whey/sludge ratio of 2.56.

Keywords: Chese whey, Biogas, Lactose, Capillary Zone Electrophoresis.

2399 PRELIMINARY DETECTION OF NATIVE LIPASE PRODUCING MICROORGANISMS FOR BIODIESEL PRODUCTION

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Lipase producing microorganisms (LPM) may catalyze the hydrolysis or transesterification of triacylglycerols to alkyl esters of fatty acids (biodiesel).
The main objective of this work was to detect LPM in oil and grease contaminated environments for future applications in biodiesel production from rapeseed oil.
Samples from contaminated soil (with rapeseed oil) from an industrial facility and contaminated soil (with salmon grease) near to a fish wastewater treatment plant were collected. The samples were first inoculated at 150 rpm and 30 ºC in a liquid enriched selective medium (70 g/L polypeptone, 1g/L $NaNO_3$, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4$ $7H_2O$, and 30 g/L rapeseed oil). Then, agar was added to the same medium for counting and isolating the colonies at 20 ºC (solid medium). Finally, the isolated colonies were grown in a minimal media at 20 ºC (10% soil extract, 0.02% urea, 1.5 % agar and 0.1% rapeseed oil emulsified with 10% tween 80). All media contained rapeseed oil as the sole carbon source. Samples from contaminated soil with salmon grease showed a total degradation of rapeseed oil in liquid media, probably due to the presence of different colonies inoculated in the solid media (six compared with only two in contaminated soils with rapeseed oil). To test lipase enzymatic activities, the isolated colonies were inoculated in the minimal solid media. Only colonies from soil contaminated with salmon grease formed a clear zone in the agar. Isolated colonies from soil contaminated with salmon grease showed a clear zone inclusively in the solid media. This fact may prove the high activities of these enzymes.
This work showed that soil contaminated with rapeseed oil or fish grease are possible sources of LPM, which could be used for future applications in biodiesel production such as whole-cell biocatalysts that unlike commercial lipase, requires no purification or complicate immobilization methods, minimizing biodiesel production process costs.

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2404 DETERMINING WASTE LIPIDS STABILITY AND POSSIBLE EFFECTS IN BIODIESEL PRODUCTION

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Waste lipids are a sustainable raw material alternative for biodiesel production, avoiding excessive use of agricultural soil. However, this raw material can be degraded in a short time of storage, affecting biodiesel production process and quality. The aim of this work was to investigate the possible degradation of waste frying oil (WFO) and animal fat (AF), monitoring parameters that could affect the biodiesel quality.
Primarily, WFO was filtrated, while AF was first liquefied at 40 °C and then filtrated. Both, WFO and AF were characterized and were stored at 22 °C in a dry and dark environment for monitoring. The following parameters were measured consecutively during three months: acid value, free fatty acid (FFA), peroxide index, kinematic viscosity at 40 °C and iodine value.
For WFO, the following parameters increased: acid value in a 16%, FFA in a 17%, peroxide index in a 34% and viscosity in a 7%. Only, iodine value was diminished in a 22%. For AF, all the parameters increased less than 10% during the three months of storage.
These results correlate with WFO and AF degradation. The increment in peroxide index by oxidation reactions of WFO, could produce a most complete combustion of biodiesel by increasing oxygen content. However, in AF the peroxide index did not increase as it is composed by saturated organic compounds. In addition, the acid value could negatively affect the basic catalyst through saponification reactions. However, using a biological catalyst, an increasing FFA value could improve the transesterification reaction yield.
Polymerization reactions between triglycerides, increasing viscosity and diminishing iodine value could affect the biodiesel quality increasing the cloud point of the produced biodiesel.
In conclusion, the characteristics of waste lipids used as raw material for biodiesel production changed by degradation reactions during its storage. These variations could positively or negatively affect biodiesel quality. Under the experimented conditions, the stability of WFO seems to be much lower than AF, for its possible use in biodiesel production after three months of storage.

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2539 IMPROVEMENT OF ANAEROBIC BIO-HYDROGEN GAS PRODUCTION FROM ORGANIC SLUDGE WASTE

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Microbial hydrogen gas production from organic matters stands out as one of the most promising alternatives for sustainable green energy production. Based on the literature review, investigation of anaerobic bio-hydrogen gas production from organic sludge waste using a mixed culture has been very limited.
The objective of this study was to assess the anaerobic bio-hydrogen gas production from organic sludge waste under various conditions.
Using a suspended-growth, mixed, mesophilic, anaerobic culture, batch assays were performed to investigate the effects of 2-bromoethanesulfonic acid (BESA) which is known as an inhibitor of methanogenesis, gas purging for lowering the hydrogen partial pressure in the fermentation reactor, and pH on anaerobic bio-hydrogen gas production from municipal sludge waste as a sole carbon source. Apart from hydrogen, methane, gas production, chemical oxygen demand (COD), and volatile fatty acids (VFAs) were also monitored. Initial BESA concentrations higher than 10 g/L resulted in severe inhibition of methanogenesis up to 92%. However, all BESA amended cultures did not result in an accumulation of hydrogen gas and only resulted in the hydrogen gas production up to 0.8% which was normalized to the total COD processed (i.e., VFAs-, H_2, and CH_4-COD) from sludge waste. Among various gas purging methods at different purging rates, gas purging through the liquid medium at 2 L/min purging rate resulted in both a significant increase of bio-hydrogen gas production (7.1% to the total COD processed) and a decrease of methane gas production (94.4% lower than that of the control culture) for the BESA-amended cultures. However, gas purging did not result in a significant increase of bio-hydrogen gas production for all cultures without amendment of BESA. Initial pH was found to have a profound effect on bio-hydrogen gas production. The BESA-amended cultures under liquid purging condition at the initial pH 5 resulted in 2.1 and 2.3 times higher bio-hydrogen gas production than those at the initial pH 7 and 9, respectively. The findings of this study could be applied in the design of a high rate bio-hydrogen gas production system.

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2541 MICRODRIVE - A RESEARCH PROGRAM ON SUSTAINABLE BIO-ETHANOL AND BIOGAS SYSTEMS

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MicroDrivE – Microbially Derived Energy – is a thematic research program on sustainable biofuel production at the Faculty for Natural Resources and Agriculture (NL), Swedish University of Agricultural Sciences (SLU). The program has the following long term goals: To maximise the energy yield of ethanol and biogas processes, improve overall process economy through development of novel co-products, and to minimise environmental impact. The program strategy is: To integrate research projects on biopreservation, enzymatic pre-treatment of plant feed stock, ethanol fermentation, bioprocessing and fermentation of ethanol process by-products, biogas fermentation, and the re-circulation of plant nutrients in biogas digestates. Initially, the focus will be on using cereal grains and sugar beets as feed stock, later straw and other cellulosic biomass sources will be explored.
MicroDrivE presently involves 16 scientists, post-docs and PhD students with specialist competence in microbiology, molecular biology/enzymology and natural products chemistry. The scientists supervise MSc thesis students doing 20 week-projects, commonly in joint projects with our industrial partners (Syngenta seeds, Chematur Engineering, Jästbolaget, Medipharm, Tekniska verken/Svensk Biogas and Sala Heby Energi). MicroDrivE is funded by the NL-faculty (50%), our industrial partners, the Swedish Farmers Research Foundation (SLF) and the National Energy Board. The research program started March 1, 2007, and is planned to run until 2013, with an annual budget exceeding 0.9 M USD. MicroDrivE welcomes international cooperation!

2880 SOLID SUBSTRATE ANAEROBIC FERMENTATION OF MUNICIPAL AND JUICE FRUIT-INDUSTRY SOLID WASTES FOR THE PRODUCTION OF BIOHYDROGEN

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Use and abuse of fossil fuels has lead to decreased supply, increasing energy costs and negative environmental and health impacts. Thus, renewable and environmentally-friendly sources of energy are attracting increased attention in recent years. Hydrogen has been targetted as the fuel of the future because of its high calorific heat and clean combustion. Moreover, biological production of hydrogen has been identified as a cost-effective alternative. Particularly, anaerobic hydrogenogenic fermentation processes based on undefined microbial consortia and cheap biomass or wastes are a promising approach. In such processes, several microbial groups that consume H_2 coexist with the H_2-producing microbes. So, it is paramount to process feasibility to find techniques to inhibit the H_2-consuming microorganisms, such as the methanogenic archaea to cite one of the most important groups. It has been reported a variety of methanogenesis inhibitors, inter alia: acetylene, bromo-ethanesulphonate (BES), heat-shock pretreatment and low pH. On the hand, near 18.2 Gkg/year of municipal solid wastes and 146 Gkg/year of solid cakes from the fruit juice-industry are generated in Mexico, posing a challenge to solid waste management and disposal. Thus, the objective of this research was to evaluate the effect of two operational strategies based on pH as a way to inhibit methanogenesis, for batch, biohydrogen production from a mixture of organic fraction of municipal solid wastes (OFMSW) and organic cake from the fruit-juice industry (JIW). The first strategy consisted of setting initial pH of the solid substrate culture with further free fermentation course (FpH), whereas the second strategy consisted in intermittently controlling pH at 6.0 (or intervention) during the course of the fermentation (IpH).
The experimental design consisted of setting the two treatments (FpH or treatment 1, and IpH or treatment 2) and several controls (only inoculum with no substrate for background hydrogen production, moisture/ionic force control with sodium chloride solution). The process was the so-called solid substrate anaerobic hydrogenogenic fermentation with intermittent venting (SSAHF-IV). The inoculum for all treatments and controls was solids from a methanogenic solid susbrate anaerobic digester. Substrate was a mixture of 77% OFMSW and 33% of cake/bagasse of fruits from a juice industry (mainly orange and Apple). The pH for bioreactors in treatment 1 was set at 6.5 at the beginning of the run with no further intervention whereas pH in treatment 2 was initially set at 6.15 and further intermittent interventions with buffer were made to keep pH ca. this value as the fermentation proceeded.
Here we present results for the first cycle of SSAFH-IV. Cumulative H_2 productions were 4.45 and 1.65 mmolH_2/minireactor for the FpH and IpH strategies. Lag times were shorter than 72 for both treatments. Time for reaching H_2 plateau was 200 h for IpH and more than double of that for FpH. Organic metabolite profiles indicated that organic acids accounted for most of the overall amount of dissolved COD. Solvent proportion was higher in solids of bioreactors of the FpH than those of the IpH units, which was consistent with a lower H_2 production. In conclusión, pH as a means of inhibiting methanogenesis in batch SSAFH-IV was shown to be successful. A distinct advantage of IpH strategy over FpH strategy was determined.

2881 RE-FERMENTATION OF SPENT SOLIDS FROM DARK FERMENTATION ALLOWS FOR A SUBSTANTIAL INCREASE OF HYDROGEN PRODUCTION FROM THE ORGANIC FRACTION OF MUNICIPAL SOLID WASTE

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In the last 10 years, interest on biohydrogen has resurrected, particularly the research on dark fermentation of solid wastes. In effect, in a context of scarce and expensive fossil fuels, hydrogen can be considered the best energy alternative because it can be produced by biological means, it has the highest energy density, it is versatile since can be used both as a primary or secondary energy source, it is compatible with electrochemical and combustion-based energy conversion processes, and it is environmentally-friendly since water is its main combustion product and no aggressive pollutants are generated. The objective of this work was to evaluate the effect of eliminating soluble metabolites (by washing) from spent solids that had been subjected to a previous fermentation, on the hydrogenogenic re-fermentation of such solids.
The process chosen was the so-called mesophilic, batch, solid substrate anaerobic hydrogen fermentation with intermittent venting (SSAHF-IV). The substrate was a model organic fraction of municipal solid waste (OFMSW). The OFMSW was first subjected to SSAHF-IV of four cycles, where acetylene was the inhibitor of methanogenesis in the first cycle. Once it was determined the absence of hydrogen production in the fifth cycle, the spent solids were washed and dried for future use. Washed spent solids were then fed to a second SSAHF-IV, where the inhibitor of methanogenesis was pH at level 6. Inocula for both SSAHF-IV were solids from a methanogenic solid substrate digester. In the first SSAHF-IV, a cumulative 16.6 mmole H_2/reactor was obtained. Releases on hydrogen partial pressure first by intermittent venting and afterwards by flushing head-space of reactors with inert gas N_2 allowed for further hydrogen production in a second through fourth incubation cycle, with no new inoculum nor substrate nor inhibitor. After the fourth cycle, no more H_2 could be harvested. Interestingly, accumulated hydrogen in 4 cycles was 100% higher than that produced in the first cycle alone. At the end of incubation, partial pressure of H_2 was near zero and high concentrations of organic acids and solvents remained in the spent solids. So, since approximate mass balances indicated that there was still a moderate amount of biodegradable matter in the spent solids we hypothetized that the organic metabolites imposed some kind of inhibition on further fermentation. Spent solids were washed to eliminate organic metabolites and they were used in a second SSAHF-IV. Two more cycles of H_2 production were obtained, with a cumulative production of ca. 2.4 mmole H_2/mini-reactor. As conclusión, washing of spent solids of a previous SSAHF-IV allowed for an increase of hydrogen production by 15 a 20% in a second run of SSAHF-IV, lending to the validation of our hypothesis.

2885 MICROBIAL FUEL CELLS: A PROMISING ALTERNATIVE FOR POWER GENERATION AND WASTE TREAMENT

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The current energy crisis has launched a renewed interest on alternative energy sources and non-fossil fuels. One promising technology is the direct production of electricity from organic matter or wastes in microbial fuel cells (MFC). A MFC can be envisioned as an bio-electrochemical reactor that converts the chemical energy stored in chemical bonds into electrical energy via the catalytic activity of microorganisms under anoxic conditions. In addition to electricity generation, a MFC may significantly decrease the organic load of the effluent, acting as a wastewater treatment unit. In spite that research on MFC has increased in the last years, several issues remain to be solved. In this work, we present a critical review on the state-of-art of MFC that includes the following topics: (i) types of MFC and relative advantages and disadvantages (i.e., 2-compartment MFC, and one compartment cells, arrangement the electrodes, etc.); (ii) types of microorganisms and substrates used in the MFC (iii) effects of operational conditions on cell performance (batch versus continuous, temperature, salinity, etc.) (iv) advances on and effects of construction materials and membrane materials son MFC performance; and (v) MFC applications, such as the common electricity generation, hydrogen production in hybrid MFC, and use as biosensors. Power output of MFC can be limited by high values of its internal resistance (Ri). So far, Ri has been minimized by the extensive use of Pt for connectors and cathode catalyst. Yet, Pt is very expensive and research on more economic new materials and catalysts is a dynamic area of development. Also, clever cell/electrode configuration and arrangement can decrease Ri. We have found that experiment with pilot or demonstration MFC are very scarce, most studies focus on lab scale MFC. This is a drawback for technology progress and implementation. Regarding the biocatalysts, most MFC research has been concentrated in pure cultures as biocatalysts. As of organic substrate, fortunately there is an increase of studies with actual or synthetic wastewaters that helped overcoming the shadows of doubt on results obtained with pure organic substances such as glucose. From our perspective, the main challenges that should be addressed by R&D in the future are: (i) to increase power density and coulombimetric efficiency by changes in cell design and configuration, among other changes; (ii) to extend application of MFC to effluents polluted with organic toxicants; (iii) to develop new materials for connectors, construction, and catalysts that are as efficient as or more efficient than Pt, and less expensive; (iv) to foster the development of the MFC assemblage engineering in order to expand MFC application to higher voltages and intensities. In our perception, MFC technology will be a significant component of the contribution of biological energies to the sustainable development of our modern societies.

2889 EFFECT OF INOCULUM TYPE ON PERFORMANCE OF A MICROBIAL FUEL CELL FED WITH ACIDOGENIC EXTRACTS FROM DARK FERMENTATION OF WASTES

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Microbial fuel cells (MFC) have been successfully applied to a variety of dilute organic effluents for simultaneous electricity generation and effluent treatment. So far, the use of pure strains seems to predominate in previous reports in the literature. In contrast, up to now less is known on the use of microbial consortia. The aim of this work was to determine the effect of type of inoculum (methanogenic and sulphate-reducing consortia) on the performance of a lab scale one chamber microbial fuel cell (MFC) during the batch treatment of a synthetic acidogenic extract from solids previously subjected to dark hydrogenogenic fermentation.
A one chamber, lab scale MFC was built with plexiglass. The MFC was inoculated with either a methanogenic (M-In) or sulphate-reducing (SR-In) inoculum from complete mix reactors. The MFC was loaded with a model extract concocted with a mixture of organic acids and mineral salts. Initial COD of cell liquor was ca. 700 mg/L and biomass contents were ca. 220 mg/L VSS. MFCs were batch-operated for 50 h at 37 ºC. The circuit of each MFC was fitted either with a 33 or 10 kΩ external resistance in order to be consistent with the Theorem of Jacobi.
All variables showed a better performance using SR-In than using M-In. The maximum, open circuit potential of the MFC loaded with M-In was 0.4 V, whereas the one loaded with SR-In was 0.7 V. On the other hand the maximum potential of the resistance-loaded MFC was 0.26 and 0.46 V with a M-In and SR-In, respectively. Average cell potentials of 0.10 (M-In) and 0.37 V (SR-In), were registered during the 50 h of operation.The anodic average power density of 12.31 mW m^{-2} using S-In, was about 12 times higher than the power density of a MFC loaded with M-In (1.04 mW m^{-2} anode). Power densities recorded were lower than values reported recently in the open literature. Meanwhile, values of VMFC were comparable to those found in the literature for other MFC, while IMFC resulted lower than those observed for MFC treating dilute organic wastewaters. Organic matter removal was low to moderate: 25% using a M-In and almost double (43%) in the MFC loaded with a SR-In. This results was consistent with very low values of the coloumbic efficiency. Both parameters could be increased by increasing the time of operation (because at the end of the run the mayority of the organic substrate was still available) and by lowering the internal resistance of the cell. Finally, the MFC seeded with SR-In outperformed the MFC loaded with a M-In.

2894 BATCH TESTS OF A MICROBIAL FUEL CELL FOR ELECTRICITY GENERATION FROM SPENT ORGANIC EXTRACTS FROM HYDROGENOGENIC FERMENTATION OF ORGANIC SOLID WASTES

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Hydrogenogenic fermentative processes of organic solid wastes produce spent solids that contain substantial concentrations of low molecular weight organic acids and solvents. The spent solids can be extracted with wastewater to give a stream containing concentrated, degradable organic compounds. Therefore, the extract from spent solids has the potential for further production of energy when fed to a microbial fuel cell. So, the objective of this work was to evaluate the production of electricity and general performance of a lab scale microbial fuel cell (MFC) for the batch treatment of extracts from spent solids generated in fermentative hydrogenogenic process. A one-chamber MFC was built plexiglass 78 mm long (between electrodes) and 48 mm internal diameter. Electrodes were a circular anode made of stainless steel plate 1 mm thickness and a cathode made of a sandwich of 3 circular layers (from inside to outside): proton exchange membrane (Nafion 117), flexible carbon-cloth containing 0.5 mg/cm^2 platinum catalyst, and a perforated plate of stainless steel 1 mm thickness. The cathode was in direct contact with atmospheric air on the metallic plate side. The MFC was inoculated with 143 mL inoculum from a methanogenic, complete mix reactor. The MFC was fed 7 mL of a model extract concocted with a mixture of acetic, propionic and butyric acids as well as acetone and ethanol and mineral salts. The COD of the model extract was ca. 16 g COD/L. The MFC was batch-operated for 50 h at 37 ºC. Initial and final pH were 7.66 and 8.32, respectively. Internal resistance of the MFC was found to be 33 kΩ so, the circuit of the MFC was fitted with a 33 kΩ external resistance. The maximum, open circuit potential of the MFC was 0.4 V whereas the maximum potential of the resistance-loaded MFC was 0.12 V during the first 20 h of operation. Current intensity maximum (3.7x10-3mA) occurred during the same period. Afterwards, both variables and power decreased with time with drops around 20% for VMFC and IMFC and 40% for power. Values of VMFC were comparable to those found in the literature for other MFC, while IMFC and power resulted significantly lower than those observed for MFC treating dilute organic wastewaters. This difference with previous reported results could be ascribed to our dilute inoculum (200 mg VSS/L), longer distance between electrodes in our MFC, high organic load of the feed, and lack of catalyst in the anode, among other reasons. Interestingly, the power density per unit area of anode was in the mid range of published data. Organic matter removal of cell liquor was low to moderate ~25%, which was consistent with very low values of the coloumbic efficiency. Both parameters could be increased by increasing the time of operation, since at the end of the run 75% of the organic substrate was still available. Further experiments are undergoing in order to look for increased power and power density of our MFC. Our results seem to indicate that MFC could be used to further tapping energy from solid wastes, increasing electricity yields from a practically inexhaustible and cheap resource.

2906 AN ENRICHMENT AND ACCLIMATION PROCEDURE TO OBTAIN PHOTOHETEROTROPHIC CULTURES FOR H_2 PRODUCTION FROM ORGANIC EFFLUENTS

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Production of H_2 via photoheterotrophic is an attractive alternative, due to the capacity of photoheterotrophic organisms to convert the organic matter of effluents into H_2. The objective of our work was to develop a protocol for selecting undefined mixed cultures of photoheterotrophic microorganisms with the capability of producing of H_2. Wild inocula were used; the sources were (i) sediments form a lagoon of the Los Azufres Michoacán, Mexico, (ii) sediments from a geyser of the Los Azufres Michoacán, México and (iii) sludge from a channel of wastewaters Río de los Remedios, Mexico City, Mexico. After the enrichment process, the production of H_2 of these mixed cultures were compared against that of the photoheterotrophic strain *Rhodopseudomonas palustris DSM-127*.
Four Winogradsky columns were loaded with 15 inocula and 50 g/L sawdust as organic carbon source. The columns were illuminated with tungsten lamps at 1.5klux for nearly 3 months. After biofilm and color development, samples were taken from the section that showed purplish color. These samples were transferred to tubes or serum bottles for further enrichment in 10 passes. Afterwards, four sequential tests of production of H_2 they were carried out. Two enrichments (3S and 6A) were selected after the 3rd test. The fourth batch test evaluated the effect of the following variables: *(i)* type of illumination (*either fluorescent or tungsten*), *(ii)* light intensity (6.5 or 3.5klx) and *(iii)* two cultivation media (Pfenning and Rhodospirilaceae) where the carbon source was a mixture of low molecular weight organic acids and solvents. The above mentioned two enrichments and the pure strain *R. palustris* were examined. The highest accumulated production of H_2 was ~ 2.6 mmol H_2/L for the mixed cultures under 3.5 klx, tungsten illumination and medium Rhodospirilaceae, a value superior to that obtained for the pure culture *R. palustris*. The mixed cultures also presented a criptic growth pattern (in two phases), that lead to extended H_2 production.
In conclusion, the enrichment and acclimation procedure developed in this work to obtain photoheterotrophic cultures from wild inocula was successful. Moreover, preliminary batch tests showed that selected enrichments had higher cumulative H_2 production than the axenic culture *R. palustris*. The significance of our work revolves around three contributions: *(i)* it provides important information on the use of hydrogenogenic photoheterotrophic mixed cultures since little has been previously reported in the open literature, *(ii)* it is the first comparative study between photoheterotrophic mixed cultures and a photoheterotrophic pure strain, and *(iii)* it expands the application of photoheterotrophic processes to complement other biohydogen-producing processes, leading to increased H_2 yields from organic substrates, particularly those coming from the fermentation of solid wastes.

2907 A TWO-STAGE BIOHYDROGEN PROCESS FOR ENERGY GENERATION FROM MUNICIPAL SOLID WASTES

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Energy supply and disposal of solid wastes are two big challenges that great cities face at the present time. Several experts have shown that hydrogen is the fuel of the future, due to their high energy content (three times more than that of the gasoline) and its clean combustion. The biological production of H_2 is a feasible and attractive alternative, due to the great metabolic flexibility that present a wide variety of microorganisms, that could thrive on a great carbon source range to produce H_2 including the organic matter present in the organic fraction of the municipal solid wastes (OFMSW). The objective of this work was to implement a two-stage biological process for H_2 production. The stage 1 consisted of a dark fermentation of the OFMSW that mainly produced H_2 in the biogas. This stage also generated great amounts of organic and solvent acids in the spent solids. This organic matter was the substrate for the stage 2 of the process, which consisted of a photoheterotrophic fermentation. The latter produced an additional amount of H_2, increasing this way the overall H_2 yield of the biological system.

The reactors of the stage 1 were fed a typical OFMSW and operated as anaerobic, acidogenic fermentation in solid substrate mode, semi-continuous regime at mass retention time of 21 days. The stage 2 consisted of a submerged, anaerobic photoheterotrophic fermentation using an enriched consortium of photoheterotrophic microorganisms. The reactors of 100 mL were batch operated regarding the liquid phase whereas the gas phase was intermittently gassed-out with argon. They received continuous artificial illumination at 3.5 klx.

The reactors of the stage 1 removed an average of 48.7 ± 2.0% of volatile solids and produced 755 NmL/kg·d of biogas with an average content of 50% v/v of H_2. The first stage produced fermented solids that were extracted with municipal wastewater (1:3 wet weight:volume). The extract contained a significant amount of low molecular weight organic acids and solvents (~ 92% of acids and ~ 8% of solvents). The extract was used as the substrate for the second stage. In the second stage of the process an average initial rate of 3.4 μmol H_2/L·h was obtained. Most importantly, a high accumulated production of H_2 (2530 μmol H_2/L) and significant removals of the organic and solvent acids (i.e., 67, 55, 50, and 75% for acetic acid, propionic, butiric and butanol respectively) were observed.

In conclusion, our concept of a process in two stages of biological production of H_2 shows a great potential to increase the conversion of OFMSR to H_2 by 15 to 20%. Undoubtedly, our system points out to an attractive contribution to the solution of contemporary challenges of supplying low-cost and clean energy as well as disposal of municipal solid wastes.

2204 PRODUCTION OF ETHANOL AND BIOMASS STARTING TO PRESENT LACTOSE IN THE MILK WHEY

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Milk whey is a by-product of the milk industry, a highly polluting waste due to the quantity of COD and BOD that it contains. The contamination caused by milk whey is mostly due to its lactose content. The fermentation of milk whey to ethanol is a possible road to reduce the polluting effect. Presently, the production of ethanol from the lactose present in milk whey is evaluated in batch experiments using as first stage the β-galactosidase enzyme produced by *Kluyveromyces lactis* to carry out the hydrolysis of lactose, followed by the fermentation of sugars by yeasts isolated from pulque (alcoholic drink obtained from agave). The effect of the initial concentration of lactose (10 and 15%), pH, different concentrations of nitrogen source and trace elements on the alcoholic fermentation was studied. The biomass production, consumption of sugar, production of ethanol and global yields of the process were determined. The obtained results indicate that the maximum yield of ethanol is observed after the 48 h of fermentation. The media with yeast extract was observed to have the biggest influence in the production of ethanol while a pH of 6 allowed the highest generation of biomass. Ethanol formation from whey increased with increasing concentration of sugar.

2371 USE OF SEVERAL WASTE SUBSTRATES FOR CAROTENOID-RICH YEAST BIOMASS PRODUCTION

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Carotenoids are industrially significant pigments produced in many bacteria, fungi, and plants. Carotenoid biosynthesis in yeasts is involved in stress response mechanisms. Thus, controled physiological and nutrition stress can be used for enhanced pigment production. Huge commercial demand for natural carotenoids has focused attention on developing of suitable biotechnological techniques including use of liguid waste substrates as carbon and/or nitrogen source. In this work several red yeast strains (*Sporobolomyces roseus, Rhodotorula glutinis, Rhodotorula rubra*) were enrolled into a comparative screening study. To increase the yield of these pigments at improved biomass production, several types of exogenous as well as nutrition stress were tested. Each strain was cultivated at optimal growth conditions and in medium with modified carbon and nitrogen sources. Synthetic media with addition of complex substrates (e.g. trast extract) and vitamin mixtures as well as some waste materials (whey, rape pressing, potato extract, molasses, sugar syrup) were used as nutrient sources. Peroxide and salt stress were applied too. The production of carotene-enriched biomass was carried out in flasks as well as in laboratory fermentor. Molecular changes in yeast cells on genome, proteome and metabolome level were studied using PFGE, 2D-GE and LC/MS. The best production of biomass was obtained in inorganic medium with yeast extract. In optimal conditions tested strains differ only slightly in biomass production. All strains were able to use most of waste substrates. Salt stress and combined salt/peroxide stress led to about 2-times increase of beta-carotene production both in R.glutinis and S.salmonicolor. As the best producer of enriched biomass strain Rhodotorula glutinis CCY 20-2-26 was found (35 g per liter OF biomass enriched by cca 6 mg/l of carotenoids annd 22 mg/l of ergosterol). Such dried carotenoid-enriched red yeast biomass could be directly used in feed industry as well as in pharmacy as nutrition supplement.

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2410 DEVELOPMENT OF A NON CONVENTIONAL ADSORBENT FROM PETROLEUM COKE FLY ASHES

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This study was carried out to produce, characterize and evaluate a low-cost adsorbent from fly ashes and an organic polymer (*lignimerin*), being both residues of national interest in Chile. Fly ashes were obtained from a petroleum coke power plant and the organic polymer was recovered from Kraft pulp mill wastewater. The adsorbent development consisted of the coating of the petroleum coke fly ashes with the organic polymer and a heating treatment at 550 °C for 2 h.
A complete characterization of the adsorbent material was performed to determine the chemical composition, physical parameters and crystalline forms. The results indicate that the detected crystalline phase minerals in the adsorbent are calcite and anhydrite, which corresponded with their chemical composition, being the major element calcium with a value of a 32.5% (w/w). In relation to the physical properties, a 75% of the produced adsorbent presented a particle size higher than 3.55 mm, with a specific surface area (BET) of 21.5 m2/g and a medium pore diameter of 20.77 Å. The cationic exchange capacity (CEC) of the adsorbent was determined, indicating a value of 46 cmol+/kg, a higher value compared to each raw material. The major exchangeable cations were Na+ (165 cmol+/kg) and Ca^{2+} (52 cmol+/kg), which is related to the obtained results of CEC for petroleum coke fly ash and *lignimerin*.
Batch test were performed to evaluate copper (Cu^{2+}) and zinc (Zn^{2+}) removal from aqueous solutions. The results indicated that the developed adsorbent presented more affinity for copper than for zinc, reaching the equilibrium time in 180 minutes with a removal efficiency about 90% for copper, whereas for zinc the equilibrium time was reached in 60 minutes with a removal efficiency close to 30%. The adsorption isotherms showed a maximum adsorption capacity of 5.91 mg/g for copper (Cu^{2+}) and 0.66 for zinc (Zn^{2+}). The obtained isotherm data was adjusted to the Langmuir and Freundlich models, resulting the Langmuir model the one that better describes the adsorption of these heavy metals onto the produced adsorbent.

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2553 AGRICULTURAL AND FOOD PROCESSING BYPRODUCTS FROM THE BALEARIC ISLANDS: KEY AND TRADITIONAL PRODUCTION PROCESSES

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The amounts of residues and byproducts, obtained from agricultural processes, in the Balearic Islands has undergone a marked increase during the last years. For economics as well as environmental reasons, there is a continuous pressure to exploit such residues and to identify products with attractive properties and with potential markets. Within this context, this study was focused on several key and traditional sectors of paramount importance for the agrifood industry of the Balearic Islands. In particular, the wine and olive oil sectors, both awarded with a denomination of origin (DOP) and, also, traditional sectors related to characteristic products of the Mediterranean Islands such as potatoes, oranges, apricots and almonds.

The aim of this study was, on the one hand, to define the actual situation of the agri-food byproducts produced by the above sectors, and, on the other hand, to evaluate its potential applications, not only in the food sector but also in the energetic field.

The wine sector, which includes two DOPs (DOP Binissalem and DOP Pla I LLevant), and different protected geographical indications (IGPs) known as "Vins de la Terra" (Illes Balears, Serra de Tramuntana, Illa de Mallorca, Illa de Menorca, Eivissa, i Formentera), produces a large amount of residues/byproducts. These amounts have increased considerably during the period of 1999-2007, reaching in 2006 the amount of 2500 tones. This has been probably due to the success of the Balearic wines, and, also, to the important increase in the number of new vineyards. The main byproducts related to the wine making are the stems and the grape pomace. These might represent up to 55-60% of the collected grapes.

The olive oil sector, which is also protected by a DOP (Oli de Mallorca) since 2004, has also experimented a marked increase in the production of byproducts during the last years, over 780 tones of byproducts were produced in 2007. In this case, these byproducts might represent up to 80-85% of the collected olives. The byproducts produced by other typical sectors of the Balearic agrifood industry such as potatoes, apricots, almonds and oranges has also been evaluated. Almonds with 9000 tones, and oranges and potatoes with 5000 tones each must also be considered. The potential application of these byproducts, as raw materials, for the obtention of dietary fibre concentrates with antioxidant properties has been assessed. Moreover, bioethanol production, using these lignocellulosic sources as raw materials, is actually being investigated.

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2555 HIGH VALUE CO-PRODUCTS FROM WINE BYPRODUCTS (II): POLYPHENOLS AND ANTIOXIDANT ACTIVITY

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The by-products of the grape/wine industry have recently attracted considerable interest as important sources of high-value antioxidants. These can be extracted from stems, such as resveratrol, and from grape pomace which contains polyphenols, procyanidins and anthocyanins.

The aim of this study as to evaluate the total amount of polyphenols (TP) and the antioxidant activity (AOA) of wine by-products obtained from six red grape cultivars (Merlot, Tempranillo, Sirah, Manto Negro, Cabernet Sauvignon and Callet) and three white grape cultivars (Chardonnay, Premsal Blanc and Macabeu) broadly used for wine making in the Island of Mallorca. By-products: stems and grape pomaces of the above grape varieties were evaluated for their AOA using four different methodologies (DPPH- and ABTS- radical scavenging capacities, FRAP assay (ferric reducing ability of plasma) and the Rancimat test on sunflower oil).

By-products from both, red and white grape varieties, contained important amounts of TP. In particular, stems from varieties such as Merlot and Callet contained over 2500 mg/L gallic acid equivalents (GAE), reaching, for the Manto Negro variety, up to 3000 GAE. In general, stems from white grapes exhibited higher TP values than stems from white grape varieties. The same observation could be detected for grape pomaces. In all cases, TP content of grape pomaces was lower than those from stems, probably due to the reported presence of large amounts of resveratrol in this latter tissue. All the MeOH extracts showed significant AOA, with some differences between the methods employed. Nevertheless, stems from Manto Negro variety was the most active sample in all tests. In general, red grape varieties exhibited higher AOA than extracts from white grapes.

Overall, the results suggest that either stems or grape pomaces from the varieties analysed could be excellent sources in order to obtain phytochemical extracts with potential use in the preparation of functional food ingredients, nutraceuticals and biopharmaceuticals.

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2589 BIOTECHNOLOGY FOR THE PRODUCTION OF CUAGUAYOTE (*Jacaratia mexicana*). SOURCE OF INDUSTRIAL PROTEASES

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Caricaceae is a well known family of plants, particularly from the use that has been given to papain (from papaya tree) as a source for bioactive products and industrial enzymes like papain and chymopapain. Another interesting member of this family is the plant named cuaguayote (*Jacaratia mexicana*) which has been studied by our group as a source of a novel protease, mexicain, a cystein protease which has proved to be a very powerful enzyme with great stability, properties that allows it to be a good candidate for several industrial uses like the production of modified peptides with enhanced nutritional and functional properties from meat, fish and plant proteins. *Jacaratia mexicana* is very similar to the papaya tree; in Mexico it has been used in the preparation of regional foods and in ethnomedicine as antihelmynthic; the plant grows wildly in tropical forest regions (Balsas basin) in Mexico; it also plays a significant role in the maintenance of the habitat of a great diversity of species. The potential in the development of economical alternatives makes the study of *J. mexicana* a detonating factor for regional development, specially in the isolated zones where it grows. A good alternative for its propagation, utilization and to restore degraded lands, is the use of biotechnology tools like plant cell culture.

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2872 SLUDGE VALUATION COMING FROM WASTE WATER TREATMENT PLANT AND APPLICATION TO THE CERAMIC INDUSTRY

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Given the world-wide environmental situation in which we are, takes importance more and more, not only the minimization and the solid remainder treatment and liquid effluent but also and more and more its valuation, this it is the case that appears next and in which we are working at the present time. By the geographic situation in which we are located, in the Jaen area, the ceramic industry has a great economic importance and it as well presents/displays a great environmental advantage, because the ceramic products are generally very heterogeneous, since they consist of clay raw materials with a very wide-range overall composition [1]. For this reason is a suitable industry for the valuation and use of diverse remainders, among which are sludge coming from the waste water treatment plant. [2]
In our work it is tried more concretely:

- Reception and drying of sludge coming from the waste water treatment plant.
- Characterization of the sludges and clay by means of ICP-MS, TG, DSC.
- Characterization of ceramic products selecting different compressive strength (3000, 5000, 7000 y 10000 kg) evaluated improve the resistance in green of the compact ones.
- Comparative analysis of the ceramic products prepared taking into account the composition, compressive strength.
- Finally the ceramic materials were sintered. This sintering process consists of thermal treatment for coherently bonding particles in order to entrance the strength and the other engineering properties of the compacted particles. The thermal heating destroys organic remainder and stabilizes inorganic materials and metals by incorporating oxides from the elemental constituent into a ceramic-like material. [3]

In the table 1 the composition and humidity of used sludges is presented. At the moment we are working in the study of the variation of the properties of the mixed test tubes according to % wt. of sludges used, selection of the best percentage and application to real materials.

Table 1

COMPOSITION	Majority (% > 1)	Minority (%<1)	Humidity
	Na, Mg, Ca, Fe,	K, Mn, Zn, [Si, Cr, Ni, Cu, Sn, Ba, Pb...] *	85,56%

(*)Minor of 10-2

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2873 PREPARATION AND PROPERTIES OF NEW CERAMIC PRODUCTS USING ASH FROM BIOMASS-FIRED GENERATION STATION IN CLAY MATRIX

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Sintered ceramics have been widely used in the construction sector. Increasing demands to the generation of more electric power has resulted in construction of thermal power plants worldwide and the generation of large combustion waste. An increasingly urgent problem for the immediate future is recycling of the by-products and waste materials occurring at biomass-fired generation stations. In Spain, considerable amounts of ash are generated daily in the thermal biomass plants. These wastes occupy much space on the industrial plants, and only portion can be utilized for industrial purposes, the remainder being directly discharge into large dumps or landfills. Hence from the environmental and economic viewpoints there is a need to search for new possibilities for their utilization. The ceramic industry consumes large quantities of natural raw materials. Ash is presented as a fine dust so it can be directly incorporated into ceramic body. Therefore, ash is a good candidate for the ceramic industry as a raw material resource [1-3].
This work has as its objective to evaluate the effect of incorporation of ash from thermal biomass station as raw material on the properties and microstructure of the clay matrix use to fabricate construction materials as bricks. An addition of ash increased the content of mullite and markedly improves the properties of stoneware products [4]. In this study, ash was added to clay and it was sintered using conventional powder processing based on power compaction and firing at different temperatures, without the addition of any additives. For this study the individual components was characterized: ash and clay. So, for the fabrication of bricks, compositions were prepared with addition of 5, 10, 15 and 20 wt % waste ash in a clay body. Ash can be directly incorporated into ceramic paste with almost no pre-treatment. Only can be carried out in a dry state and was sift to a fine dust. The samples were sinterized at temperatures of 900, 1000 and 1100 ºC. The composition and mineralogical properties of ash was studied for X-ray diffraction (XRD) and Fluorescence X-ray (FRX),the typical chemical composition of ash is SiO_2, Al_2O_3, CaO, Fe_2O_3 and other oxides with a high content in quartz and mullite. Afterwards we subject its properties as suction and absorption of water, compression assay, porosity, density and thermal behaviour, so as microstructure by scanning electron microscopy.

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2367 ENVIRONMENTAL ANALYSIS OF SHORT CHAIN ALIPHATIC CARBOXYLIC ACIDS BY A SEMI-DIRECT FLUORIMETRIC METHOD

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Aliphatic carboxylic acids are ubiquitous in the ecosphere and their environmental analysis are of great interest in many research topics : soil and agriculture, aquatic ecosystems, ground and drinking waters, aquifers, atmosphere, wastes and residues. Volatile Fatty Acids (VFA) -acetic, propionic, butyric and valeric acids- are generally analyzed in off-line measurements by chromatographic methods (ion-exchange, ion pair and gas chromatographies, or capillary zone electrophoresis), often coupled with preconcentration and distillation steps. However, in some applications (anaerobic digestion processes, for instance), it is important to quickly assess concentrations of these products.
Aims of this study were thus to develop a rapid method for determination of VFA in various environmental samples with no need of column separation. The analytical protocole developped is based on a specific derivatization of the carboxylic acid group by an amine fluorescent tagging reagent, followed by a direct spectrofluorometric measurement of the corresponding amide fluorescent derivatives; amidation reaction yields are improved by use of a carbodiimide-based condensing reagent.
Optimized analytical procedure is as follows : i) activation of carboxylic group by N'-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC); ii) fluorescent labelling step with N-(1-naphthyl)ethylenediamine (EDAN); iii) organic phase separation and iv) direct spectrofluorimetric quantification of VFA at λ_{ex} = 335 nm and λ_{em} = 396 nm, expressed as acetic-equivalents.
Spectral responses of various carboxylic acids (VFA and other carboxylic acids) and potential interferences induced by organic compounds (humic substances, sugars, alcohols, amines and Polycyclic Aromatic Hydrocarbons-coming from burned soils) or by mineral compounds (major anions and some heavy metals known to be acting as quenching agents) found in the environment are presented. Validations (intercomparison measurements with High Pressure Ionic Chromatography/Electrochemical Conductivity) and analytical calibrations (linear calibration ranges, regression equations and detection limits) of our method are assessed with various environmental samples (rivers, water-extracted soils and composts, water-diluted oxidized animal by-products and sewage sludge samples).
This rapid and direct determination of VFA in aqueous samples has proven its ability to detect concentrations of VFA between 0.5 and 100 mg.l^{-1}, in very different aqueous samples. The next step of this research will consist in automating this procedure and use it in microplate assays.

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2437 THE USE OF DIFFERENT CONCENTRATIONS OF GINGER ROOT OIL TO IMPROVE THE MATING PROPENSITY OF THE MEDFLY OF THE STERILE MALES

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The Sterile Insect Technique is an efficient technique used to control fruit flies such as Medfly *Ceratitis capitata* (Wiedemann) (Diptera: *Tephritidae*). Unfortunately, this technique involves mass breeding of huge quantities of target insects in the factory and sterilizing the males by exposing thm to low doses of radiation. This procedure reduces the mating propensity of the sterile males which are the active agent of the technique. It was reported that the exposition of the sterile males to ginger root oil [*Zingiber officia*l Roscoe (Zingiberaceae)] improves the mating propensity of the sterile males in comparison with the non exposed ones. In this study, we exposed irradiated males at two different doses (100 and 110 Gy) to different concentrations of the ginger oil (20 µl; 50 µl; 80 µl; 0.25 ml; 0.5 ml; 1 ml) the third day after emergence. This study shows that the irradiated males at 100 Gy are more competitive in comparison with those irradiated at 110 Gy with a mating propensity percentage respectively of 60 and 35%. Regarding the exposition dose of the ginger oil, 1 ml gave the best mating propensity (42%) in comparison with the other concentrations.

2467 A TWO-STEP ELECTRO-DIALYSIS METHOD FOR DNA PURIFICATION FROM POLLUTED METALLIC ENVIRONMENTAL SAMPLES

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To construct libraries from metagenomic environmental samples, highly pure DNA is required. However, isolating good quality DNA from contaminated environments is often complicated, as polysaccharides and polyphenols abundant in certain samples (soils have mainly been studied) are difficult to eliminate using standard DNA extraction protocols. These and other compounds co-precipitate with DNA and can interfere with subsequent analytical reactions. To increase both the yield and purity of DNA, various improvements to standard protocols have been reported. Resinous and aromatic compounds from soil sources can be eliminated using cation-exchange resins and detergents, activated charcoal, polyvinylpyrrolidone (PVP) and hydroxyapatite. There is little information, however, on the isolation of DNA from other difficult samples such as oxidized metallic environments. Biological metallic corrosion has been studied by direct cultivation of the organisms present in the biofilm or direct scratching of the surface for the posterior extraction of the DNA. The importance of obtaining highly pure DNA at the beginning of the extraction process in order to not dilute rarely represented organisms has been demonstrated. For this reason we developed a method involving only two extraction steps, reasoning that fewer manipulations will help to preserve the under represented taxa in the sample. This method requires the direct lysis of the cells contained in the sample by physical (freezing/thawing) and chemical (detergents and salt) means, and electro-dialysis of the released DNA. Direct electro-dialysis does not require phenol-chloroform-isoamyl alcohol (PCI) washes or ethanol precipitation. We also compared different quantities of samples, as well as polluted vs. non-polluted sources. In all cases, yields and quality of the isolated DNA were adequate for PCR, restriction enzyme digestion and cloning reactions. Compared to one reported method, and one commercial method, which did not allow isolation of sufficient DNA, or produced DNA unsuitable for manipulation by molecular techniques, our method was efficient and relatively fast. This technique can be also used for DNA isolation from pure cultured organisms.

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2511 PERSPECTIVES ON THE DEVELOPMENT OF BIOPESTICIDES FOR VEGETABLE PROTECTION

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Vegetable production requires the development and diffusion of effective alternative pesticides, including biopesticides. The term biopesticide refers to bacteria that promote plant growth by control of deleterious organisms. The present work stated the isolation and identification of *Pseudomonas* spp. from tomato and pepper rhizosphere soil and their study as potential antagonist agents of fungal pathogens. The isolates were identified by means of the API 20NE system (bioMèrieux®SA, Marcy-l'Etoile, France) and were tested against *Alternaria alternata*, causal agent of leaf blight, Sclerotium rolfsii, causal agent of southern blight and *Fusarium solani*, causal agent of root rot. Dual culture antagonism assays were carried out on 25% Tryptic Soy Agar (TSA), King B medium and Potato Dextrose Agar (PDA) in order to determine the effect of the isolates on the germination of the phytopathogens; the inhibition percentage of micelial growth was estimated in the same culture media. The influence of three concentrations of FeCl3 (50, 100 and 200 μM) upon the observed antagonism against *A. alternata* was tested; possible caseinolytic, chitinolytic and cellulolytic activities were also investigated. Four of the rhizosphere isolates belong to the species Pseudomonas putida, four to *Pseudomonas fluorescens*, one to *Ralstonia pickettii* and one to *Pseudomonas aeruginosa*. All of the strains significantly inhibited *A. alternata* and the higher activity was observed in 25% TSA medium. *A. alternata* inhibition on King B decreased with the augment of iron concentration in the medium. The higher antagonistic effect upon *S. rolfsii* and *F. solani* was observed on King B medium. Protease production was detected in 30% of the strains; neither cellulase nor chitinase were produced by any of these strains. In vitro antagonism studies showed a promising level of activity, mainly against *A. alternata* and *S. rolfsii*. At the present time, both pathogenicity and antagonism assays are being performed in tomato plants in order to promote the development and application of microbial fungicides for vegetable production.

2544 *Ziziphus mauritania* FRUIT PULP EXTRACT: AN ANTAGONIZER OF THE TOXIC EFFECT OF CHEMICAL PESTICIDES.

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The use of chemical pesticides is an integral part of the agriculture to get better produce. But at the same time the use of pesticides beyond a certain limit shows toxic effects on the body. The oxidative stress in the body is one of the mechanisms by which the pesticides result in toxic effects. The oxidative stress is known to result in the cell cyotoxicity as well as involved in the process of ageing. On the other hand the role of antioxidants in the health is well documented now, The antioxidant activity of the edible compounds has been found to be related to their tumorocidal, antiageing and also their immunomodulatory activity .The present study was conducted to find out the antioxidant activity of an edible part of *Ziziphus mauritiana*, and further to test it as an antagonizer of the pesticidal effect in vitro. Lipid peroxidation, catalase, and Superoxidase dismutase and GSH enzymes activity as well as H_2O_2 were checked in vitro in the presence and absence of the extracts. Results revealed that extract addition diminished the effect of pesticides oxidative stress. The results paralleled that of the immunomodulatory activity of the pesticides and extracts. It is concluded that the antioxidant activity of aqueous extract of pulp of *Z. mauritiana* can be employed to reduce the oxidative stress caused by the pesticides /other oxidative stress causing agents e.g. irradiation, chemicals and in turn their toxic effects e.g. Hepatotoxicity.

2588 SUSTAINABLE AGROINDUSTRIALIZATION OF MEXICAN PLANTS

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Mexican biodiversity constitutes the main strength for the sustainable development of a competitive agroindustrial sector. The scientific knowledge, the conservation and rational use of its natural resources may contribute to the environment protection and fulfill the requirements for the economical and social development in our country. México has been widely recognized as a megadiverse country due to his great variety in ecosystems, species, and genes. Nevertheless, this abundance has not rendered benefits into a competitive agro-industrial development, but in a concerning situation characterized by an accelerated loss in the biodiversity, a consequent deterioration in environment coupled with an increased disappearance of the formerly ethnical diversity, (people that holds the traditional knowledge of medicinal and food plants) and conservation strategies of their natural resources. The present review outlines the obtained results from an ample research program, focused on the sustainable industrialization of Mexican species of the *Bromelia* and *Carica* genera. These species can be considered as multi-purpose medicinal and food plants that generate income and may enhance environmental services, by reducing pressure on wild plants and by helping to stabilize and enrich soil in dry and degraded areas, thus promoting sustainable land management. The achieved results include isolation and characterization of new proteases with a high potential utility in industrial applications; development of technical uses in food processing; agronomical methods for an intensive primary production for industrialization; and experiences in the restore of degraded areas in the tropical dry forest in Morelos, Mexico. The result of these experiences has lead us to conclude in a proposal that involves a multifunctional production strategy for the sustainable exploitation of Bromelia and Carica Mexican plants; i.e. the production of biochemical industrial products (i.e. industrial proteases and lipases, fiber, or food) and biodiversity and environment conservation.

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RESEARCH ACTIVITIES R+D PROGRAM AMONG RESEARCH GROUPS FROM MADRID REGION.

NATURAL RESOURCES AND ENVIRONMENTAL TECHNOLOGIES AREA

EIADES Program subsidizes by Consejería de Educación de la Comunidad de Madrid (2006-2009) is composed by 8 Research Groups from Madrid Region and 9 Companies which work in this industry, apart from other national and international research groups, that deal with the following objectives:

- Remediation y restoration of soils contaminated with heavy metals and/or organic compounds.
- Application of different physico-chemical and biological remediation technologies "in situ"
- Application of an useful system to evaluate the environmental impact of the remediation process.

The Program proposes to develop innovative scientific tools to tackle these challenges. A methodology to assess the environmental impact of industrial activities on soils and of the remediation projects themselves according to the risk analysis techniques will be developed applying last generation scientific development (PRA Probabilistic Risk Assessment, Biotch-DTA, biotechnology-based –Direct-Toxicity Assessment)
The application of different remediation strategies such as electrokinetic, bioremediation and phytoremediation will be considered in relation to the size and type of contaminant, characteristics of the ecosystem and the future use of the soil. With the aim to evaluate the efficiency of the remediation processes, an ecotoxicological analysis system will be developed to quantify the ecosystem remediation in terms of cost/profit.
Work scenarios

a) Old landfill
b) Abandoned mines
c) Natural or agricultural soils affected by contamination sources

RESEARCH ACTIVITIES.

Characterization of contaminated soils.

- Study of new contaminated sites: analysis of organic and inorganic contaminants.
- Physicochemical and biological soil characterization.
- Monitoring of the ecotoxicological diagnosis of the contaminated sites in the Madrid region. Study of Bioindicators

Remediation Technologies.

- Bioremediación.
- Phytoremediación
- Electrokinetic technologies

Evaluation of the decontamination processes.

- Molecular techniques to control decontamination processes.
- Characterization of decontaminating mechanisms in plants
- Biomonitoring of the contaminants in soil-plant system

Field experiments.

- Evaluation of the environmental impact.
- Proposal of remediation technologies.
- Modelling of the soil decontamination processes in soils. Evaluation of times and costs.

Technologies application to contaminated sites according to Spanish legislation. (Real Decreto 9/2005).

TRAINING AND DISSEMINATION ACTIVITIES.

- Researchers training.
- Postgraduates courses.
- Organization of International Workshops.
- Participation in Official Postgraduates Programs (Master and Doctorate)
- Participation in Business Forum
- Dissemination of activities performed (scientific and technical journals conferences…)

EIADES-CM

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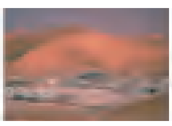
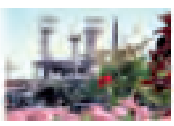

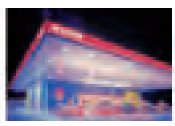


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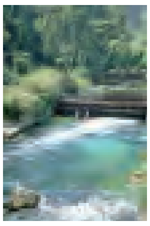

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