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Preface

Environmental Biotechnology and Environmental Engineering are two faces of a modern, valuable, and indispensable scientific and technical coin. The growing significance and awareness of environmental problems, caused especially by use of fossil resources in connection with industrial pathways of production, depletion of finite natural resources, mismanagement of renewable resources, etc., have led to the development of both disciplines. They have their own historical roots, i.e., one has blossomed from Biotechnology and the other has grown from the old Civil and Sanitary Engineering. Yet, They have developed in full-fledged branches of knowledge and specialization, and at the same time they complement each other.

Regarding Environmental Biotechnology, its contributions span from environmentally-friendly and cost effective "end-of-the-pipe" solutions to environmental pollution and problems (bioremediation of soils and aquifers, biological waste treatment), to the development of sustainable alternatives for their prevention and alleviation, such as the replacement of fossil fuels by biohydrogen and methane from wastes and futuristic "biorefineries". Biotechnology has the potential of a reduction of operational and investment costs for the design and operation of more sustainable processes based on microbes and other living organisms as agents. Yet, so far the sustainability of technical processes is more the exception than the rule. In this regard, Environmental Biotechnology is a serious candidate to provide substantial advances in the near future

On the other hand, Environmental Engineering has developed several significant fields of research and applications (everything matters in Environmental Engineering; natural sciences and social sciences are as significant to its practice as classical engineering skills); some of them partially overlap with Environmental Biotechnology (for instance, biological waste treatment), whereas other subjects are original and cover issues that Environmental Biotechnology can not, and have proved to be of use to other branches of knowledge. With respect to this, we would like to highlight a significant contribution of Environmental Engineering that has transcended to other fields of Engineering and Technology: sound Environmental Engineering has designed the essential framework of System Engineering Analysis applied to environmental issues, also known as Life Cycle Analysis (LCA) and other denominations. The contemporary history of industry and technology has sadly taught us that new technological solutions and new processes derived from Environmental Biotechnology (and from other fields of knowledge) should be examined under the light of LCA and environmental impact analysis before attempting their implementation. Very often, a precipitated and immature application of a new product or process has led to adverse impacts on health and the environment that have become technical, ethical and economic burdens on modern 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.

In 2003, a group of pioneering biotechnologists in Mexico led by Dr. Hector M. Poggi-Varaldo, Dr. Fernando Esparza-García and Professor Elvira Ríos-Leal, accompanied by a constellation of international scientists such as Dr. Isabel Sastre-Conde from Spain, Dr. Hervé Macarie from France, Dr. Franco Cecchi and Dr. Paolo Pavan from Italy, Dr. E. Foresti from Brazil, Dr. Irene Watson-Craik from Scotland, Dr. Jose Luis Sanz from Spain, and others, identified a gap in the dissemination of both Environmental Biotechnology and Environmental Engineering. This was particularly true for developing countries, although the situation in developed countries was not much better.

On the one hand, there were several international and regional events dealing with Biotechnology but no international event was devoted to Environmental Biotechnology. At most, Environmental Biotechnology has one or two sessions in a Biotechnology Congress. On the other hand, most regional Environmental Engineering events showed a strong commercial component that negatively competed with the exchange of advanced knowledge and the formation of research networks. Moreover, Environmental Biotechnology and Environmental Engineering are two dynamic drives with a strong interaction, and the scientific community could obtain several advantages from their joint diffusion. In short, there was a need for an international event dedicated to both disciplines, with a strong vocation for serious dissemination of scientific and technological knowledge, as well as research networking.

The synthesis to this diagnostic was to launch a new event focused on both disciplines. In this way, the First International Meeting on Environmental Biotechnology and Engineering was born and held in 2004 in Mexico City. This first event was co-organized by the Dept. of Biotechnology and Bioengineering of CINVESTAV del IPN in Mexico, the IRD of France, the IMIA from Spain, the Mexican Polytechnic Institute (IPN) from Mexico, the National University of Mexico (UNAM, México), the University of Hidalgo (UAEH, México), among others. The event was backed-up by a diverse International Scientific Committee that had the contributions of outstanding scientists and professionals from Brazil, Italy, Spain, Scotland, France, and Mexico.

After the Second International Meeting on Environmental Biotechnology and Engineering also held in Mexico City, Mexico, in 2006, we had the satisfaction to see that the 3rd International Meeting on Environmental Engineering held in Palma de Mallorca had exponentially grown and matured. Its outreach was multiplied by a factor of 10 compared to that of the 1st IMEBE. The Organizing Committee led by Dr. Isabel Sastre-Conde should be congratulated for the success and resonance of the third version of this event. This fact is a confirmation of the original diagnostic: the scientific community was avid of an international event with the characteristics of the IMEBE.

Now, the name of the event has been changed from Meeting to Symposium, in order to reflect the increases on both quantity and quality of our event. So, in 2014, the name of the event is the Fourth International Symposium on Environmental Biotechnology and Engineering.

This Book of Abstracts Environmental Biotechnology and Engineering-2014 contains the edited abstracts of the contributions presented in the 4ISEBE and it is both a reference

and a reminder. It is a reference of fine research and works on Environmental Biotechnology and Environmental Engineering, for personal and Library consultation, since several copies of the books will also be distributed among the main Universities of the countries that have participated in the event. Furthermore, the book is a reminder of the efforts that we should still make in order to improve our environment and quality of life, as well as the commitment in further continuing the dissemination and exchange of these efforts in the coming 5th ISEBE.

If you want to know more on any subject of these abstracts, we recommend the reading of the CD-ROM book "Environmental Biotechnology and Engineering-2014". The later contains the complete contributions of most abstracts published in this Book of Abstracts.

We want to acknowledge all authors of the works presented in the 4ISEBE. Also, we express our gratitude to the support to 4ISEBE from the CINVESTAV del IPN and its Department of Biotechnology and Bioengineering, CONACYT (Mexican Council of Science and Technology of Mexico), the Institut de Recherche pour le Développement (IRD) and IMBE from France, the American Chemical Society, la Fundacion Semilla from the Baleares Islands, Spain, the Mexican Society of Biotechnology and Bioengineering (SMBB), the Mexican Association of Solar Energy (ANES), and a constellation of Mexican private companies, among others. Without their varied contributions and support, the 4ISEBE would have not happened. We are also very grateful to Ms Ana Lucía Castro-Ríos for her excellent work in the production of the CD-ROM books of 4ISEBE. Finally, we are very grateful to the members of the Scientific Committee who have evaluated the nearly 300 abstracts submitted to 4ISEBE, as well as to the Organizing Committee for the logistics and the work performed around the 4ISEBE.

We look forward to meeting all of you and as well as a stream of new participants in the next 5th ISEBE in 2016. Keep posted.

The Editors

Chapter 1.
Renewable and Alternative Energies and Biorefineries

A TECHNO-ECONOMIC EVALUATION OF BIO FUEL PRODUCTION FROM WASTE OF STARTING DATES IN SOUTH ALGERIA

Insaf Mehani* (1); Bouchekima Bachir (2)

(1) Laboratoire LENREZA Université Kasdi Merbah Ouargla
(2) Laboratoire LENREZA Université Kasdi Merbah Ouargla

Renewable energy, including bio energy are an alternative to fossil fuel depletion and a way to fight against the harmful effects of climate change. It is possible to develop common dates of low commercial value, and put on the local and international market a new generation of products with high added values such as bio ethanol. Besides its use in chemical synthesis, bio ethanol can be blended with gasoline to produce a clean fuel while improving the octane.

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*Author for all correspondence

ETHANOL PRODUCTION FROM ENZYMATIC HYDROLYSATES OF *Agave lechuguilla* PRETREATED BY AUTOHYDROLYSIS

Oscar H. Ortiz-Mendez (1); Leopoldo J. Rios-Gonzalez* (1);
Jose A. Rodríguez-de la Garza (1); German Aroca-Arcaya (2)

(1) Biotechnology Department, Chemistry Faculty, Universidad Autonoma de Coahuila, México;
(2) Environmental Biotechnology Lab. of Biochemistry Engineering Faculty, Pontificia Universidad Catolica de Valparaiso, Chile

In recent years the demand for ethanol has increased worldwide, resulting in a demand of fermentation facilities that comply and with regulations and need. Presently, ethanol is mainly obtained from food crops, which causes a rise of food prices. In recent years the use of lignocellulosic biomass as a source of fermentable sugars has received increased attention due to its low cost and abundance, avoiding with this competition with food supply. Furthermore, the combination of pretreatment of the lignocellulosic material with an efficient and cost-effective enzyme blend is being examined to obtain fermentable sugars. One of the most promising biomass sources for ethanol production in México is the genus Agave. The genus Agave traditionally includes about 166 species, being *Agave lechuguilla* the most abundant in Northern region of Mexico. Currently *Agave lechuguilla* is one of the species of choice for fiber production in Mexico. The aim of the present work was to assess ethanol production by *Saccharomyces cerevisiae* from enzymatic hydrolysates obtained from *Agave lechuguilla* previously pretreated by autohydrolysis. Pretreatment process was optimized using a 1 gallon high pressure Parr reactor under the following conditions: 1) Time (5, 8, 10, 12, 15, 20, 30, 45 and 60 min) and 2) temperature (150, 160, 170, 180, 190 and 200 °C) with different severity factors (R_f), 1:6 w/v ratio (substrate:water) and 200 rpm for all cases. Substrate obtained after optimization of the pretreatment was subjected to enzymatic hydrolysis with commercial cellulase, Accellerase 1500 (Genecor, USA). Enzymatic hydrolysis was performed using a enzyme load of 100 FPU (Filter Paper Units) per gram of glucan in sodium citrate buffer (pH 4.8) at 50 °C, 150 rpm for 48 h and 20% (w/v). The enzymatic hydrolyzate was used for ethanol fermentation using *Saccharomyces cerevisiae* ATCC 4126. The hydrolyzate was supplemented with 10 g/L of yeast extract and 20 g/L of peptone at pH 5.5. Fermentation was carried out at agitation of 100 rpm for 10 h at the temperature of 30 °C. Results obtained during optimization of pretreatment process showed that glucan present in the substrate was mostly preserved and also an increase in digestibility was observed for the case of recovered substrate at 190 °C for 30 min corresponding to 4.127 Rf. Enzymatic hydrolysis of recovered substrate showed a maximum yield of 72%, with a glucose concentration of 59 g/L, obtaining 25.4 g/L of ethanol during fermentation process by *S. cerevisiae* representing a 90 % conversion value according to theoretical ethanol yield. Results obtained in the present work demonstrate the potential of *Agave lechuguilla* as biomass stock for ethanol production.

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*Author for all correspondence

BIOLOGICAL PRETREATMENT OF *Agave lechuguilla* BY *Phanerochaete chrysosporium*

Ricardo Reyna-Martínez (1); Thelma K. Morales-Martínez (1); Leopoldo J. Rios-Gonzalez* (1); Jose A. Rodríguez-de la Garza (1) Julio C. Montañez-Saenz (2)

(1) Biotechnology Department, Chemistry Faculty, Universidad Autonoma de Coahuila, México;
(2) Department of Chemical Engineering, Chemistry Faculty, Universidad Autonoma de Coahuila, México;

Lignocellulosic materials are an attractive raw material for the production of fuel ethanol due to their extensive distribution, renewable character, and availability in great quantities at low cost. Basically, they consist of cellulose, hemicellulose, and lignin. Early research regarding the use of *Agave lechuguilla* (*Agavaceae* family), a plant widely distributed in Northern Mexico, has shown the potential of this plant for ethanol production. Pretreatment, as the first step towards conversion of lignocellulose to ethanol, makes up one-third of the total production costs and remains one of the main barriers preventing commercial success. A benign alternative to harsh chemicals is microbial pretreatment, which employs microorganisms especially fungi and their enzymes systems to breakdown lignin present in lignocellulosic biomass. Fungal pretreatment has been previously explored, and recently has received renewed attention as a pretreatment technique for enhancing enzymatic saccharification and fermentation of lignocellulosic biomass to ethanol. *P. chrysosporium* is one of the most investigated white rot fungus for pretreatment because of its high growth rate compared to many other basidiomycetes, exceptional oxidation potential, and efficiency for lignin biodegradation.

The objective of this study was to evaluate and optimized fungal pretreatment (lignin degradation) of *Agave lechuguilla* in solid state fermentation by *P. chrysosporium*. Pretreatment was carried out in 50 ml Erlenmeyer flasks, using previously pretreated (milled and dehydrated) *Agave lechuguilla* biomass. Biomass chemical composition was as following; cellulose 21 %, hemicellulose 7.7 % and lignin 13.5 %, kirk medium was added according to humidity levels. An orthogonal experimental design (L9(3^4)) was used to optimize the pretreatment conditions. Nine pretreatments were carried out to evaluate the effect of humidity (40, 60 and 80%), temperature (30, 35 and 40 °C), incubation time (10, 15 and 20 days) and inoculum concentration (4, 5 and 6 µl of spore suspension). Results showed a maximum lignin degradation of 29% by *Phanerocheate chrysosporium*, with no cellulose lost during the process. Incubation time had a statistically significantly higher influence during lignin degradation (63%), followed by temperature (32%), meanwhile humidity and inoculum concentration had no statistical significance. Finally, results showed that under optimum conditions 41% lignin degradation can be achieved.

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*Author for all correspondence

CHARACTERIZATION AND IMPROVEMENT OF FUNGAL XYLANOLYTIC ENZYME ACTIVITIES FOR DEPOLYMERIZATION OF VEGETABLE BIOMASS

Kary Haro-Pérez (1); Ainhoa Arana-Cuenca (2); Aldo González-Becerra *(1)

(1) CBM-SO; Madrid- España.
(2) UPP-DB; Hidalgo- México

The process of obtaining ethanol by degradation of plant biomass arouses a great interest, because it allows degrading agricultural and municipal wastes. The bioethanol derived from this biomass is called second-generation bioethanol given that it does not affect human or animal food. Usually this biomass is degraded by physical, chemical and physico-chemical mechanisms that are contaminant processes.

In the study performed in our laboratory we use ligninolytic enzymes (Laccases, Manganese-Peroxidases and Manganese Independent Peroxidases), in order to release cellulose, hemicellulose and lignin phenolic compounds using the basidiomycetous fungus *Pleurotus ostreatus*. Ligniolytic enzymes were extracted using 0.2M acetate buffer and MilliQ water because the pH affects the enzymatic extraction, modulating their concentration. The measurement of enzymatic activity was performed using the oxidation of 2- 6 Dimethoxyphenol, resulting more activity in MilliQ water extraction.

Knowing the level of delignification of the biomass used, the main experiment of this preliminary study was performed to obtain xylanolytic enzymes from the TRP1 strain (*Trichoderma pleurotum*) which is able to compete for the substrate with industrial mushroom cultures. Another main objective was to find the regulatory gene of this activity (gene *XlnR*) to modify and increase the capacity to depolymerize cellulose and hemicellulose in hexoses and pentoses, getting more free sugars to be used to produce biofuels by fermentation with native yeasts that are available in our laboratory. The process to search *XlnR* gene was carried out by molecular analysis (differential screening), using the kit PCR-SelectTM cDNA Subtraction Protocols. The result of these analysis resulted in three fragments of proteins being characterized (**H9BVB7** H9BVB7_ TRIHA; **H9BVC0** H9BVC0_ TRIHA and **G9MF07** G9MF07_HYPVG) and four proteins with a determined function and complete sequence (**H9BVB7** H9BVB7_ TRIHA; **H9BVC0** H9BVC0_ TRIHA and **G9MF07** G9MF07_HYPVG).

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* Author for all correspondence

USAGE OF THE AGRO-INDUSTRIAL RESIDUES AND WASTES FOR LIGNOCELLULOSIC BIOFUELS COPRODUCTION UNDER AN ADVANCED BIOREFINERY SCHEME

Salvador R. González-Vaca; Víctor Sevilla-Güitrón; Martín Murguía, Arturo Sanchez*

(1) Centro de Investigación y de Estudios Avanzados del IPN, Unidad Guadalajara de Ingeniería Avanzada, Zapopan, Jalisco.

This work presents a comparative analysis of an advanced biorefinery scheme (ABS) for biofuels (i.e. ethanol, biogas and biohydrogen) co-production and electricity cogeneration against a conventional biorefinery plant (CBP). Agro-industrial residues and wastes from the cheese and tequila industries as well as crop residues are used under muti-feedstock strategies. The analysis is based on calculations of the total production cost (TPC) and the end-use energy ratio (EER). Feasible conditions are established for achieving acceptable TPC in mid-size agriculture sectors.

The ABS flowsheet consists of seven stages (pretreatment, dark fermentation, enzymatic saccharification, alcoholic fermentation, separation, waste water treatment and cogeneration) and is fed by wheat straw, cheese whey, vinasses (these last two with high COD) and some other raw materials. The products are lignocellulosic bioethanol and electricity. The produced biogas and biohydrogen are fed to the cogeneration stage for electricity production. The CBP flowsheet is fed only with wheat straw and does not consider biohydrogen production using all monosaccharides in alcoholic fermentation. The steady state mass and energy models as well as equipment capital were solved with state-of-the-art simulation tools for the 100 – 2,000 ton DB/day capacity interval and polysaccharides content (pc) (35% -70%) of the main feedstock (i.e. wheat straw). All other feedstocks were adjusted proportionally. The Net Present Value (NPV) technique was employed for the economic evaluation. The EER defined as the ratio of energy produced (steam, electricity and chemical energy of ethanol) and total energy consumed in the process, was calculated using data from the energy balances.

A quasi-linear section was obtained for 2,000, 1,000 and 500 ton DB/day capacities and 45%, 55%, and 70% pc respectively. The results in this area are not larger than 20% of the minimum TPC obtained (1.28 USD/l etOH for 2,000 ton DB/day and 70% pc) with an EER 100%. Results for the best TPC for ABS were compared against the CBP TCP results ($0.93 USD/l etOH and EER 110%). The paper discusses the operational, technological and economic conditions giving rise to these results. Although ABS TCP is 38% higher than the CBP counterpart, the ABS could be a solution for the treatment of agro-industrial residues and wastes since it take advantage of biofuels production, reducing its high COD index and providing an income from wastes and residues than will certainly help to pay the investment of treatment equipment.

*Author for all correspondence

HYDROGEN ROLE AS A PART OF CO_2 HYPER-COMBUSTION REACTION MECHANISM

José Carlos Hernández-López*; José Ángel Dávila-Gómez

UAM-AZCAPOTZALCO, Depto. de Energía, México, D.F., México.

Within the hydrogen investigation project by the Sustainable Technologies Area on hyper-combustion with CO_2, which would substitute air as oxidizer, it was designed and built a cannon type shot probe to introduce fuel / air mixtures, and proceed to their ignition through an electric arc. Construction of the prototype required design and additional set up of a circuit of generation and maintenance of electric arc, as well as the use of specialized laboratory equipment for feeding the device and obtaining final gases samples to be analyzed.

This work presents the results of tests performed during the starting operation using LP gas as fuel and air from the ambient as oxidizer; the tests contemplated the study of fuel / air ratio burning range –proposed in the initial phase of experimentation to verify the correct operation of the device–; and their characterization with regard to the observations made.

The test of device was performed using 60 ml volume standard mixtures at 1 bar absolute pressure of LP gas / air; the results show the acceleration of the combustion to detonation and the existence of a specific combustion range; noting responses with characteristic odors, different sounds and divers delays of time.

Gases remaining from these tests were analyzed by gas chromatography, showing the presence of hydrogen (H_2) as part of the performing mechanism; so –besides to the expected causes of the "failed tests"–, by the existence of deposits of carbon at electrode, spark plug; therefore there are evidences of a primary wet gas reaction mechanism, along with other secondary reactions (e.g. Bosch and Sabatier).

As a general conclusion resulting from this work is that hydrocarbon fuels and, in particular, hydrogen, participates in the reaction mechanism, based on their composition and the oxidizer used, in such a way that its specific studio is potentiated completely through this explanatory work; additionally, that the observance of the detonation phenomenon, highlights its importance. Preliminary tests with LPG/CO_2 mixtures provide strength to this evidence.

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*Author for all correspondence

POTENTIAL OF *Jatropha curcas L* HUSK TO PRODUCE BIOGAS

Eric Houbron*; Elena Rustrián; Gerardo Ramírez; Carla Arellano; Víctor Buendía; José Rivera.

Universidad Veracruzana – Laboratorio de Gestión y Control Ambiental, Orizaba, Veracruz.

In this study the potential of *Jatropha curcas L* husk to produce biogas was estimated. In a first stage the raw material is characterized by determining the composition in percentage of cellulose, hemicellulose, lignin, moisture, ash and organic matter using techniques established by TAPPI. In a second stage, acidic hydrolysis is conducted using H_2SO_4 (concentration of 0.13% v/v) and alkali (concentrations of: 5.7%, 7% and 10%) using NaOH at 120 °C. The response variable was the generation of reducing sugars which are estimated by the DNS technique and the solubilization of organic matter through the soluble COD technique. Additionally, the content of total and ammonia nitrogen, and ashes were characterized. In the third stage, methanogenic potential tests were performed on both hydrolyzed using glucose as reference and an initial concentration of 2,000mgCOD/L. The tests were performed in duplicate with a ratio So/Xo 0.25 using anaerobic sludge at temperature of 35°C and constant agitation of 150 rpm. The methane was measured by volume displacement performed by a Flask Mariotte system equipped with a CO_2 trap filled with NaOH 3N. In these tests were performed determinations for soluble COD, pH, and initial and final TSS and VSS.

The results show a composition of *Jatropha curcas* husk of cellulose 57.13%, 2.99% of hemicellulose, and 19.87% of lignin, 7.85% moisture, 6.65% ashes and 93.34% organic matter. By acidic hydrolysis, a concentration of reduced sugars of 4.61 g/L, and 5.11 g/L of COD was determined while by alkali treatment could obtain 1.66 g/L of reduced sugars and 18.14 g/L of COD. Regarding of the methanogenic potential, a conversion yield of 87% of CH_4 from the acid hydrolyzate and a conversion yield of 46.88% from the alkali hydrolyzate was achieved. These results demonstrate that the *Jatropha curcas L* husk presents an interesting potential for methane generation after acid treatment. Furthermore, the anaerobic digestion not necessarily required the release of reducing sugars as solubilized COD was completely transformed.

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*Author for all correspondence

CINÉTICA DE INHIBICIÓN DE PRODUCCIÓN DE HIDRÓGENO MEDIANTE FERMENTACIÓN OSCURA: UNA REVISIÓN

Perla X. Sotelo-Navarro (1); Sylvie J. Turpin-Marion (1); Rosa M. Espinosa-Valdemar (1); Alethia Vázquez-Morillas (1); Margarita Beltrán-Villavicencio (1); Héctor M. Poggi-Varaldo*(2)

(1) UAM-A, México DF, México
(2) CINVESTAV- IPN, México DF, México;

Durante décadas, el uso y abuso de los combustibles fósiles ha causado la contaminación del suelo, aire y agua. En la búsqueda de soluciones a esta problemática, se han propuesto combustibles alternativos, uno de ellos es el hidrógeno, al que se considera un combustible limpio ya que no tiene carbono, azufre o nitrógeno y además presenta un alto poder calorífico. Los métodos de producción convencionales de hidrógeno son el reformado con vapor de metano y otros hidrocarburos, la oxidación parcial no catalítica de combustibles fósiles y la auto-reformación térmica. Esos métodos son todos procesos intensivos de energía que requieren altas temperaturas (> 850 ºC). La producción biológica de hidrógeno es una alternativa a los métodos anteriormente mencionados y puede realizarse mediante biofotólisis a partir de algas y cianobacterias o fotofermentación de compuestos orgánicos. Una opción atractiva de producción biológica, es la fermentación oscura, ya que se puede generar de forma continua y a altas tasas de producción comparado con los otros procesos biológicos y es viable realizarla empleando residuos como sustrato.

Sin embargo, el proceso de fermentación oscura enfrenta límites termodinámicos y cinéticos. En relación a los últimos, en este trabajo se revisa críticamente los modelos cinéticos aplicados al proceso, tales como los de Gompertz, Monod, Han-Levenspiel y Andrew, enfatizando el análisis de los factores de inhibición que se han identificado para el proceso. Entre los modelos de inhibición de la fermentación oscura para producción de hidrógeno de los cuales se conocen diferentes modificaciones predominan aquellos de inhibición por sustrato. Por el contrario, la modelación de la inhibición por producto (efectos inhibidores debidos a la propia acumulación de hidrógeno y productos de fermentación como ácidos orgánicos y solventes) es menos estudiada. Por otro lado, en caso de la inhibición por ácidos orgánicos, se ha modelado el efecto de un puñado de ácidos orgánicos (tres), y por separado. Si bien loable, no representa la inhibición de la hidrogenogénesis real, puesto que en esta última se genera una mezcla de varios ácidos orgánicos y solventes, lo cual puede tener efectos inhibitorios sinergísticos (o de otro tipo) sobre la cinética de producción de H_2. Por lo tanto se recomienda más investigaciones en este aspecto, lo que supone que un modelo basado en inhibición multivariables para la producción de hidrógeno y de metabolitos dará un mejor ajuste que los modelos basados en los casos para un solo producto.

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*Author for all correspondence

ENVIRONMENTAL AND ECONOMIC SUSTAINABILITY ANALYSIS OF LIGNOCELLULOSIC BIOETHANOL PRODUCTION

G. Magaña (1); D. R. Gómez (1); M. Solís; A. Sánchez* (1)

(1) Centro de Investigación y de Estudios Avanzados del IPN, Unidad Guadalajara de Ingeniería Avanzada, Zapopan, Jalisco.

In the present work a method to analyze the environmental and economic sustainability of lignocellulosic bioethanol production processes, is proposed. This method enables to analyze the impacts of prospective processes such as bioethanol and electricity coproduction. Since there are no commercial production plants in operation, but only pilot and demonstration facilities, most of the current analysis depend on data difficult to trace.

The proposed method is based on a process engineering approach and is composed by four main elements: the conceptual design (comprises a process flow sheet, mathematical models and an economic analysis), the application of the Process Analysis Method (PAM, providing a set of well supported indicators), the indicator analysis (consists on examining the indicator-results to extract conclusions when comparing different schemes) and the weighting process (integrates the indicator results into a global one *using dimensional functions* and *scaling factors*).

A comparison of four different schemes for lignocellulosic bioethanol production is presented as case study. The systems named PETA 3.0 and PETA 3.2 are single product plants while BIOREF 3.0 and BIOREF 3.2 are multipurpose biorefineries. All systems coproduce lignocellulosic ethanol and electricity. The BIOREF schemes have an extra stage (dark fermentation) to produce biohydrogen which is fed to the cogeneration stage aiming to increase the production of electricity. The 3.2 schemes include energy integration and water recirculation.

A total of 11 indicators were generated (6 for the environmental and 5 for the economic domain) and calculated. The indicator analysis showed that BIOREF emits 31% more GHG than PETA due to the large amount of CO_2 produced in the dark fermentation stage. Besides, the discharged water of BIOREF has lower quality owing mostly to the aggregates of the dark fermentation stage. Also, monosaccharides availability on the alcoholic fermentation stage represents a higher bioethanol production for PETA schemes which is translated into bigger impact on the yield and contribution to the country's economy indicators. The 3.2 schemes present less impact in the environmental domain mainly due to water recirculation whilst economically; energy integration contributes in a reduction about 4% and 2% on Total Production Cost (TPC) for PETA and BIOREF, respectively.

Regarding the weighting analysis, BIOREF 3.0 scheme is the least sustainable due to the great amount of CO_2 produced and the lower bioethanol production. Water recirculation and energy integration contributes on making PETA 3.2 the most sustainable scheme together with the highest bioethanol production and the lowest TPC.

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*Author for all correspondence

POTENTIAL OF *Pennisetum* sp. GRASS FOR BIOETHANOL PRODUCTION

Eric Houbron* (1); Elena Rustrián (1); Carla Arellano (1); Gerardo Ramírez (1); Nallely Díaz (1).

(1) Universidad Veracruzana – Laboratorio de Gestión y Control Ambiental, Orizaba, Veracruz.

In this study the potential of *Pennisetum sp.* grass to produce bioethanol was estimated. In a first stage the grass is characterized by determining the composition in percentage of cellulose, hemicellulose, lignin, moisture, ashes and organic matter using techniques established by TAPPI. In a second stage, acidic hydrolysis is conducted using H_2SO_4 (concentration of 0.13% v/v) and alkali (concentrations of: 5.7%, 7% and 10%) using NaOH at 120 °C. The response variable was the generation of reducing sugars which are estimated by the DNS technique and the solubilization of organic matter through the soluble COD technique. Additionally, the content of total and ammonia nitrogen, and ashes were characterized. In a third stage for the bioethanol production were used *Saccharomyces* strains (SC 42, SC 326, SC 327, SC 710 and SK 718) and *Kluyveromyces* (KLC 22, KL 668 and KL837), fermentations were conducted for both hydrolyzed monitoring the consumption of substrate by the DNS and COD techniques, cellular growth and pH. Ethanol quantification was performed by chromatography using CG 6820 Agilent Tecnology chromatograph equipped with a capillary column DB-35ms and flame ionize detector (FID). The oven conditions were: 55°C for 5 minutes, ramp 12°C/minute to 200°C, after the run the temperature was increased at 280°C for 4 minutes.

The results of *Pennisetum sp.* showed a composition of 37.99% cellulose, 42.58% of hemicellulose and 19.43% of lignin, 7.09% moisture, 13.76% ashes and 86.24% of organic matter. By acidic hydrolysis, a concentration of reduced sugars of 8.89 g/L, and 26.06 g/L of COD was determined while by alkali treatment could obtain 1.12 g/L of reduced sugars and 7.47 g/L of COD. Regarding the ethanol production, only strains SC42, SC327, SK710, KL668, and KL837 were able to produce ethanol by acid treatment, with the following results: 1g/L, 2 g/L, 7 g/L, 6 g/L and 6g/L of ethanol, respectively. Due to the low production of sugar during the alkali treatment, it was not possible to obtain by this means ethanol. These results demonstrate that the *Pennisetum sp* grass presents an interesting potential for the ethanol production using acid treatment.

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*Author for all correspondence

EVALUATION OF METHANE EMISSIONS AND BIOENERGETIC POTENTIAL IN COMARCA LAGUNERA OF NORTHERN OF MÉXICO

Itzcóatl Muñoz-Jiménez (1); Inty Omar Hernández De Lira (1);
Lilia Ernestina Montañez-Hernández (1); ***Nagamani Balagurusamy**** (1)

(1) Laboratorio de Biorremediación, Escuela de Ciencias Biológicas, Universidad Autónoma de Coahuila, México. Email: bnagamani@uadec.edu.mx; bnagas@gmail.com

Anaerobic digestion (AD) is a well-established technology for the production of biogas and it has been demonstrated that it represents an efficient method for the treatment of the organic fraction of different types of wastes. Methane (the final product of AD) is one of the principals greenhouse gases (GHS), having a warm potential 21 times more than carbon dioxide (CO_2). However correct management of wastes can help to reduce the negative effects of methane on global warm because of its potential to generate electric energy. *Comarca Lagunera* in North México is well known as one of the principal cattle activity zones of México, and one of the principal milk producers in Latin America. According to the last population census of livestock done in 2011 by Secretary of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA), Región Lagunera has a total of 427, 874 heads of livestock just destiny to the milk production. That data equals in terms of livestock manure production to 8.5 Ton day^{-1}, however, biomethane potential and bioenergy generation of these wastes has not been evaluated.

The aim of this study was to estimate the methane emissions in *Comarca Lagunera* and their potential of bioenergy production in terms of electricity, based on the Guidelines for National Greenhouse Gas Inventories of Intergovernmental Panel on Climate Change (IPCC, 2006). Using an anaerobic treatment for the four principal manures in the region: Beef Cattle (137,157 heads), Pigs (76,727 heads), Poultry Broilers (98,272, 028 heads) and Dairy Cattle (427, 874 heads), we worked with 600ml batch reactors with a working volume of 150 ml. Inoculum was obtained from an interior of an working anaerobic biodigester of a local farm. The total time of the study was 75 days.

Based on the Guidelines for National Greenhouse Gas Inventories of IPCC, we estimated and concluded that the potential of methane emissions in the region is 2.426 Gg CH_4 that equals to 50.946 Gg CO_2 Eq. Based on our data, the bioenergy potential using an anaerobic treatment for the livestock manure was calculated on approximately 16.61 TJ, which can generate 4.61 GWh of electricity.

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*Author for all correspondence

PRODUCCIÓN DE LÍPIDOS EN LA BIOMASA DE *Trichoderma* sp. CON UN CULTIVO ESTACIONARIO Y EXTRAÍDOS CON TRES TÉCNICAS PARA LA OBTENCIÓN DE BIODIESEL

Daniel Vélez-Martínez (1); Ma. Remedios Mendoza-López (1);
Jesús Samuel Cruz-Sánchez (1); Rosalba Argumedo-Delira*(1)

(1) SARA-UV, Xalapa, Ver., México;

Los biocombustibles son combustibles de origen biológico obtenidos de manera renovable a partir de restos orgánicos provenientes de la biomasa de origen vegetal y animal principalmente, para obtener como productos biogás, hidrógeno, bioetanol, biobutanol y biodiésel. Hablando particularmente del biodiésel, este se produce a partir de los ácidos grasos contenidos en los aceites vegetales o grasas animales esencialmente. Sin embargo, la producción de biodiésel a partir de las fuentes antes mencionadas no satisface su creciente demanda, compromete la seguridad alimentaria y tiene un alto impacto ambiental, por lo cual otras fuentes de aceites deben ser exploradas. En los últimos años se ha prestado mucha atención a la exploración de los aceites microbianos, que son producidos por algunos microorganismos oleaginosos, tales como las microalgas, levaduras, bacterias y hongos filamentosos. Considerando lo anterior la presente investigación tuvo como objetivo evaluar la producción de lípidos en la biomasa del hongo *Trichoderma* sp. H-1 con un cultivo estacionario a 25 °C y extraer los lípidos con tres técnicas para la generación de biodiésel a partir de la reacción de transesterificación, utilizando como catalizador BF_3.

Los resultados muestran que con la técnica de extracción de lípidos modificada de Blight y Dyer (1959), Folch *et al*. (1956) y Ramos *et al*. (2010) se obtuvo el 18.4 %, 10.3% y 17.1% de lípidos respectivamente. Con lo que respecta a la reacción de transesterificacion efectuada a los lípidos obtenidos con las tres técnicas antes mencionadas se encontró que el componente mayoritario del biodiésel conseguido presenta diferencias para los lípidos extraídos con Ramos *et al*. (2010), ya que el éster metílico del ácido palmítico (40.8%) es el componente mayoritario, mientras que para técnicas de extracción de Folch *et al*. (1956) y Blight y Dyer (1959) el componente mayoritario fue el éster metílico del ácido linoléico con 41.2 y 37.6% respectivamente.

Las condiciones de cultivo y las técnicas de extracción de lípidos utilizadas son un factor determinante para la obtención de biodiésel a partir de lípidos fúngicos, por lo tanto la modificación de algunas de estas condiciones podría incrementar su eficiencia y viabilidad.

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*Author for all correspondence

GENERACIÓN SIMULTÁNEA DE METANO, HIDRÓGENO Y ENERGÍA ELÉCTRICA EN BIOCELDAS ANAERÓBICAS ACOPLADAS DE CULTIVOS MIXTOS

José Diego Bárcenas-Torres* (1); ***Ismael Arroyo-Tena*** (1); Eliel R. Romero-García (1); José Fernando Covián-Nares (1); Gerardo Marx Chávez-Campos.

(1) ITM, Morelia, Mich., México.

Se construyeron biopilas generadoras de energía eléctrica a partir de dos cultivos microbianos generadores de hidrógeno-metano, colocando en el ánodo cultivos autóctonos de *Bacillus subtilis* aislados a partir del ensilado de maíz en su etapa de metabolismo fermentativo, y en el cátodo un consorcio microbiano metanogénico previamente caracterizado. Inicialmente los reactores fueron alimentados diariamente con 15 ml de una solución de 30 g/l de sacarosa para el cultivo hidrogenogénico y 15 ml de una solución de 20 g/l de acetato de potasio para el cultivo metanogénico hasta obtener los mayores rendimientos de degradación. Posteriormente fueron acoplados en una conformación de biopila en reactores de 250 ml, acondicionados con sensores que median temperatura, pH, oxígeno disuelto, voltaje y corriente acoplados a una tarjeta electrónica recolectora de datos, y analizados en el software NI Labview.

Se probó la influencia de la agitación y la temperatura en un diseño experimental 2^2 sobre el incremento de las variables de eficiencia, demostrando condiciones óptimas a 100 rpm y 37 °C, con constante de reacción de K= 0.00165 l/mg·h, y una tasa específica de consumo de sustrato q= 0.0485 h^{-1}.

Las eficiencias de generación de energía eléctrica (I_{e-}), producción de biogás (P_{CH4}; P_{H2}), degradación de materia orgánica (θ_{DQO}) y coulombica (ε_{Cb}) fueron determinadas cuando se suministraron diferentes concentraciones de sacarosa y acetato de potasio en la alimentación y manteniendo condiciones estables de pH, temperatura y resistencia externa (150 kΩ). Los resultados demuestran mayores rendimientos ($I_{e-} = \varepsilon_{Cb}$; P_{CH4}; P_{H2}; θ_{DQO}) cuando se alimentan sacarosa en el cátodo y acetato en el ánodo. De manera interesante se obtuvo una menor generación de ácidos orgánicos y mayor generación de butanodiol cuando se suministra acetato en ambos reactores y una notable disminución de la generación de metano sin afectar los rendimientos de generación de voltaje y corriente.

La incorporación de dos reactores acoplados en un sistema hidrogenogénico-metanogénico es capaz de generar energías integrales simultaneas a partir de sustratos simples, lo que la convierte en una alternativa prominente para el aprovechamiento de efluentes líquidos contaminantes.

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*Author for all correspondence

CARACTERIZACIÓN CINÉTICA Y METABÓLICA DE CULTIVOS AISLADOS DE ENSILADO, PRODUCTORES DE ELECTRICIDAD

José Diego Bárcenas-Torres*(1); ***María de Carmen Cano-Correa*** (1);
Adriana del Ángel Guzmán-Telles (1).

(1) ITM- Div. Ing. Qca. y Bqca, Morelia Mich. México;

Con el aislamiento de dos microorganismos con características diferentes, de una muestra de ensilado de maíz, se pudo obtener una producción significativa de electricidad (150 mV), dichos microorganismos fueron sometidos a una serie de técnicas microbiológicas para su identificación morfológica macro y microscópicamente, encontrando que se trataba de dos tipos de microorganismos Gram positivos capaces de generar esporas, su caracterización fue realizada mediante dos metodos: i) bioquímica realizada mediante el test API 50 CH (Biomérieux), encontrando que pertenecían al género Bacillus con un porcentaje de similitud de 76 % con *B. mojavensis* y de 93 % con *B. subtilis* para el primer y segundo cultivo respectivamente, y ii) Una comparación del gen 16S rDNA de ambos cultivos mediante aislamiento e identificación de secuenciación directa por extracción del DNA, amplificación por PCR y secueciación. En otra etapa subsecuente se realizaron cinéticas de consumo de sustratos fácilmente asimilables en condiciones tanto aerobias como anaerobias, obteniendo que ambos microorganismos presentaban mayores eficiencia en condiciones anaerobias en la asimilación del acetato de sodio (*B. Mojavensis*, 99.35%; *B. subtilis* 43.02%).

Finalmente se evidenció la capacidad de los cultivos aislados en la generación de bioelectricidad (hasta 150 mV) cuando se opera una pila de combustible microbiano en condiciones anaerobias y alimentando acetato como única fuente de carbono, conteniendo a *B. subtilis* en la sección catódica y a *B. mojavansis* en la sección anódica del biorreactor.

Con esto se concluye que es una alternativa altamente promisora para la producción de electricidad y el saneamiento de aguas residuales y con ello atacar dos de los problemas más graves que afectan a la sociedad hoy en día.

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*Author for all correspondence

ENERGY ANALYSIS OF BATCH AND SEMI-CONTINUOUS DARK FERMENTATION OF AGRICULTURAL WASTES

Paula Natalia Robledo-Narváez (1,2); Héctor M. Poggi-Varaldo*(1)

(1) CINVESTAV- IPN, Depto. De Biotecnología y Bioingeniería, GBAER, México DF, México;
(2) ITSTB, Maestría en Ciencias de los Alimentos y Biotecnología, Tierra Blanca, Ver.

Los residuos sólidos agroindustriales (RSA) de bagazo de caña (BC) y piña (RP), tienen un alto contenido de celulosa y hemicelulosa (C+H), lo que los hace candidatos a ser usados para la generación de H_2 por fermentación oscura (FO). El estudio en México de la FO para el tratamiento de la mezcla de BC y RP, a lo que denominamos FORSA, para la obtención de bioH_2 podría significar un gran avance en la gestión del uso de RSA especialmente en la región de la Cuenca del Papaloapan. Esta es una región de gran importancia agrícola de México debido a que generan más de 4.5 millones y 0.5 millones de toneladas/año de BC y RP, respectivamente (INEGI, 2005). Por otro lado, se podría llenar un vacío existente en la aplicación de métodos más eficientes para el tratamiento de estos en varias regiones de México y en el extranjero. Dado que los RSA contienen típicamente 35-45% de celulosa y 20-30% hemicelulosa, se tienen cantidades anuales totales de 2.25 millones de toneladas de celulosa y 1.5 millones de toneladas de hemicelulosa en la región de la Cuenca del Papaloapan, que son sustratos atractivos para ser sometidos a FO.

La Secretaría de Energía (2010) reporta que para el 2009 el requerimiento energético de México fue de 9250.7 PJ, de los cuales el 6.9% (638.3 PJ) se abasteció por energías renovables. A su vez la biomasa suplió el 3.8% de esa demanda energética, es decir 24.26 PJ. Si consideramos la C+H de FORSA como glucosa el aporte energético que podría obtenerse empleando estos residuos, y considerando un rendimiento de H_2 de 1.5 molH_2/mol, un factor de rendimiento de 8.8 molH_2/Kg(C+H), la entalpia de combustión del H_2 143 KJ/g, la FORSA a nivel nacional generaría hasta 36.3 PJ. Con esto se aumentaría el porcentaje de energía suplida a través de la biomasa de un 3.8 a un 5.7%. Como este trabajo solo se enfoca a los residuos generado en la cuenca del Papaloapan, se tendría una producción de H_2 de $3.3X10^{10}$ molH_2/año equivalente a 8.05 PJ/año supliendo un tercio de la energía requerida a nivel nacional a través de la biomasa.

Se efectuó el balance energético para la FO en lote, donde se comparó la energía obtenida por generación de H_2 con los insumos de energía debidos a calefacciones varias, energías de bombeo y mezclado. Se observa que la energía requerida ($1.86x10^{11}$ J) es mayor que la energía que aporta la combustión del H_2 producido ($1.32x10^{11}$ J). Sin embargo, haciendo algunas consideraciones de escalamiento podrían solventarse algunas desventajas que favorezcan el balance energético. Por otro lado, la complementación del proceso con una segunda etapa fotofermentativa de generación de más bioH_2 o fermentación metanogénica para producción de CH_4, puede hacer que el balance energético sea completamente positivo.

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*Author for all correspondence

BIOHYDROGEN PRODUCTION IN A SERIES PROCESS BASED ON DARK AND PHOTOFERMENTATION OF AGRICULTURAL WASTES

Paula Natalia Robledo-Narváez (1,2); Héctor M. Poggi-Varaldo*(1)

(1) CINVESTAV- IPN, Depto. De Biotecnología y Bioingeniería, GBAER, México DF, México;
(2) ITSTB, Maestría en Ciencias de los Alimentos y Biotecnología, Tierra Blanca, Ver.

En el presente trabajo, se desarrolló y evaluó un proceso biológico con etapas en serie. El proceso se basó en fermentación anaerobia en sustrato sólido hidrogenogénico con venteo intermitente (FASSH-VI), como primer etapa, seguido de fotofermentación hidrogenogénica (FFH) de los extractos acidogénicos de la primer etapa. La FASSH-VI usó la fracción orgánica de residuos sólidos agroindustriales (FORSA) como sustrato porque tiene potencial hidrogenogénico dado su perfil de contenidos moderado a alto de celulosa y hemicelulosa. Se evaluó el efecto del contenido de sólidos totales inicial y pH inicial en la alimentación sobre la producción de biohidrógeno en FASSH-VI. Los resultados indican que conforme aumenta la cantidad de sólidos totales se tienen un efecto negativo en la producción de hidrógeno. Se confirma que el intervalo de pH que favorece la producción de hidrógeno es 6.0-6.5.

Los extractos de sólidos gastados generados durante la FASSH-VI de la FORSA, resultaron ricos en ácidos orgánicos y en menor concentración de solventes. Por tanto, fueron un sustrato factible para una segunda etapa fotoheterótrofa productora de hidrógeno. En la FFH se empleó un consorcio con atractiva capacidad de producción de hidrógeno (G3), enriquecido en bacterias púrpuras no sulfurosas (BPNS). Este cultivo tuvo un desempeño similar a la cepa pura *Rhodopseudomonas palustris* (control) en la producción en lote de H_2 utilizando extractos con diluidos 1:10 de la fermentación hidrogenogénica en sustrato sólido. Dichos extractos diluídos presentaron una concentración inicial de 9.83 g DQO/L. La etapa de FFH significó una producción adicional de H_2 promedio del 65 y 61%, en relación a lo producido en la FASSH-VI (3.03 $mmolH_2/kg_{ss}$); es decir 1.97 $mmolH_2/kg_{ss}$ para *Rhodopseudomonas palustris* y 1.85 $mmolH_2/kg_{ss}$ para el consorcio G3. En el tiempo operado en la fotofermentación no se presentó metano aún cuando el extracto de los sólidos gastados no fue esterilizado.

Concluímos que el consorcio enriquecido G3 fue tan efectivo como la cepa pura para la producción de $bioH_2$ por FFH, lo que permite pensar en un proceso más económico puesto que no se esterilizaría el extracto a usarse en la FFH por G3.

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*Author for all correspondence

PRODUCCIÓN DE ISOBUTANOL EN *Escherichia coli* A PARTIR DE SEMILLA DE AGUACATE

Viridiana Villa-Islas* (1), Agustino Martínez-Antonio (1)

(1) CINVESTAV- Unidad Irapuato. Departamento de Ingeniería Genética. Km. 9.6 Libramiento Norte Carr. Irapuato-León C.P.36821 Irapuato, Gto. México Tel: (462) 623 96 00 Fax: (462) 624 58 46

El aumento de las poblaciones aunado al decremento en las fuentes de energía fósil, han llevado a las naciones a la búsqueda de energías alternativas, una de ellas es la producción de biocombustibles. El butanol y su isómero isobutanol ofrecen mejores características que el etanol para su uso como combustibles, por lo que en el presente trabajo se habilitó a la bacteria *Escherichia coli* para producir isobutanol mediante la vía de los 2-cetoácidos. Para lo cual, se expresaron dos enzimas heterólogas de *Saccharomyces cerevisiae*, una descarboxilasa ARO10 y una deshidrogenasa ADH7. Como fuente de carbono se utilizó semilla de aguacate hidrolizada, con el fin de aprovechar este desecho agroindustrial y producir un compuesto de valor agregado.

Con esta estrategia a escala de laboratorio, se logró alcanzar a las 35 horas una concentración de 22.9 μM de isobutanol en el medio de cultivo.

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*Author for all correspondence

GROWTH OF *Chlorella vulgaris* AND *Nannochloris oculata* IN EFFLUENTS OF TILAPIA FARMING FOR THE PRODUCTION OF FATTY ACIDS WITH POTENTIAL IN THE PRODUCTION OF BIOFUELS

Yesica I. Ferrer-Álvarez (1), Luis A. Ortega-Clemente* (1), Ignacio A. Pérez-Legaspi (1), Martha P. Hernández-Guevara (1), Paula N. Robledo-Narváez (2), Elvira Ríos-Leal (3), Héctor M. Poggi-Varaldo (3)

(1) ITBOCA, DEPI. Boca del Río, Ver. Mexico;
(2) ITSTB, Depto. Inv., Tierra Blanca, Ver. México;
(3) CINVESTAV- IPN, México DF, México;

The use of microalgae in the wastewater treatment, and their biotechnological exploitation for the production of biofuels, is a potential environmental application, since the microalgal growth takes place in the presence of CO_2, light and other nutrients such as nitrogen and phosphorus available in effluents, which allows its further use. Some species of microalgae, stand out due to its lipid composition and fatty acid profile suitable for the production of biofuel.

During the present study was conducted a factorial 2^3 experimental design, which assessed three factors: i) two species of microalgae (*Chlorella vulgaris* and *Nannochloris oculata*), ii) two culture media, wastewater of tilapia farming "WTF" and Basal Bold medium "BBM" and iii) two types of lighting (Multi-LEDs lamps and white light), at an intensity of continuous light of 79.88 µmol m^{-2} s^{-1}. The Microalgae were inoculated in 16 photobioreactors (8 L capacity) in 6 L of medium (WTF or BBM), at an initial concentration of 1.0 X 10^6 cells mL^{-1} at 20 ± 2 °C for 10 days. The above to determined the cell density, production of biomass, productivity of lipids and fatty acids.

Increased cell density on average, as well as the greater productivity of biomass observed in treatments was the treatment in which remained *C. vulgaris* in BBM and Multi-LEDs lighting (8.83 x 10^7 cells mL^{-1} and 0.0854 $gL^{-1}d^{-1}$, respectively). The major production of lipids was obtained in the exponential phase of *N. oculata* cultivated in Multi-LEDs lighting in both treatments (BBM with 58 % and WTF with 52 %). The culture of both species that were maintained in WTF generated lipid increase productivity (2-18 $mgL^{-1}d^{-1}$) compared to the cultivated BBM. The fatty acids palmitic, stearic, oleic, linoleic, linolenic acid and eicosanoic (C16-C20) were presented in both species of microalgae in concentrations between 26 to 74 %. Based on the results of the present study, we conclude that the cultures of *N. oculata* and/or *C. vulgaris* in WTF illuminated with Multi-LEDs are an economical and sustainable alternative for the production of biodiesel. Since you can get up to 58% of lipids, with an optimal fatty acid profile of up to 74% of total fatty acids.

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*Author for all correspondence

PRODUCCIÓN EN CONTINUO DE HIDRÓGENO EN UN REACTOR UASB: EFECTO DE LA VELOCIDAD DE RECIRCULACIÓN DEL EFLUENTE

María E. Hernández–Rojas* (1); Sergio Morales–Hernández (2); Eder Argueta (2); Sandro Báez–Pimiento (3); Gloria M. Trejo–Aguilar (4); José A. Dávila–Gómez (2)

(1) Dpto. Ciencias de la Alimentación, Universidad Autónoma Metropolitana–Lerma, C.P. 52006, Edo. de Méx, México;
(2) Dpto. de Energía, Universidad Autónoma Metropolitana–Lerma, C.P. 02200, México DF, México;
(3) Universidad Nacional de Colombia–Sede Manizales–Facultad de Ingeniería y Arquitectura–Departamento de Ingeniería Industrial–Grupo de Investigación en Innovación y Desarrollo Tecnológico–Kilómetro 7, vía al Aeropuerto, Manizales, C. P. 4–72–Colombia;
(4) Dpto. Biotecnología, Universidad Autónoma Metropolitana–Iztapalapa, México DF. México.

Un reactor anaerobio de flujo ascendente con manto de lodos (UASB) con recirculación fue operado para la producción fermentativa de hidrógeno empelando como sustrato melazas diluidas. La temperatura del reactor fue de 35°C. El inoculo mixto fue obtenido de un reactor UASB utilizado para el tratamiento de aguas residuales provenientes de una procesadora de frutas y verduras. El inoculo se enriquece en bacterias productoras de hidrógeno calentándolo a 120°C por 1h. El pH de alimentación fue ajustado a 7.05 con Na_2CO_3 para mantener el pH en el reactor a un valor constante de 5.0. El efluente fue reciclado a través de bomba conectada en la base inferior del reactor y en la parte superior en la zona de sedimentación del reactor. Dos condiciones de velocidad de recirculación fueron probadas 0.36 y 0.24 mh^{-1}. La velocidad de carga orgánica volumétrica fue 9.3 y 8 g $DQOL^{-1}d^{-1}$, para cada una de las velocidades de recirculación.

El reactor fue operado en continuo por un periodo de 90 días. El efecto de aumentar la velocidad de ascenso del efluente incremento el porcentaje de degradación de la DQO para la misma carga orgánica de 9.3 g $DQOL^{-1}d^{-1}$. El efecto de disminuir la velocidad de ascenso aumento la degradación de DQO debido a que hubo mayor tiempo de contacto de los microorganismos con el sustrato. Aunque para los carbohidratos totales la degradación disminuyo de 95 a 90% para una menor velocidad de ascenso en la recirculación, esto se debió a que los carbohidratos son un sustrato rápidamente biodegradable a diferencia del resto de la materia orgánica que conforma la DQO. La composición de hidrógeno en el biogás se mantuvo entre 10 y 20% en todas las condiciones de trabajo, la producción de metano se vio inhibida los primeros 40 días de operación del reactor y posteriormente la presencia de metano persistió entre 2–4% a pesar del pre–tratamiento del inoculo y de las condiciones de operación de pH bajo de 5 del reactor. La velocidad de producción de hidrógeno fue de mayor a velocidad de ascenso alta, siendo de 78 $mLH_2L^{-1}d^{-1}$. La velocidad de recirculación es un factor a considerar para incrementar la producción de hidrógeno en reactores en continuo.

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*Author for all correspondence: E–mail: m.hernandez@correo.ler.uam.mx , Tel. 728 282-7002 ext.1013

HYDROGEN AND POLYHYDROXYBUTYRATE (PHB) PRODUCTION OF A PHOTOHETEROTROPHIC MIXED CULTURES USING ORGANIC ACIDS

Victor Franco-Franco, Victor Jurado-Marban, Axayacatl González-García, Inés García-Peña*

Bioprocesses Department, Unidad Profesional Interdisciplinaria de Biotecnología, Instituto Politécnico Nacional, México D.F., México

Biological hydrogen (H_2) production is one of the most promising sources of energy because of the well-recognized advantages. The H_2 production by dark fermentation has been study during the last decades. The potential use of waste material during this process makes it as a suitable technology for providing energy with simultaneous waste treatment and bioremediation. However, its main drawback is the low H_2 yields and the sub-products (Volatile Fatty Acids, mainly butyric and acetic) accumulation. Hybrid fermentation technology which combined dark fermentation and photo-fermentation process might be one of the most promising routes to improve the H_2 yield. Some photo-heterotrophic bacteria are capable of converting acetic, lactic and butyric acids to H_2, carbon dioxide (CO_2) and others products as polyhydroxybutyrate (PHB) under anaerobic conditions in the presence of light.

Three mixed phototrophic cultures (C_2, C_4 and C_5) were evaluated for the production of H_2 and PHB when feeding with acetic and butyric acids in batch test. All phototrophic cultures were able to grown on acetate and butyrate. When growing under non-limiting condition, all cultures showed similar values for specific growth rate (0.006 h^{-1}) and biomass yield (0.23 g/g).

H_2 was produced only under completely ammonia limiting condition and C_2 showed the highest H_2 content (74%, v/v) on the gas phase and the highest H_2 production yield. A molecular analysis (pyrosequencing) showed that the lower H_2 production on cultures C_4 and C_5 could be consequence of a higher microbial diversity. On the contrary, culture C_2 showed less diversity, determining that *Rodoseudomonas palustris* was the predominant microbial population (67%) at genus level.

Keywords: Hydrogen, PHB, Phototropic cultures

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*Author for all correspondence P.O. Box 07340, México City, México.Ph.: (+52) 5557 296000 ext 56474, Fax: (+52) 5557 296000 ext 56305, e-mail: egarciap@ipn.mx.

BIOHYDROGEN PRODUCTION FROM CRUDE CHEESE WHEY AND FRUIT VEGETABLE WASTE BY CO-DIGESTION IN BATCH AND CONTINUOUS SYSTEMS

Gómez-Romero, J (1); García-Peña, E.I.* (1)

(1) UPIBI-IPN, México DF, México;

The co-digestion process of crude cheese whey (CCW) with fruit vegetable waste (FVW) for biohydrogen production was investigated in batch and continuous systems, in stirred 1.8 L bioreactors at 37°C. Five different C/N ratios (7, 17, 21, 31, and 46) were tested in batch systems. While, in continuous system eight conditions were evaluated, hydraulic retention time (from 60 to 10 h) and organic load rate (from 21.96 to 155.87 g COD/L d).

Data in batch tests showed a maximum specific biohydrogen production rate of 10.68 mmol H_2/Lh and a biohydrogen yield of 449.84 mL H_2/g COD at a C/N ratio of 21. In continuous co-digestion system, the optimum hydraulic retention time and organic loading rate were 17.5 h and 80.02 g COD/L d, respectively. Under these conditions, the highest volumetric production hydrogen rate (VPHR) and hydrogen yield were 11.02 mmol H_2/L h, 800 mL H_2/COD, respectively. A pyrosequencing analysis showed that the main microbial population at the initial stage of the co-digestion consisted of *Bifidobacterium*, with 85.4% of predominance. Hydrogen producing bacteria such as *Klebsiella* (9.1%), *Lactobacillus* (0.97%), *Citrobacter* (0.21%), *Enterobacter* (0.27%), *and Clostridium* (0.18%) were less abundant at this culture period. The microbial population structure was correlated with the lactate, acetate, and butyrate profiles obtained. Results demonstrated that the co-digestion of CCW with FVW improves biohydrogen production due to a better nutrient balance and improvement of the system's buffering capacity.

Key words: Biohydrogen, Cheese Whey, Co-digestion, Fruit Vegetable Waste

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*Author for all correspondence

ESTUDIO PRELIMINAR DEL COMPORTAMIENTO VOLTAICO DE CELDA DE COMBUSTIBLE MICROBIANA, EMPLEANDO SEDIMENTOS DE LA REGIÓN SEMIDESÉRTICA DEL ESTADO DE COAHUILA

Juan Antonio Ugalde-Medellín; M. Fernanda Rodríguez-Flores;
Mónica M. Rodríguez-Garza*; José A. Rodríguez-de la Garza; Yolanda Garza-García;

Biotechnology Department, Chemistry Faculty, Universidad Autónoma de Coahuila, México

Actualmente el mundo vive no solo una crisis energética, sino que también sufre una crisis ambiental, a causa de la contaminación provocada por la desmesurada actividad industrial, incluyendo a la industria química, por lo que una alternativa novedosa es el empleo de microorganismos exoelectrogénicos, en sistemas de celda de combustible microbiana, como biorremediadores entrando no solo a subsanar una problemática sino varias.

Una celda de combustible microbiana o CCM es una tecnología emergente, destinada a la producción de energía eléctrica directamente de la biodegradación de materia orgánica por bacterias. En este tipo de sistemas los microorganismos producen energía a partir de compuestos orgánicos e inorgánicos por medio de reacciones de óxido reducción, generando así electrones los cuales liberan al medio extracelular para que sean capaces de llegar a un aceptor final, en este caso, un electrodo toma el papel de dicho aceptor permitiendo que los electrones liberados circulen a través de un circuito electroquímico, generando corriente eléctrica. En cuanto a la capacidad de generación de energía se conoce que el uso de consorcios o cultivos mixtos son capaces de generar densidades de energía mayores que aquellas cámaras que utilizan cultivos puros, además se han buscado estrategias para incrementar la potencia voltaica de las CCM pero los estudios en su mayoría se han centrado en el diseño mismo de la celda o en el tipo de sustrato utilizable.

El objetivo de este estudio es el de evaluar una serie de celdas combustible microbianas montadas a partir de consorcios microbianos obtenidos de sedimentos de cuerpos de agua con características y capacidades únicas y propias de la región semidesértica del estado de Coahuila. Empleando para este estudio un diseño de celdas de dos cámaras en H, previamente utilizado y cuyos rendimientos al trabajar con lodos anaerobios generaron resultados favorables. El enriquecimiento de microorganismos exoelextrogénicos sobre electrodos de acero inoxidable cubiertos de grafito se realizó en forma de batch alimentado, durante 5 semanas, usando el mismo medio al usar un sistema de CCM. Los resultados preliminares de trabajo en la celda muestran evidencia de la formación de biopelícula exoelectrogénica.

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*Author for all correspondence

MICROBIAL FUEL CELL FITTED WITH AN ALTERNATIVE PROTON EXCHANGE MEMBRANE TREATING LANDFILL LEACHATES

Giovanni Hernández-Flores (1); Omar Solorza-Feria (1); Juvencio Galíndez-Mayer (2); Elvira Ríos-Leal (1); María T. Ponce-Noyola (1); Tatiana Romero-Castañón (3); Héctor M. Poggi-Varaldo*(1)

(1) CINVESTAV - IPN, México DF, México;
(2) ENCB-IPN, México DF, México;
(3) IIE, Cuernavaca, Morelos, México.

Up to now, due to their outstanding properties Nafion (*NF*) is the membrane of choice for usual in Microbial Fuel Cells (*MFC*s). Yet, its commercial price is up to US $ 1470/m^2 and it determines the *MFC* total cost.

Therefore, the aims of this work were: (*i*) to test an alternative proton exchange membrane (*PEM*) and (*ii*) to treat landfill-leachates on *MFC* performance in long batch process.

The tests were carried out using a new organic membrane (*NOM*) and *NF-117* as a target to compare results. The fuel used was a very recalcitrant influent, actually leachate from Mexico City sanitary landfill, mixed with an inoculum previously enriched in electrochemically-active bacteria, with a total chemical oxygen demand (*COD*) of 2 g/L. The *MFC*s were operated simultaneously along 15 days (d) and two electrochemical characterizations were performed, at 0 and 8 d, to set the adequate load resistance.

At the beginning, the first characterization indicated a remarkable superiority of *NF-117* over *NOM*. The values for maximum volumetric power ($P_{v,max}$) were 9 and 1100 mW/m^3 for *NOM* and *NF-117*, respectively. On the other hand, the internal resistances (R_{int}) were 650 y 350 Ω for *NOM* and *NF*, respectively. However, after 8 d of operation, a new *MFC* characterization was carried out, unexpectedly, the *NOM* outperformed the *NF-117*. The $P_{v,max}$ depicted by *MFC* using *NOM* as *PEM* in the *MFC,* reached 22.5 W/m^3 in contrasted with 8.6 W/m^3 showed when using *NF* as *PEM* in the *MFC*.

Regarding the R_{int}, their values were also better to *NOM* than when using *NF*, 41 and 80 Ω, respectively. In the first period of the batch operation (0 to 8 d), the average volumetric powers (P_Vs) were 14 and 5 W/m^3 for *MFC* fitted with *NOM* and *NF*, respectively. In the second period (8 to 15 d), when the external resistances were readjusted, the P_Vs reached values of 18.9 and 5.1 W/m^3 for *NOM* and *NF*, respectively. In the batch operation, the *MFC* fitted with *NOM,* the characteristics and performance with time, improved attributed to an in-cell enrichment process or acclimation of inoculum. The *COD* removals at the end of the batch operation (15 d) were ~40% and 30% for *NOM* and *NF*, respectively. In this study, the *NOM* hold promise as *PEM* for replacing the more expensive *NF-117* membrane in *MFC*s.

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*Author for all correspondence

PRODUCCIÓN DE HIDRÓGENO A PARTIR DE *Chlorella* sp. Y *Chlamydomonas sp.*

Erica Mariana Hernández-Hernández (1); Roxana Olvera Ramírez* (2);
Claudia A. Cortés Escobedo (3)

(1) ENCB-IPN, México D.F., México;
(2) ENCB-IPN, México D.F., México;
(3) CIITEC-IPN, Azcapotzalco, México D.F., México.

La obtención de energía tiene un gran impacto ambiental, por lo que una alternativa a esta problemática son las tecnologías del hidrógeno, esto debido a la elevada densidad energética por unidad de masa del hidrógeno y es considerado un vector energético alternativo. Se ha descubierto que algunas algas verdes unicelulares y cianobacterias tienen la capacidad de generar hidrógeno molecular (H_2) fotosintéticamente, proceso al que se le llama biofotólisis. El objetivo de esta investigación fue evaluar la producción de H_2 en cultivos de *Chlorella* sp. y*Chlamydomonas* sp., empleando el medio mineral BG-11 sin azufre en cultivos en suspensión e inmovilizados en alginato de sodio.

Se obtuvieron las cinéticas de crecimiento de ambas cepas durante el cultivo en medio mineral BG-11, valorando el efecto de la inmovilización celular sobre su crecimiento. Posteriormente se realizaron pruebas de producción de hidrógeno, sometiendo a estrés por ausencia de azufre en el medio de cultivo, anaerobiosis y luz continua durante 10 días. Las muestras gaseosas fueron inyectadas en un espectrómetro de masas de diferencial electroquímico (DEMS) para evaluar por masa la presencia del gas. .

Como resultado se observó que la inmovilización influye en ambas cepas sobre la velocidad de crecimiento pero no afecta su sobrevivencia ni reproducción. El análisis de las muestras gaseosas mostró resultados positivos de producción de H_2 para *Chlamydomonas sp.* en ambas condiciones. Sin embargo *Chlorella sp.* mostró señales menos evidentes en cultivos suspendidos y señal nula de producción de H_2 en condiciones de inmovilización. Por lo que probablemente su potencial de producción es menor. Adicionalmente *Chlamydomonas sp.* resultó ser más resistente a las condiciones de estrés durante más tiempo. El uso de la técnica de inmovilización facilita la manipulación de los organismos sin afectar la producción de hidrógeno, por lo que realizando las adaptaciones necesarias, este procedimiento de obtención de hidrógeno puede escalarse en un foto biorreactor y acoplarse a celdas de combustible.

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*Author for all correspondence

A NEW CARBON FROM NATURAL SOURCE TO BE APPLIED AS NANOCATALYSTS SUPPORT FOR THE OXYGEN REDUCTION REACTION

José L. Reyes-Rodríguez (1); Sathish Kumar-Kamaraj (2); Omar Solorza-Feria*(1)

(1) CINVESTAV- IPN, México DF, México;
(2) Universidad Politécnica de Aguascalientes, Aguascalientes, México.

Currently one of the most efficient ways to achieve better dispersion of catalytic materials is support them in a porous carbon matrix. Carbon, whether used in amorphous form as Vulcan, or in order form as graphite, nanotubes, or graphene is a material that stands for good electronic conductive properties, moderate stability to acids-alkalis, particle size and pore size related of course to its large surface area.

However most of the carbon produced industrially comes from burning fossil fuel in large quantities which they are directly responsible for global warming and other environmental problems. A future, when reserves of mineral combustibles are depleted surely we will use hydrogen as an energy carrier in fuel cells and this cells will need catalyst supported on carbon, therefore is necessary to research for new sources of production of carbon to meet the demand.

In this work it is propose the implementation of a novel carbon (pending patent), obtained from a natural source more friendly with environment, to be used as a support nanoparticles with catalytic activity towards the reduction reaction of oxygen in order to apply them in PEM Fuel Cells. The obtained carbon was subjected to different physico-chemical treatments in order to functionalize the surface to generate anchoring sites that allow better interaction with the metal particles and to achieve better dispersion. Once carbon were functionalized, Pt nanoparticles obtained by chemical reduction with $NaBH_4$ was incorporate. The material was evaluated by electrochemical methods as cyclic voltammetry, CO-stripping and rotating disk electrode to determine the effect of treatment on the catalytic activity of the platinum and thereby to establish a synthesis protocol. The work will complement studies of TEM, SEM, XRD, Raman, FTIR and BET area. All the results are compared with Pt/Vulcan Carbon pre-treated.

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*Author for all correspondence

MAGNETITE DECORATED WITH PLATINUM AS A CATALYST FOR THE OXYGEN REDUCTION REACTION. A POTENTIAL CANDIDATE AS CATHODE FOR PEM FUEL CELLS

Luis López-Sosa (1); José Luis Reyes-Rodríguez (1); Fernando Godínez-Salomón (1); Omar Solorza-Feria*(1)

(1) CINVESTAV- IPN, México DF, México.

Nowadays one of the greatest challenges in the field of fuel cells is the development of new catalytic materials towards oxygen reduction reaction (ORR) which must have higher activity than Platinum and a high durability. It is known in the scientific literature that when Platinum alloys are formed with other transition metal element is able to improve the catalytic properties of the material compared with platinum alone. This improvement in catalytic activity is due to a change in the internal conformation involving structural processes of expansion and contraction of the crystal lattice. Disruption of these structural parameters produce a moderate oxygen adsorption on the surface of the nanoparticles to perform electron transfer during the reduction of oxygen. Today core shell structures are the most attractive to be used as catalysts in fuel cells since they involve the formation of a nucleus of an abundant non-noble metal decorated by a thin shell of Platinum. The addition of the second metal as a core reduces the amount of used platinum, thereby decreasing material costs, but also ensures that the platinum atoms remain on the surface, thereby making better use of the noble metal during the reduction.

But getting obtaining a core-shell configuration is a challenge that a lot depends on the synthesis conditions and reagents. In our research group have attempted to obtain such structures with an established methodology that has given encouraging results for materials based on Ni@Pt and Co@Pt. Iron is a neighbor to the other two elements and was proposed for use in an attempt to form a core-shell Pt; however, due to its high ability to bind to oxygen and form oxides characterization results for EDS and XRD indicated that indeed a magnetite-platinum alloy was obtained. Results of electrochemical studies with RDE show a significant catalytic activity towards ORR at least twice the commercial 20%Pt-Etek. Magnetite was synthetized by chemical reduction of iron nitrate with $NaBH_4$ in presence of TBAB as capping agent. Pt shell was obtained by Galvanic replacement on the nanoparticles surface. Nanoparticle was supported on Vulcan carbon previously functionalized. In order to evaluate the effect of the thermal treatment a small amount of the catalyst was carried to 400°C in N_2/CO atmosphere.

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*Author for all correspondence

INTEGRATION OF A BIOREFINERY MODEL FOR THE PRODUCTION OF HYDROGEN, METHANE, ENZYMES AND HYDROLYSATES FROM THE ORGANIC FRACTION OF MUNICIPAL SOLID WASTES

Carlos Escamilla-Alvarado (1); Héctor M. Poggi-Varaldo* (1);
M. Teresa Ponce-Noyola (1); Elvira Ríos-Leal (1); Fernando Esparza-García (1);
Josefina Barrera-Cortés (1); Jaime García-Mena (1); Ireri Robles-González (2);
Noemi Rinderknecht-Seijas (3)

(1) CINVESTAV- IPN, México DF, México;
(2) Nova Universitas, Oaxaca, México;
(3) ESIQIE-IPN, México DF, México.

A lab-scale biorefinery model was developed and tested. It comprised four stages for the production of hydrogen, methane, holocellulolytic enzymes and hydrolysates (H-M-Z-S).The hydrogen-producing process (H-stage) was fed with the organic fraction of municipal solid wastes (OFMSW). This stage generated organic purges namely fermented solids (FS), which were fed to the other stages of the biorefinery. The methanogenic stage (M-stage) produced methane from the FS. For the enzymes production (Z-stage), *Trichoderma reesei* MCG 80 was used. In the saccharification process (S-stage), the enzyme extract from Z-stage was used for the production of sugars from FS.

In the H-stage, the hydrogenogenesis in semi-continuous process had productivity over 200NmL H_2/kg_r/dat thermophilic regime and 21 d mass retention time. In the batch process the yield was over 1 980µmolH_2/g VS at thermophilic conditions without the addition of phosphate salts nor nitrogen in the form of activated sludge. The productivity in M-stage was 2 000NmL CH_4/kg_r/d at thermophilic regime. In Z-stage, the FS at 1 %VS gave 1.46 FPU/mL and 0.98 IU_{CMC}/mL. Using the fermenters at fed-batch regime and fed with FS at 1.5 %VS, 2.06 FPU/mL and 1.23 IU_{CMC}/mL were obtained. In S-stage, saccharification efficiencies were up to 65 $\%_{VSb}$ for the FS. After 72 h of saccharification it was possible to retrieve and recycle the enzyme extract.

From the analysis of the experimental data, it was established that the best configuration for the biorefinery model was a series-parallel process.

The main contributions of this work were: *(i)* the technical bench-scale demonstration of a 4 stages biorefinery model based on an inverse cascading approach, and *(ii)* the use of the FS from H-stage as a building block for other bioproducts (i.e. methane, enzymes and hydrolysates).

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*Author for all correspondence

ANAEROBIC DIGESTION FULLY ENHANCED BY MERGING THE BIOMETHANATION OF SYNGAS FROM SOLID DIGESTATE

Serge R. Guiot* (1); Ruxandra Cimpoia (1); Rony Das (2); Silvia Sancho Navarro (3)

(1) Energy, Mining and Environment Portfolio, National Research Council Canada (NRC). Montreal, Canada H4P 2R2;
(2) Department of Civil Engineering, University of British Columbia, Vancouver, Canada V6T 1Z4;
(3) Department of Microbiology, Infectiology and Immunology, Université de Montréal, Montreal, Canada H3C 3J7.

The synthesis gas (syngas) resulting from biomass gasification is usually steam reformed and purified into methane, so to be used as renewable natural gas (RNG). Catalytic processes normally involve high pressure and temperature, may be problematic when impurities are present and tend to have low product specificity. An alternative to circumvent these disadvantages is to use microorganisms to convert the syngas main compounds (carbon monoxide, CO, and H_2) into RNG (biomethanation). Activity tests performed under varying CO partial pressures with specific metabolic inhibitors (bromoethanesulfonate and vancomycin) showed that CO was converted mainly to acetate, which was further transformed to methane. At high CO partial pressure, CO conversion shifted to hydrogen, which was then used to reduce CO_2 into methane. Thermophilic conditions (55-60 ºC) provided the anaerobic sludge with a CO-biomethanation potential significantly larger (five-fold) than under mesophilic conditions (35ºC), so long as the gas-liquid transfer was alleviated. The methane yield was near the stoichiometric yield i.e. 25% (vol CH_4/vol CO). Hence the overall CH_4 yield of a treatment chain made of biomass gasification followed by AD for syngas biomethanation would be around 0.3 Nm^3 CH_4/kg dry volatile or organic solids (VS) gasified.

Then the syngas biomethanation potential was studied via co-digestion with food waste (FW). A 2 L mesophilic completely stirred tank reactor (CSTR) was operated in the semi-batch mode with an average hydraulic retention time (HRT) of 28 days, and a FW organic loading rate of 1 g VS per liter of reactor (RXR) and per day. The co-digestion methane potential was not significantly affected by CO at a 0.2 atm partial pressure (p_{CO}); however, higher p_{CO} resulted in delay and relatively decreased methane production. In the CSTR a maximum CO conversion rate (12 mmol CO/g VS.d) occurred at a CO inflow of ¼ mol/L_{RXR}.d, a p_{CO} of 0.2 atm, and a 0.04 d^{-1} gaseous HRT. Under these conditions, the VS destruction efficiency could attain 85%, and the conversion yield, 0.47 L_{STP} CH_4/g VS destroyed.

Food waste is usually quite easily amenable to AD, however biosolids or manure are significantly less biodegradable, with a non-digested residue (solid digestate) of 50 to 40%. The digestate may be dewatered mechanically to produce a cake with up to 30% solids, depending among other things on the undigested fiber content. When digestate cannot be traded as a soil amendment or fertilizer, it could be further dried, then gasified to produce syngas. Integration of such syngas methanation to existing AD facilities could therefore maximize the energy potential of organic residues while minimizing the processing steps. For instance, in case of a biosolids 50% conversion by conventional AD, the CH_4 yield would be at the most 0.25 Nm^3/kg VS. With gasification of the digestate (the other 50% of VS) and processing of the resulting syngas in the AD system, an additional 0.15 Nm^3/kg VS could in theory be expected.

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*Author for all correspondence

CARACTERIZACIÓN Y PRODUCCIÓN DEL BIODIESEL A PARTIR DE ACEITE DE COCINA USADO Y EVALUACIÓN DEL DESEMPEÑO Y EMISIONES DEL MOTOR DIESEL CON MEZCLAS B5, B10 Y B20

Víctor H. Castillo Barragán* (1); Ricardo M. Aguilar Valdivia (1); Alejandro Torres Aldaco (1); Helen D. Lugo Méndez (1); Raúl Lugo Leyte (1)

(1) UAM-I, Div. CBI., México DF, México.

En México se consumen anualmente 2,500 millones de litros de aceite vegetal, y la mayoría de estos aceites después de utilizarse, son vertidos en las alcantarillas, provocando problemas graves de contaminación de aguas y suelos; incrementando los costos de tratamiento de las aguas residuales en forma considerable.

Cuando se utiliza biodiesel en un motor, el dióxido de carbono (CO_2) liberado durante la combustión se compensa con el CO_2 capturado por las plantas con que se produce biodiesel; y considerando el ciclo de vida de las emisiones de gases de efecto invernadero el petro diesel tiene altas emisiones respecto del biodiesel a partir de aceite residual.

En este trabajo se realizó la caracterización del aceite, biodiesel y las mezclas de biodiesel-diesel B5, B10 y B20. El biodiesel empleado se elaboró a partir de aceites residuales de cocina, con un proceso transesterificación, por medio de catálisis básica homogénea utilizando metanol (CH_4) e Hidróxido de Sodio (NaOH) al 1% en masa como catalizador. Se evaluaron las propiedades de índice de acidez, yodo, éster peróxido, humedad, punto de nube, densidad y poder calorífico inferior; para compararlos con las normas americana y europea. También se evaluó el desempeño del motor diésel utilizando las mezclas B5, B10 y B20; los resultados se compararon con lo obtenido al utilizar diésel PEMEX.

El biodiesel obtenido cumple con lo establecido por la norma ASTM D-6751. En el motor las mezclas biodiesel-diesel presentan una mejora en su desempeño mecánico utilizando B20 y una mejora en las emisiones de CO_2 utilizando B10 respecto a lo obtenido utilizando diésel PEMEX.

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*Author for all correspondence

MEMBRANES IN MICROBIAL FUEL CELLS: A REVIEW

Giovanni Hernández-Flores (1); Omar Solorza-Feria (2); Juvencio Galíndez-Mayer (3); Elvira Ríos-Leal (4); María T. Ponce-Noyola (5); Noemí Rinderknecht-Seijas (6); Tatiana Romero-Castañón (7); Héctor M. Poggi-Varaldo*(1)

(1) CINVESTAV - IPN, México DF, México;
(2) CINVESTAV - IPN, México DF, México;
(3) ENCB-IPN, México DF, México;
(4) CINVESTAV - IPN, México DF, México;
(5) CINVESTAV - IPN, México DF, México;
(6) ESIQIE-IPN, México DF, México;
(7) IIE, Cuernavaca, Morelos, México.

Currently, most of the Microbial Fuel Cells (*MFC*s) are devices designed and focused to produce electrical energy and waste treatment. We can describe a *MFC* constituted mainly by the following items: anode, cathode, microorganisms, substrates and proton exchange membrane (*PEM*). Usually, the role of a *PEM* is to divide the electrodes, the anode from the cathode in a *MFC*. Regarding the costs, the use of membranes could represent around the 40% of the *MFC* total cost.

The aim of this work was to critically review the state of the art on membranes in *MFC*s. The scope of this review includes (*i*) the different membranes used in *MFC*s, (*ii*) costs and performance of typical membranes used, (*iii*) configuration of membranes in *MFC*s, (*iv*) membraneless *MFC*s, and (*v*) perspectives on *PEM*s.

An ideal membrane separator must have some characteristics such as high proton conductivity, biocompatibility, good mechanical properties, low oxygen transfer, low permeability to substrate, good chemical and thermal stability, and low cost. The most common membrane used is Nafion®, a perflourinated membrane, due to their excellent proton conductivity, nevertheless Nafion is too expensive. Nafion belongs to the type of cation exchange membranes, also known as *PEM*s.

In order to reduce the costs, other types of proton exchange materials have been tested in *MFC*s such as salt bridge, anion exchange membranes, microfiltration and ultrafiltration membranes, bipolar membranes, among others. However, studies focused on low cost and or natural polymers for *PEM* are still scarce. Alternatively, in some works, the *MFC*s have been operated without a membrane. Yet it was found that the coulombic efficiency substantially decreased.

Because of the type of membrane affects the performance and the total cost of the *MFCs,* is necessary to increase efforts to develop new, more economic membranes that exhibit good properties and allow for good cell performance at the same time.

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*Author for all correspondence

EFFECTS OF DIFFERENT CULTURE SYSTEMS ON PRODUCTIVITY OF *Nannochloropsis oculata* GROWN IN

Rosario Martínez-Macías (1); Patricia Ruiz Ruiz (2); Edna Meza-Escalante (1); Denisse Serrano-Palacios (1); Ana María Rentería-Mexía (2); Pablo Gortáres-Moroyoqui (2); Gabriela Ulloa-Mercado*(2).

(1) ITSON, Departamento de Ciencias del Agua y Medio Ambiente. Cd. Obregón, Sonora México.
(2) ITSON, Departamento de Biotecnología y Ciencias Alimentarias. Cd. Obregón, Sonora, México.

The microalgae *Nannochloropsis oculata* has received priority attention due to its lipids content for biodiesel production and due to its use as food in aquaculture. It has been proposed as a commercial source for the dietary, due to its high amount of lipids, omega-3 fatty acids, proteins, carbohydrates, and vitamins. Nonetheless, the best use of its oils is certainly the transformation into biofuels such as biodiesel. Growing conditions have a great influence on lipids and biomass productivity. The aim of this study was to evaluate the effect of different operation mode of bioreactors. Fed batch tubular reactor (FBTR) and semi-continuous reactor (SCR) were operated under completely defined conditions to assess their effect on microalgae lipid, protein and carbohydrate productivity of the green microalgae *Nannochloropsis oculata.* Both reactors were operated for 21 days at 25°C, with light intensity of 200 µmol E m^{-2} s^{-1}, with addition of CO_2 to adjust pH to 8.5. The tubular reactor (6 L operation volume) was fed with f/2 Guillard medium (f/2GM) every 72 hours; then, the feeding was discontinued. The SCR (80 mL operation volume) was operated with a renewal rate of 30% after 11 days with f/2GM and “algal” medium (AM). The biomass was harvested and used to determinate its biochemical composition and productivity.

The results show that the AM promoted the best biomass productivity, reaching until 3 fold more that f/2GM with 0.94 g $L^{-1}d^{-1}$. In the same way, the specific growth rate was higher in AM, 0.47 ± 0.03 d^{-1}; while f/2GM reached 0.395 ± 0.01 d^{-1} in the FBTR and 0.246±0.02 d^{-1} in the SMR. However, for lipid productivity the difference was not significant, reaching 0.028 g $L^{-1}d^{-1}$ in both culture media. In composition, protein content was similar in all cultures, reaching values between 33.7 % and 34.9 % of dry weight; the highest carbohydrates content was in AM, reaching 18.5 ± 2.0 %, followed by f/2GM in FBTR; whereas in SCR carbohydrates content was 15.2 ± 1.4 % and 13.9 ±1.0% using f/2GM and AM respectively. Data showed that the highest cell density in AM in fed batch tubular reactor is due to the high amount of nitrogen and all nutrients in general. The use of nutrient rich water helps the growth of microalgae and reduces the need of endogenous nutrients. As a result, the lipid production was higher in semi continuous reactor, because the nutrients were enough. A nitrogen starvation culture can be used as a control mechanism to enhance the accumulation of lipids, while simultaneously decreasing cell division. In conclusion the main kinetic parameters were achieved in AM semi-continuous reactor, allowing a high lipids productivity, this makes microalgal lipids a potential replacement for fossil fuel and other biotechnological applications.

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*Author for all correspondence

ESTABLECIMIENTO DE UN CULTIVO HETEROTROFICO DE *Scenedesmus incrassatulus* PARA LA PRODUCIÓN DE BIODIESEL

Liliana Flórez-Miranda (1); Orlando Melchy-Antonio (1);
Rosa Olivia Cañizares-Villanueva*(1)

(1) CINVESTAV- IPN, Departamento de Biotecnología y Bioingeniería, laboratorio de Biotecnología de Microalgas. Av. IPN 2508, San Pedro Zacatenco, C.P. 07360, México DF, México.Tel.:52(55)57473800 ext. 4342. E-mail: rcanizar@cinvestav.mx

La microalga clorofícea *Scenedesmus incrassatulus* posee reconocidas aplicaciones biotecnológicas que van desde el tratamiento de aguas residuales, hasta la producción de lípidos y pigmentos carotenoides. Su crecimiento y la producción de metabolitos han sido evaluados en autotrofía y mixotrofía, pero las productividades alcanzadas son muy bajas, lo que dificulta el aprovechamiento de la microalga. El presente trabajo se enfocó en el establecimiento del cultivo heterotrófico de *S. incrassatulus* como una estrategia para mejorar la productividad de biomasa y lípidos, para lo cual se formuló y evaluó un medio de cultivo empleando glucosa como fuente de carbono. La concentración de los nutrientes se calculó con base en la composición elemental de la microalga. Como fuente de nitrógeno se evaluó, la urea, el extracto de levadura, una mezcla de urea:extracto de levadura (50:50) y urea+vitaminas (cianocobalamina, tiamina y riboflavina). La microalga se cultivó en matraces Erlenmeyer de 500 ml con 250 ml de medio de cultivo a 37 ± 2°C y 200 rpm. La concentración de lípidos en la biomasa y el perfil de ácidos grasos se determinaron al final del experimento.

La mezcla urea:extracto de levadura, mostró un efecto positivo sobre el crecimiento de *Scenedesmus incrassatulus,* alcanzando 4.98 g L^{-1} de biomasa seca, contra 4.04 y 4.22 g L^{-1} obtenidos con urea y extracto de levadura respectivamente. Al comparar el crecimiento obtenido en la mezcla urea:extracto de levadura con el del tratamiento de urea+vitaminas no se encontraron diferencias en la concentración de biomasa alcanzada 4.2 ± 0.16 g L^{-1} y 4.22 ± 0.05 g L^{-1} respectivamente. La productividad de biomasa obtenida en el cultivo heterotrófico fue 5 veces mayor a la productividad obtenida en autotrofía y 2.3 veces superior que la productividad alcanzada en mixotrofía. El porcentaje de lípidos en la biomasa fue de 11.2% y 10.1% para el tratamiento de urea+vitaminas y para la mezcla urea:extracto de levadura, respectivamente. La productividad de lípidos se incrementó más del doble en heterotrofía, con respecto a la productividad alcanzada en mixotrofía. El perfil de ácidos grasos revela que los principales acilglicéridos presentes en la biomasa son el palmitato y el linoleato, los cuales son una buena referencia para estimar la calidad del biodiesel.

En base a los resultados se concluye que el cultivo heterotrófico de *Scenedesmus incrassatulus* permitió incrementar la productividad de biomasa y de lípidos en la microalga y que de acuerdo al perfil de ácidos grasos es posible obtener biodiesel de buena calidad a partir de la fracción lipídica de esta clorofícea.

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*Autor para toda la correspondencia.

BIOHYDROGEN PRODUCTION AT INDOOR AMBIENT TEMPERATURE USING CHEESE WHEY AS SUBSTRATE: EVALUATION OF PROCESS PERFORMANCE AND DETERMINATION OF MICROBIAL COMMUNITIES

Karla M. Muñoz-Páez (1); Héctor M. Poggi-Varaldo* (1); Jaime García-Mena (2); Elvira Ríos-Leal (1); Selva Sankar (3); Alberto Piña-Escobedo (2)

(1) Dept. Biotechnology and Bioengineering, CINVESTAV- IPN, México DF, México;
(2) Dept. Genetics and Molecular Biology, CINVESTAV- IPN, México DF, México;
(3) Dept. Pharmacology, CINVESTAV- IPN, México DF, México;

Cheese whey (CW) is the main waste of dairy industry and its use as substrate for biological H_2 production is promising. It is important to evaluate H_2 production at ambient temperature in order to allow for substantial energy savings. Another important aspect in biohydrogen production revolves around the microbial communities harbored in biological reactors; knowledge in this area would foster finding relationships between process performance and biocatalysts that could be useful for improving process design and operation. Thus, the objectives of this work were *(i)* to assess the H_2 production in anaerobic fluidized bed reactors (lab scale) at ambient temperature using CW as substrate, and *(ii)* to characterize the microbial community anchored in the bioparticles of our bioreactors. Bioreactors were operated in three periods; in the first and second, sucrose was loaded at 8 and 10 g/(L.day), respectively. In the 3rd period, CW at 10 g/(L.day) loading rate was used as carbon source. The main response variables were: H_2 productivity ($NmLH_2/L_{bed}$.day) and ratio between acetic acid concentration and butyric acid concentration (A/B). The study of the microbial community diversity was made by massive semiconductor sequencing of a 16S rDNA library.

The H_2 productivity in the Period 3 (with 10 g/(L.day) of CW) was 10 fold lower than that in Period 1 (with 10 g/(L.day) of sucrose), i.e., 1 011 and 101$NmLH_2/L_{bed}$.day, respectively. In Period 3, , the H_2 production progressively decreased and finally stopped. At the same time, lactic acid was detected in the effluent, which suggested the presence of lactic acid bacteria (LAB). LABs are microorganisms related to low H_2 production due to two main effects: substrate competition and excretion of bacteriocins. The taxonomic analysis showed that the bacterial community composition in the bioparticles significantly changed after the feed with CW. With sucrose, the main genera detected were *Firmicutes* (65-70%) and *Bacteroidetes* (20-25%) whereas when CW as substrate, *Firmicutes* presence increased up to 91% and *Bacteroidetes* decreased as low as 1.7%. The presence of LAB's was confirmed; they had a little increment with the change to CW (2.8 to 6.1% of *Lactobacillales*). We concluded that the process of H_2 production using CW as substrate needs a strategy to avoid the effects of LABs. The change of substrate seemed to be related to substantial changes of the microbial community. The best performance of our bioreactors was related to a lower ratio of *Firmicutes-to-Bacteroidetes.*

.*Author for all correspondence

SYNTHESIS AND CHARACTERIZATION OF Y@PT/C CORE-SHELL FOR OXYGEN REDUCTION REACTION

Miriam M. Tellez-Cruz (1); Fernando Godínez-Salomón (1); Omar Solorza Feria* (1)

(1) CINVESTAV- IPN, México DF, México

Proton exchange membrane fuel cells (PEMFCs) are promising candidates to resolve the environmental problems for their high efficiency and low emission energy conversion. The reaction of interest in a fuel cell is the cathodic reaction of oxygen reduction (ORR), which limits the cell performance due to its slow kinetics. Platinum is the best known catalyst for ORR, but its price is very high and therefore is necessary to research for new materials with the minimum amount of Platinum. During the last years the main challenge has been to obtain catalyst with high catalytic activity for the ORR and with a larger stability. One alternative is the formation of Platinum alloys with others transition metal which improves the catalytic properties owing to geometric and electronic interactions which occurs at a structural level. The main problem for the alloys is their dissolution in acid media. The way to solve this is to form a thin shell of Platinum involving a transition metal core. With this is possible to increase the stability of the catalyst besides that Platinum remains in the surface of the nanoparticles and there is where the process of electronic transfer take place, in this way the efficient of Platinum is improved.

Recently it was proposed in our laboratory a binary system of yttrium decorated with Platinum where the first results show a higher catalytic activity and a better durability than any other Pt based binary alloy. So in this work we present the synthesis and characterization of a nanocatalyst based on yttrium decorated with platinum and supported on Vulcan carbon to evaluate its catalytic properties toward the oxygen reduction reaction in acid media.

The carbon-supported nanoparticles with an average size of 2.5 nm were synthesized by chemical reduction of the precursor salt with NaBH4 to form the core of Y in the presence of surfactant agent and the shell was deposited by galvanic displacement in the surface atoms of Y. The material was characterized by XRD, TEM, SEM and EDAX. The electrochemical performance of Pt@Y/C is evaluated by cyclic voltammetry, CO stripping and RDE.

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*Author for all correspondence

DIMENSIONADO DE UNA PLANTA DE BIOGÁS PARA PRODUCCIÓN DE ENERGÍA ELÉCTRICA MEDIANTE EL ANÁLISIS TERMODINÁMICO EN UNA TURBINA DE GAS

Juan C. Paredes-Ramírez* (1); David Sampablo-Cruz (1); Ignacio Aguilar Adaya (1); Alejandro Torres-Aldaco (1); Raúl Lugo-Leyte (1).

(1) Universidad Autónoma Metropolitana Iztapalapa, México D.F., México.

La fermentación anaerobia de la materia orgánica, es un proceso que se fundamenta en la oxidación biológica de la materia orgánica la cual es idóneo para el tratamiento de residuos mediante la actuación de un consorcio de bacterias específicas que en ausencia de oxígeno descomponen la materia orgánica en productos gaseoso o biogás, compuesto principalmente por metano y dióxido de carbono, obteniendo un gas con un poder calorífico entre 14.3 – 28.6 MJ/m^3, y un potencial energético considerable, parte del cual se obtiene un efluente con una mezcla de productos minerales (N,P, K, Ca, etc.) con gran valor fertilizante. Este gas puede producirse en condiciones anaerobias en un contenedor hermético llamado biodigestor, el cual descompondrá las excretas a través de una serie de fases; hidrólisis, acidogénesis, acetogénesis y metanogénesis, que se desarrollan de manera simultánea y secuencial.

Existen diferentes tipos de generación de electricidad a partir del biogás, se puede llevar a cabo con motogeneradores de una capacidad P_{el} entre 150 kW hasta 4000 kW. Dichos motores son ajustados para operar con diferentes gases y trabajan entre 1 500 y 1 800 RPM, en el caso de las turbinas de gas que son utilizadas también en la generación de energía eléctrica pero utilizando como combustible gas natural, y no con biogás debido al bajo poder calorífico de este biocombustible.

Se realiza un análisis termodinámico por medio de una simulación en Thermoflex de una turbina, marca Solar Saturn de un 1 MW usando como combustible biogás a diferentes concentraciones de CH_4 y CO_2, para observar las variaciones en la potencia generada por la turbina de gas, así como el análisis termodinámico de un motor Jenbacher modelo J320 de GE implementado en la generación de energía eléctrica en plantas de biogás, como en Ecoparc I, los residuos orgánicos se procesan convirtiéndose en biogás, que sirve de fuente de energía para los motores a gas. La electricidad generada se utiliza en la misma planta y se suministra a la red. Una parte de la energía térmica se utiliza como calor de proceso en los biodigestores.

Con los datos obtenidos se puede realizar un estimado del número de biodigestores, a partir del flujo de combustible que se necesita para una potencia establecida.

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*Author for correspondence: gready64@hotmail.com

ELECTROACTIVITY OF THE NiO@Pt/C CORE-SHELL NANOCATALYST FOR THE OXYGEN REDUCTION REACTION

Diana Vega-Villalobos (1); Fernando Godínez-Salomón (1); José L. Reyes-Rodríguez (1); Luis Lartundo-Rojas (2); Omar Solorza-Feria*(1)

(1) Departamento de Química, Centro de Investigación y Estudios Avanzados del IPN, Av. IPN 2508, Col. San Pedro Zacatenco, A. Postal 14-740, 07360 México D.F., México.;
(2) Centro de Nanociencias y Micro y Nanotecnologías-IPN, UPALM, Col. San Pedro Zacatenco; A. Postal 07738 México D.F., México.

Recently the use of core-shell like materials has attracted the attention to be used as catalysts for the oxygen reduction reaction (ORR) in fuel cells application. Using these structures, it's possible to improve the surface's electrocatalytic features owing to the core-shell interaction, further; a considerable diminishing of production cost could be attained because of the replacement of noble metal in the core.

In this work the physical characterization of nickel oxide nanoparticles supported on carbon (i.e. Ketjenblack), decorated with platinum, as well as, the electrochemical results for the ORR in 0.1 M $HClO_4$ are reported. The catalyst was synthesized using two step procedure, according to previous reports. Pt electroactivity observed a highly influence from nickel oxide core. Physical characterization with TEM, XRD, RAMAN, XPS and EDS reveled highly particle size distribution in a range of 2-20 nm and nickel oxide as well Pt phases in ratio 71 and 29 wt.% respectively. The presence of nickel oxide in the core, dramatically change the Pt electroactivity towards ORR, compared with those with metallic nickel in the core, which have been developed in our team. In this case, NiO@Pt showed electrocatalytic properties very similar to those of commercial Pt/C-Etek considered as reference.

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*Author for all correspondence

EFECTO DEL TRATAMIENTO TÉRMICO SOBRE LA PRODUCCIÓN FERMENTATIVA DE HIDRÓGENO A PARTIR DE CELULOSA CRISTALINA

Zulma L Alamilla Ortiz *(1)*; Ana E Escalante Hernández (2); Iván Moreno-Andrade (3); Graciela ML Ruiz-Aguilar (1); Idania Valdez Vazquez (1,3)*

(1) Depto. Ciencias Ambientales, DICIVA Universidad de Guanajuato CIS. Irapuato, México
(2) Instituto de Ecología, UNAM. México
(3) Instituto de Ingeniería, UNAM. México

Diversas comunidades microbianas (CM) complejas se utilizan en la producción fermentativa de H_2 teniendo como ventajas altas velocidades de producción, una mayor capacidad metabólica, uso de residuos orgánicos no definidos como sustrato, reducción de costos de operación al trabajar en condiciones no estériles. En general, estas CM se someten un tratamiento térmico (tt) que las enriquece de esporas de *Clostridium* y elimina al resto de poblaciones. Este tt es eficaz para transformar azúcares fácilmente fermentables. Sin embargo, existe gran un potencial en los sustratos derivados de la agricultura, que al estar formados de celulosa y hemicelulosa, su conversión requiere de la acción de varias poblaciones microbianas, por lo que, el tt podría ser inadecuado.

El objetivo del trabajo fue estudiar el efecto del tt sobre la conversión de celulosa en H_2 mediante CM. Primero, se comparó una CM compleja y una CM tratada térmicamente (CM-tt) en sus capacidades de convertir celulosa en H_2. Después, se estudió el proceso de colonización de la celulosa y competencia por sustrato en la CM-tt, teniendo como controles una CM compleja y *Clostridium acetobutilicum*. Para ambos ensayos la CM compleja provino de un digestor anaerobio. El tt fue aplicado en un baño María durante 60 min. Se utilizó celulosa cristalina (SIGMA) como sustrato, 4 g/L en medio anaerobio pH 5.5. Los reactores (volumen útil 100 mL) fueron sellados en una atmosfera anaerobia (80%N_2, 20% CO_2) e incubados estáticamente a 37 °C. El volumen y composición de biogás (GC-TCD, columna PLOT Q) se determinaron cada tercer día, el crecimiento se determinó como proteína y el consumo de celulosa como el método del fenol-sulfúrico. Además, las fibras de celulosa fueron analizadas al microscopio. Los resultados fueron sujetos de un análisis de varianza con nivel de significancia de 0.05.

Los resultados mostraron que el tt tuvo un efecto altamente significativo ($P<0.001$) y negativo sobre la velocidad de producción de biogás en comparación con la CM compleja (0.4 vs. 6.2 mL $L^{-1}d^{-1}$). También el consumo de celulosa y crecimiento fueron menores. Se encontró que la colonización de la celulosa por la CM-tt fue afectado negativamente por la presencia de otras poblaciones debido a la competencia por el sustrato. Este efecto no fue relevante en el cultivo de *C. acetobutilicum*.

Las principales conclusiones fueron que en el rango mesofílico la conversión de celulosa en H_2 fue más eficiente con una CM compleja que con una CM-tt. Sin embargo, en la CM compleja, las especies de *Clostridium* deben competir por el sustrato con el resto de las poblaciones presentes, por lo que es importante considerar y favorecer el proceso de colonización por éstas especies.

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*Autor para toda la correspondencia: valdez_idania@yahoo.com

ATRIBUTOS DE COMUNIDADES MICROBIANAS PRODUCTORAS DE H_2

Zulma L Alamilla Ortiz (1); Ana E Escalante Hernández (2);
Gustavo Hernández Guzmán (1); Idania Valdez Vazquez (1,3)*

(1) DICIVA Universidad de Guanajuato CIS. Irapuato, México
(2) Instituto de Ecología, UNAM. México
(3) Instituto de Ingeniería, UNAM. México

Los cultivos mixtos silvestres productores de H_2 (CMS-PH) son interesantes desde el punto de vista ecológico, ya que diversas poblaciones microbianas conviven y actúan en conjunto para convertir el sustrato en H_2 y subproductos. Los orígenes los CMS-PH son diversos y algunos de ellos muy diferentes entre sí: fuentes termales, zonas intermareales, compostas, digestores anaerobios y plantas de tratamiento de aguas residuales (PTAR). Hasta el momento, es poco el conocimiento sobre las relaciones ecológicas presentes, y sólo se cuenta con información sobre la estructura microbiana.

El objetivo del trabajo fue conocer los atributos de riqueza, composición y predominancia de especies en (CMS-PH). Para ello, se revisaron un total de 90 publicaciones desde 2006 a la fecha que reportaran la estructura microbiana por métodos moleculares. Se hizo un inventario de las especies reportadas en cada CMS-PH en el punto de máxima producción. Con esta información se construyó una base de datos y una matriz de presencia-ausencia de grupos taxonómicos a nivel de especie en donde se indica el origen del inóculo. Los datos de la matriz se analizaron para identificar los grupos dominantes en los distintos tipos de inóculo y también se analizaron con un método de ordenamiento conocido como UPGMA (Unweighted Pair Group Method with Arithmetic Means) el cual arroja un dendrograma que permite visualizar la similitud en composición de especies.

Se encontró que los CMS-PH derivados de digestores anaerobios tuvieron la mayor riqueza con 109 especies, de las cuales *Clostridium* y *Lactobacillus* fueron predominantes (32 y 9%, respectivamente). En segundo lugar, CMS-PH derivados de compostas reportaron 78 especies, nuevamente *Clostridium* y *Lactobacillus* predominaron (24 y 9%, respectivamente). Los CMS-PH derivados de PTAR tuvieron 68 especies con predominancia de *Clostridium* y *Thermoanaerobacterium* (32 y 8%, respectivamente). Los CMS-PH de lodos termales y zonas intermareales tuvieron la menor riqueza microbiana con 34 y 12 especies, cada una. En estas últimas, las especies predominantes fueron *Clostridium*, *Thermoanaerobacterium* y *Bacillus* (38, 11 y 3% del total, respectivamente). Fue manifiesto que en todas los CMS-PH, *Clostridium* fue altamente predominante (>30% del total). La mayor similaridad entre los CMS-PH fue con aquellos de origen de compostas y lodos termales. Las especies presentes en todos los CMS-PH fueron *Clostridium butyricum* y *Klebsiella pneumoniae*. Este trabajo proporciona información para el análisis de las relaciones ecológicas presentes en los CMS-PH. Esto a su vez, permitirá idear estrategias para operar de forma más eficiente los reactores productores de H_2 en el largo plazo.

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*Autor para toda la correspondencia: valdez_idania@yahoo.com

COMPARISON OF THE HYDROGEN PRODUCTION AND THE RELATED MICROBIAL COMMUNITY IN FLUIDIZED BED BIOREACTORS OPERATED AT TWO TEMPERATURES: INDOOR AMBIENT AND MESOPHILIC TEMPERATURE.

Karla M. Muñoz-Páez (1); Héctor M. Poggi-Varaldo* (1); Elvira Ríos Leal (1); Jaime García-Mena (2); Selva Sankar (3); Alberto Piña-Escobedo (2)

(1) Dept. Biotechnology and Bioengineering, CINVESTAV- IPN, México DF, México;
(2) Dept. Genetics and Molecular Biology, CINVESTAV- IPN, México DF, México;
(3) Dept. Pharmacology, CINVESTAV- IPN, México DF, México;

Cheese whey (CW) is a dairy wastewater that has high organic matter content and low alkalinity. The use of CW as substrate for hydrogen production could provide a positive solution to waste CW management and treatment. A significant parameter in H_2 production is the operation temperature; if the H_2 production at ambient temperature could be of the same order to that obtained at mesophilic, the energy savings would boost the economic feasibility of bioH2 from CW.

Thus, the main goals of this work were *(i)* to evaluate the H_2 production in lab scale fluidized bed reactors operated at indoor ambient and mesophilic temperature and *(ii)* to determine the microbial community anchored in the bioparticles of the bioreactors. The organic rate was 5 g CW/L. day. The microbial community was determined by massive semiconductor sequencing of a 16S rDNA library. The major response variables were: H_2 productivity ($NmLH_2/L_{bed}$.day) and Shannon-Weaver index (H'_{10}). The reactors were feed with sucrose for 10 days and afterwards the feed was changed to CW. In the stage with CW, the reactor at ambient temperature exhibited a H_2 productivity of 330 ($NmLH_2/L_{bed}$.day), whereas there was not H_2 production at mesophilic temperature. Interestingly, the pH of both reactors were very close (4.3 ± 0.17 and 4.05 ± 0.19), this pH is in a range when some authors detect that the fermentation shifted to solventogenesis. The main metabolites detected were butyric acid (64.3 %) and ethanol (7-44 %). The taxonomic analysis showed that the phyla found in the bioreactor operated at indoor ambient temperature were: *Actinobacteria* (54.4%), *Firmicutes* (26.4%), *Bacteroidetes* (13.2 %) *Proteobacteria* (5.7%) and *Cyanobacteria* (0.1%). The corresponding findings at mesophilic temperature were: *Actinobacteria* (0.9%), *Firmicutes* (87.6%), *Bacteroidetes* (8.6 %) and *Proteobacteria* (2.7%). The H'_{10} at ambient temperature was 0.48 whereas at mesophilic was 0.2, this indicate that the community at ambient temperature was more diverse than at mesophilic temperatures. *Clostridiales* was the principal order of the phylum *Firmicutes* at both temperatures. It was more abundant at mesophilic temperature than at indoor ambient temperature (86.9% and 25.5% of the total orders, respectively). It is known that these microorganisms are hydrogen producers and could be expected that the abundance of *Clostridiales* was related to H_2 productivity, but that not happen in this experiment. It is interestingly, that the phylum *Actinobacteria* was more abundant at indoor ambient temperature than at meshopilic temperature. *Bifidobacteriales* was the main order of this phylum. This type of microorganisms has been reported to coexist with hydrogen producers and they could contribute to the degradation of carbon substrates than then may be utilized by *Clostridium*. We can conclude that, on the one hand, the reactors at ambient temperature had better performance that the mesophilic ones. On the other hand, it seems that the presence of *Actinobacteria* was related to the better performance of indoor temperature reactors than mesophilic ones.

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*Author for all correspondence

SÍNTESIS Y CARACTERIZACIÓN DEL CATALIZADOR Co@Pt/C CORE-SHELL PARA LA REACCIÓN DE REDUCCIÓN DE OXÍGENO Y CELDA DE COMBUSTIBLE POLIMÉRICA

Victor H. Rodríguez-Martínez (1); José L. Reyes-Rodríguez (1); Fernando Godínez-Salomón (1); Luís Lartundo-Rojas (2); Omar Solorza-Feria* (1)

(1) CINVESTAV- IPN, México DF, México;
(2) CNMN-IPN, México DF, México;

En el presente estudio se sintetizaron y caracterizaron nanopartículas tipo núcleo-coraza Co@Pt soportadas en carbón para evaluar su desempeño como catalizador en una celda de combustible polimérica. Se obtuvieron núcleos de Co a partir de la reducción química de $CoCl_2$ con $NaBH_4$ usando TBAB como tensoactivo y un sistema de sonda de ultrasonido. Por medio de un proceso de desplazamiento galvánico se sustituyeron átomos superficiales de Co por átomos de Pt para formar una envoltura o coraza. Se exploraron dos proporciones diferentes en relación masa de los metales de interés y se soportaron en carbón Vulcan XC-72R previamente funcionalizado. Ambos materiales se caracterizaron por TEM y SEM revelando nanopartículas en un rango de 4~10 nm y el análisis EDAX confirma la presencia de las fases de Co y Pt. Los difractogramas de XRD revelan que ambos materiales presentan picos de difracción análogos a los de la fase de platino que indican que en efecto existe una coraza de Pt que envuelve al Co.

También se realizaron estudios de espectroscopia RAMAN y XPS. La caracterización electroquímica se llevó a cabo en un sistema de tres electrodos usando $HClO_4$ 0.5M como electrolito, los resultados de Voltamperometría Cíclica no mostraron segregación de la fase Co a bajos potenciales (i.e. 0.3 – 0.4 V/NHE) lo que confirma lo encontrado por XRD. Mediante la técnica EDR de película delgada se encontró que ambos catalizadores presentaron una densidad de corriente al menos tres veces superior a la del catalizador comercial 20%Pt/C Etek®. Se seleccionó el catalizador con mayor actividad específica para preparar un ensamble membrana electrodo (EME) y evaluar su desempeño en una monocelda PEMFC. Se realizó la optimización de las cargas catalíticas del material y los resultados finales mostraron un desempeño superior en mW/mg de Pt a las telas catalíticas comerciales. Pruebas mediante SEM realizadas a los EME muestran que las películas más porosas brindan un mayor desempeño en comparación a las películas más compactas.

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*Author for all correspondence

EL PRINCIPIO DE CASCADA APLICADO A LA BIORREFINERÍA DE RESIDUOS SÓLIDOS URBANOS PARA LA GENERACIÓN DE PRODUCTOS DE VALOR AGREGADO: UNA REVISIÓN

Leticia Romero-Cedillo (1); Héctor M. Poggi-Varaldo* (1); Teresa Ponce-Noyola (1); Ana Carmela Ramos-Valdivia (1); Elvira Ríos-Leal (1); Carlos M. Cerda-García Rojas (2); José I. Tapia-Ramírez (3); Jaime García-Mena (3)

(1) CINVESTAV-IPN, DBB, México DF, México;
(2) CINVESTAV-IPN, DQ, México DF, México;
(3) CINVESTAV-IPN, DGBM, México DF, México;

La energía alternativa se puede producir a través de una biorrefinería, un proceso homólogo al de una refinería de petróleo tradicional, con la diferencia de que la materia prima son residuos orgánicos de muy bajo costo, renovables y los combustibles que se generan son menos contaminantes que los combustibles fósiles. Además en una biorrefinería se generan otros productos de valor agregado como son las enzimas, ácidos orgánicos, aditivos, solventes, aminoácidos, etc. Para esto es necesario seguir el principio de cascada, que se refiere al uso de los residuos orgánicos como materia prima en procesos secuenciales para así obtener el máximo rendimiento en productos y una mayor ganancia.

En el caso de la biorrefinería HMZS (hidrógeno, metano, enzimas y sacarificación), se utiliza el “principio de cascada inversa”, para producir primero bioenergía mediante la degradación de la fracción orgánica de residuos sólidos urbanos (FORSU) durante la fermentación oscura. Mediante la integración de etapas secuenciales en la biorrefinería HMZS se ha logrado diversificar el uso de estos residuos y se han obtenido productos de valor agregado como las holocelulasas, a partir del hongo *Trichoderma reesei*. Estas enzimas a su vez, pueden ser empleadas en procesos de sacarificación para generar azúcares simples que sirven de materia prima en la producción de bioetanol, o sea más bioenergía. Sin embargo, uno de los retos que se presentan dentro de la biorrefinería HMZS es aprovechar la cantidad de celulosa disponible en los sólidos residuales que se generan en la producción de holocelulasas.

Para este fin, los pretratamientos son una herramienta necesaria para incrementar el acceso de las enzimas a la biomasa celulósica y mejorar el rendimiento en la obtención de azúcares fermentables. Los métodos comúnmente utilizados para este fin, son la explosión por vapor, organosolv, enzimático, hidrólisis ácida, tratamiento ácido diluido e hidrólisis alcalina. Este último tiene la ventaja de llevarse a cabo a bajas temperaturas y de los tratamientos químicos, es uno de los más efectivos métodos para el rompimiento de los enlaces éster entre la lignina, celulosa y hemicelulosa, además de que evita la fragmentación de los polímeros de hemicelulosa. Por lo anterior, es importante conocer la efectividad de los pretratamientos en los sólidos que contienen una cantidad importante de celulosa para ser aprovechados nuevamente en el mismo proceso de biorrefinería y explorar su potencial en la obtención de otros productos.

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*Author for all correspondence

BIOELECTRICITY PRODUCTION FROM MUNICIPAL LEACHATE IN A MICROBIAL FUEL CELL

Ana Line Vázquez-Larios (1); Omar Solorza-Feria (1) Elvira Ríos-Leal (1); M. Teresa Ponce-Noyola (1); Héctor M. Poggi-Varaldo*(1) Rosa de Guadalupe González-Huerta (2); José Tapia Ramírez (1); Carlos Cruz-Cruz (1)

(1) CINVESTAV- IPN, México DF, México;
(2) ESIQIE-IPN, Mexico D.F., Mexico

The objective of this work was to evaluate *(i)* the effect of four inocula enriched in Fe(III)-reducing (soil origin or sulphate-reducing origin), bacteria and Mn(IV)-reducing bacteria and sulphate reducing (*In-E*$_{Fe(III)-S}$, *In-E*$_{Fe(III)-SR}$, *In-E*$_{Mn(IV)}$ and *In-SR* respectively), and *(ii)* the effect of chalcogenide cathodic catalyst, for treatment and bioelectricity production from municipal leachate in a microbial fuel cell.

The *MFC-G* consisted of a horizontal cylinder built Plexiglass 90 mm long and 57 mm internal diameter. The opposing faces of the cylindrical shell were fitted with corresponding sets of an assemblage of (inside to outside) proton exchange membrane (Nafion 117), a Toray flexible carbon-cloth containing 0.5 mg/cm^2 platinum catalyst (Pt 10 wt%/C-ETEK) and 1 mg/cm^2 $Ru_xMo_ySe_z$. Each assemblage was corresponded with anodes made of granular graphite. The anode chamber volume was 100 mL. The cells were loaded with 20 mL of municipal leachate. The cells were loaded with 80 mL of four inocula enriched in (IV)-reducing bacteria and sulphate reducing.

Each face (I and II) of the *MFC-G* was characterized by separate (I and II), in series and parallel connection. Parallel connection of faces increased P_{V-max}. In general parallel connection of electrode faces significantly decreased the internal resistance of the device (R_{int}). The cells were connected in parallel for 300 h of operation. The cells were connected to an R_{ext}= 100 Ω. The volumetric power average were up to values 26 424, 25 548, 25 752 and 13 379 mW/m^3 for In-E$_{Fe(III)-S}$, In-E$_{Fe(III)-SR}$, In-E$_{Mn(IV)}$ and In-SR respectively. The high P_{V-ave} obtained for our enriched inocula could be attributed to the increasing the total electrode surface area by the application of graphite, and this, in turn, could have improved the electron transfer microbe-to-anode and feasible presence the species of electrochemically active bacteria. Our results demonstrate the promising application of graphite granules anode and inocula enriched on treatment and additional benefits of bioelectricity generation from a recalcitrant municipal leachate. This, in turn, could have improved the electron transfer microbe-to-anode. The power values achieved in this work (13 W/m^3 for the chalcogenide catalyst, and 25W/m^3 for Pt) were higher than results of MFC treating leachate in the literature. Finally, application of enriched in Fe (III) and Mn (IV)-reducing bacteria significantly improved the performance that used $Ru_xMo_ySe_z$ as a cathodic catalyst for ORR

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*Author for all correspondence

PRODUCCION DE BIOCOMBUSTIBLES A PARTIR DE ORGANISMOS FOTOSINTÉTICOS

Alejandra B. Otero-Barrera *(2);* Alma Rosa Domínguez-Bocanegra (2); Jorge Torres-Muñoz (1); Ricardo Aguilar López* (1)

(1) CINVESTAV- IPN, México DF, México
(2) TESE, Div. Ing. Qca. y Bqca., Edo. De Méx,

Las reservas de combustibles fósiles se agotan, el tratamiento de residuos es cada vez más costoso y problemático, el crecimiento poblacional requiere mayor cantidad de energía y el cambio climático es una realidad, asociado principalmente a los gases de efecto invernadero producidos por la contaminación derivada de combustibles fósiles. Estos factores, aunados a la eminente extinción de las fuentes fósiles a mediano y/o largo plazo, han originado una crisis energética global que, a su vez, ha motivado una mayor conciencia ambientalista y ha incentivado enormemente la búsqueda de biocombustibles los cuales, por su propio origen, son amigables con el medio ambiente. Nuestro país no es ajeno a esta situación y, por ende, el desarrollo de tecnologías de biocombustibles eficientes, que impacten significativa y positivamente, no sólo desde el punto de vista ambiental, sino también desde el punto de vista de generación de empleos, es una demanda de alta prioridad. Es claro que hoy en día, existen diversas alternativas a la economía del petróleo y una de ellas es energía renovable basada en la conversión de recursos agroindustriales, materia prima de segunda generación, que no representa ningún problema potencial para el abasto de alimentos, o bien el uso de biomasa de algas o micro-algas marinas, que se identifican como biomasa de tercera generación. Además, se sabe que el desarrollo y producción másica y económica de esta energía se deben fundamentar en el concepto de biorrefinería. Los sectores industriales adoptan ahora distintos enfoques de este concepto para generar productos químicos y alimenticios similares a los convencionales, así como biomoléculas, biomateriales, biocombustibles y bioenergía, sobre la premisa de conservar la seguridad alimentaria, cuidar el uso apropiado de la tierra y hacer un uso integral de los productos y subproductos de los cultivos. En México existen diversos grupos de investigación dedicados al desarrollo de biocombustibles, pero se requiere aun de nuevos y diversos enfoques que faciliten eventualmente la transferencia de tecnología. El objetivo del presente trabajo es obtener biocombustibles a partir de organismos fotosintéticos.

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*Author for all correspondence

COCONUT WATER UTILIZATION FOR BIOETHANOL PRODUCTION

Alma Rosa Domínguez-Bocanegra* *(1),* Jorge Torres-Muñoz (1),
Ricardo Aguilar-López (1)

(1) CINVESTAV- IPN, México DF, México

The development of a fermentation process using carbon sources is of great economic importance for the production of biofuels on a commercial scale as is the case of coconut water for the production of bioethanol from *Saccharomyces cerevisiae*. Coconut is used for various purposes in the food and cosmetic industry but often coconut water ends in the sewer, which is unfortunate because the coconut water has components such as sugars, vitamins, minerals, electrolytes (such as potassium, magnesium, calcium, sodium and phosphorous), enzymes, amino acids, cytokinins, and phytohormones (natural hormones). He is also very low in calories and has no fat content, which makes it an excellent food.

The aim of this study was to use coconut water as a substrate to grow to *Saccharomyces cerevisiae* and obtain bioethanol. Coconut water was obtained from a company north of Mexico City. *Saccharomyces cerevisiae* cells were grown in YM medium (1% glucose, 0.3% yeast extract, 0.3% malt extract, 0.5% casein peptone) for 24 hours at constant temperature of 28 °C and stirrer speed 150 rpm and these cells were used as inoculum for our fermentation. The experiments were conducted in 500 mL flasks with 350 mL of coconut water no nutrient was added or YM medium. The cultures were inoculated with 35 mL YM medium in the exponential phase and incubated using a gyrating shaker at 150 rpm and 28°C, for 5 day. Samples were taken every two hours and reducing sugars were quantified according Miller, 1959, dry weight, cell number, optical density and ethanol concentration using a gas chromatograph. The results obtained indicated that the coconut water turned out to be an excellent substrate for *Saccharomyces cerevisiae* to grow reaching a maximum growth of $90x10^6$ cell per milliliter at 36 hours with a substrate consumption of 95%, the maximum production bioethanol obtained at that time was 50%.

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*Author for all correspondence

APLICACIÓN DE FUNCIONES DE DECISIÓN MULTICRITERIO PARA EL ESTUDIO DE LAS CONDICIONES DE CULTIVO DE *Botryococcus braunii* UTEX 2441 EN LA SÍNTESIS DE HIDROCARBUROS

Javier Acuapan-Hernández (1); Ricardo Aguilar-López (1);
Rosa O. Cañizares-Villanueva*(1)

(1) Departamento de Biotecnología y Bioingeniería, CINVESTAV- IPN, México DF, México.

Actualmente el estudio con microalgas se ha intensificado, debido a que son organismos fotosintéticos con capacidad de sintetizar productos de interés comercial tales como: suplementos alimenticios, fitohormonas, biocombustibles entre otros. Así mismo, muchas de ellas pueden ser consumidas directamente en la dieta de peces y camarones.

Sin embargo, la optimización de las condiciones de cultivo de estos organismos para lograr las aplicaciones antes mencionadas, es todavía un reto. El objetivo de la presente investigación fue aplicar *funciones de decisión multicriterio* mediante la implementación de un diseño *Plackett-Burman*, para encontrar las condiciones de cultivo que permitieran obtener una mayor síntesis de compuestos lipídicos (conocidos como hidrocarburos) en la microalga clorofícea *Botryococcus braunii* UTEX 2441.

En este estudio se encontró que las interacciones entre variables como intensidad luminosa, composición del medio de cultivo (principalmente a niveles bajos de nitrógeno) y fotoperiodo, mejoraron significativamente la producción de estos compuestos ($p<0.05$), los cuales representaron el 35% del peso celular seco. Un análisis de cromatografía de gases acoplado a masas, demostró que aproximadamente el 70% de los compuestos lipídicos obtenidos son hidrocarburos de cadenas hidrocarbonadas C-29 a C-31, los cuales pueden ser destilados para obtener combustibles con potencial para mitigar los efectos del cambio climático.

Los resultados anteriores indican que el empleo de ciertas herramientas estadísticas, tales como las *funciones de decisión multicriterio* ayudaron a encontrar las variables que tienen un efecto positivo tanto en el crecimiento como en la síntesis de lípidos por *Botryococcus braunii* UTEX 2441. Lo anterior permitió conocer el efecto de tres variables que intervienen en el cultivo autotrófico de esta microalga tales como fotoperiodo, intensidad luminosa y composición del medio de cultivo, las cuales podrían ser útiles en la producción de lípidos a nivel de fotobiorreactor.

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*Author for all correspondence

HYDROGEN TECHNOLOGIES IN A SUSTAINABLE HOUSE

Rosa de Guadalupe González Huerta* (1)

(1) ESIQIE-IPN, Laboratorio de Electroquímica y Corrosión, UPALM, CP 07738, D.F. México

In Mexico, around 33 % of greenhouse gas emissions are due to the electrical power consumption in the household. The implementation of renewable energy is currently growing in Mexico at a moderate pace, some government programs encouraging investment in these technologies begin to appear. Renewable energy supply electric power to the grid so they cause a reduction in the cost of current high electricity tariffs.
Providing reliable, environmentally friendly, and affordable energy has been a goal for many countries throughout the world. The rising consumption of energy and falling accessibility of natural resources are increasing the cost of electricity. In addition, as the industry develops, greenhouse gases are becoming a threat to the natural ecosystem.

Therefore, renewable energy has received more attention recently. Nowadays the growing energy demand for the production of goods and services in the world and the need to produce it in a clean and sustainable way has generated in recent years a strong global interest in developing new technologies and materials. Extensive research to achieve the best approach to generate clean energy has been largely transformed from "scientific competence" to "scientific cooperation" in order to achieve social and environmental well-being, since the best way to generate sustainable energy is to harness the weather of each region and integrate key systems such as solar photovoltaic and other renewable energy sources such as hydrogen through fuel cells.

In the case of Mexico, the generation of greenhouse gas emissions is due to motor vehicle sector and the electrical power consumption by industry and household. The environment has also been badly damaged by the oil industry in different regions of the country. On the other hand, a factor of great importance in Mexico is the rapid increase in energy prices, being gasoline and electricity tariffs an important key factor to get a virtual stable economy in the country, but in the last 10 years it seems that actual economic instability in energy is jeopardizing national development. Among the principal electric power consumers in Mexico, household users represent 33% of total consumption. This work presents a demonstration of the use of a PV/fuel cell hybrid power system to supply electricity to a mobile house.

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*Author for all correspondence e-mail: rosgonzalez_h@yahoo.com.mx

OXYGEN REDUCTION NANOCATALYSTS IN PEMFC-LI IONS BATTERIES AN HYBRID ELECTRIC VEHICLE PROTOTYPE

Omar Solorza-Feria* (1)

(1) Depto. Química, Centro de Investigación y de Estudios Avanzados del IPN., Av I.P.N. 2508, Col. Zacatenco. 07360 D.F., México

Nowadays catalysts based on carbon supported nanocatalysts are materials of great interest to be used as electrodes in polymer electrolyte membrane fuel cells (PEMFC); however the common problem in alloys is the easily degradation of the non-noble elements of the materials in acidic media, decreasing significantly its stability and performance. Currently intense investigation has been focused on the synthesis of core-shell nanocatalysts, where cores of different non-noble metals have been decorated with Pt and Pt-based compounds. This procedure could deeply reduce the amount of Pt used; decreasing the catalyst cost and improving catalytic activity towards oxygen reduction reaction (ORR), in relation to benchmark Pt/C catalysts.. In this communication I would like to talk about the chemical synthesis and physical and electrochemical characterizations of different nanocatalysts for the oxygen reduction reaction in PEM fuel cells. Results conducted to assume that the enhanced activities are related with changes on geometrical and/or electronic properties owing to M-Pt interactions. In first instance, for example Co cores were obtained from the chemical reduction of $CoCl_2$ with $NaBH_4$ using TBAB as surfactant and in an ultrasound probe system. Subsequently through a galvanic displacement process Co atoms were replaced by Pt atoms to make a shell. TEM and SEM analysis have showed highly dispersed nanoparticles with size averaged between 4-10 nm. XRD diffractograms showed peaks related to Pt phase little shifted to higher angles compared with pure Pt nanoparticles used as reference. Electrochemical results show that both catalysts presented superior current density, of at least three folds of specific activity than of commercial 20% Pt/C Etek® catalyst, however the mass activity is still below obtained by the commercial catalyst, which we must continue to work in order to eradicate this problem attributed to agglomeration of nanoparticles. The catalyst with higher specific activity was selected for preparing a membrane electrode assembly (MEA) and their performance evaluated in a PEM single cell. Optimization of catalytic materials loading was carried out and final results showed superior performance in maw/mg Pt to commercial catalysts. The SEM analysis showed that MEAs of more porous films provide higher performance compared to the most compact films. Finally I will present the design and manufacture of 350W PEMFC prototype incorporated in a hybrid two places electric vehicle operated with H2/O2 and Li ion batteries.

Key words: hydrogen, oxygen, fuel cells.

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*Author for all correspondence

SELECTION GUIDE FOR HYDROGEN STORAGE MATERIALS

Karina Suárez Alcántara* (1)

(1) UNAM-IIM Morelia, Antigua carretera a Pátzcuaro 8710, Col. Ex-hacienda de San José de la Huerta, Morelia, Michoacán, 58190, México

Hydrogen as energy vector can solve a lot of economical, environmental or even geopolitical problems. Ideally hydrogen will be produced locally from water and available-renewable energies; then stored until further use. The hydrogen storage is a huge challenge not resolved. From the materials or chemistry science point of view, several materials can indeed store hydrogen. From the practical or engineering point of view, several issues are not satisfied: i.e. economics, safety, efficiency, etc. This work presents a review of the advantages and disadvantages of several hydrogen storage materials. Also it is presented an obvious but necessary check list of the practical issues to consider when selecting a material for a practical application.

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*Author for all correspondence

NOVEL SYNTHESIS PATHWAY OF ZnS/ZnO/M (M=Ag,Cu,Ni) COMPOSITES FOR THE PHOTOCATALYTIC REDUCTION OF CO_2 UNDER SIMULATED SOLAR LIGHT

Miguel A. Valenzuela* (1); Patricia Vázquez (1,2); Jorge Aguilar (2); Daniel Bahena (3)

(1) Lab. Catálisis y Materiales. ESIQIE-Instituto Politécnico Nacional. Zacatenco, 07738, México D.F., México. mavalenz@ipn.mx, Tel: 57296000, ext. 55112.
(2) Escuela Superior de Física y Matemáticas-Instituto Politécnico Nacional. Zacatenco, México D.F., México.
(3) Lab.Avanzado de Nanoscopía Electrónica(LANE). Cinvestav-Instituto Politécnico Nacional, México D.F., México.

Photocatalytic CO_2 reduction and fixation have had a growing interest in the last decade. These processes can be considered as methods of green utilization of carbon dioxide to produce fuels or chemicals. The reduction of CO_2 takes place as any photocatalytic process, which includes light absorption by the semiconductor, separation of the photogenerated charges, transportation to the surface and the catalytic reactions of oxidation and reduction with electrons and holes, respectively.

In the present research we developed a new experimental procedure to synthesize ZnO deposited with co-catalysts type Ag, CuO and NiO, via the use of commercial ZnS impregnated and thermally treated with the corresponding metal (Ag, Cu, Ni) precursors. After a carefully oxidative treatment of ZnS in presence of the metal precursors, it was partially converted to ZnO decorated with metal nanoparticles (Ag) or metal oxides (CuO or NiO). The characterization was carried out by thermal analysis (DTA/TGA), X-ray diffraction, UV-Vis diffuse reflectance, Photoluminescence, Transmission electron microscopy (TEM and HRTEM) and X-ray photoelectron spectroscopy (XPS). The photocatalytic activity of the composites ZnS/ZnO/MO were evaluated in the reduction of CO_2 in presence of water and isopropyl alcohol as hole scavenger under visible light. Methane and hydrogen were the main products obtained after 8 h of irradiation time, although other unidentified compounds were also detected during gas chromatography analysis.

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*Author for all correspondence

THE PHOTOVOLTAIC CONVERSION; MATERIALS, STRUCTURES AND SYSTEMS

Yasuhiro Matsumoto* (1)

(1) Electrical Engineering Department, Research Center for the Advanced Studies of the National Polytechnic Institute, CINVESTAV-IPN, México D.F.

In today's world, more than 80% of our energy consumption depends on the combustion of fossil fuels. Therefore the pollution and global warming continues. Renewable sources of energy such as solar, has an enormous potential and advantages compared to hydrocarbons in general.

The solar cell harnesses solar radiation, a virtually inexhaustible resource to generate electricity without emitting pollutants. Photovoltaic conversion takes place without noise, friction, moving parts and without the dangers of explosion, i.e. virtually clean and safe system.

In different continents, there have been basic research of semiconductor materials and associated technological development of solar cells for 60 years. Photovoltaic conversion is now a mature technology with commercially competitive. In recent years and in some countries, photovoltaic systems reached parity (levelized cost of electricity) with the interconnection scheme using the electricity networks. Global production of photovoltaic modules increased the rate of 38% annually over the past 10 years. It reached 37 000 Mega-Watts of output during 2013 and the accumulation of nearly 140 thousand Mega Watts. This is equivalent to provide electricity to more than a 30 million homes. In Mexico already starts some relevant magnitudes of applications, so-called "mega projects" or "solar farms". In this talk, will be detailed the way to get polysilicon, the raw material for solar cells, as well as the principles of operation of the solar cell, its state of the art and cell types, including solar concentration. Furthermore will be discussed different applications at National and International levels.

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*Author for all correspondence

PROSPECTIVE ELECTRICAL ENERGY DEMAND: A SIMPLE MODEL

Arturo Morales-Acevedo* (1)

(1) CINVESTAV del IPN. Electrical Engineering Department

Knowledge of the future energy demand in the world, in a region, or even in a single country, is an important tool for planning energy production and establishing an energy policy. This energy demand will have to be satisfied by an optimum mix of the available energy sources, taking into account the restrictions imposed by future economic and social changes towards a sustainable world. Therefore, a macro model with only a few variables that can be predicted in a global way is needed; i. e. without a detailed analysis for each of these variables. In this work, for predicting the energy demand, the global variables affecting it have been defined first. It has been established that social, economic and technological development aspects can be taken into account by means of three important variables: population growth rate, gross domestic product per capita and energy intensity. As an example case, this model has been applied to estimating possible scenarios of the electrical energy demand in Mexico up to 2050. Based on these results, it will be shown that some estimates have been exaggerated because they do not take into account the evolution of the electrical energy efficiency which directly affects the respective energy intensity. In other words, it will be clear how important the energy efficiency is for planning the future electrical energy demand and also a portfolio with increased renewable energy participation.

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*Author for all correspondence: amorales@solar.cinvestav.mx

PROSPECTIVE ELECTRICAL ENERGY DEMAND: A SIMPLE MODEL

Hernando Romero-Paredes Rubio* (1)

(1) Departamento de Ingeniería de Procesos e Hidráulica, Universidad Autónoma Metropolitana-Iztapalapa

En esta platica presento un panorama energético nacional de frente al desarrollo de las aplicaciones de las energías renovables (ER). Presento el balance de energía haciendo énfasis en las renovables y su evolución en el tiempo de los últimos años. De cara al futuro, la reforma energética es un reto para incrementar la incidencia de las FRE y, bajo algunas consideraciones, se analiza su probable evolución de acuerdo a lo que se puede prever de las leyes secundarias. En este contexto, hago la observación de la propuesta para la creación de La Ley de Transición Energética (LTE). Esta Ley deroga, si se aprueba, la Ley para el Aprovechamiento de las Energías Renovables y el Financiamiento de la Transición Energética (LAERFTE) y la Ley para el Aprovechamiento Sustentable de la Energía (LASE). Sin embargo las propuestas a esta ley puede ser en sí misma una oportunidad que México no debería dejar pasar para impulsar y enfatizar las aplicaciones de las ER. La iniciativa privada nacional tiene un reto interesante, pues con sustento en la LTE puede aprovechar la coyuntura para impulsar el uso de energías limpias donde los sistemas de cogeneración también están incluidos. Y dejo, a nivel de conclusión la siguiente pregunta: ¿México podría convertirse en líder mundial en sustentabilidad energética?

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*Author for all correspondence: hrp@xanum.uam.mx

EL DESARROLLO DEL MERCADO DE LA ENERGÍA SOLAR EN MÉXICO Y LOS RETOS PARA SU FORTALECIMEIENTO

Alberto Valdés Palacios* (1)

(1) Asociación Nacional de Energía Solar

El desarrollo del mercado fotovoltaico en México se ha visto fuertemente favorecido por la interconexión de sistemas a la red eléctrica. Históricamente, hasta 2011, se tenían registros de 50 MW instalados en nuestro país, mientras que para mayo del 2014 se tienen ya, entre sistemas en operación y en desarrollo, la cantidad de 1,122 MW. De acuerdo con la Comisión Reguladora de Energía, entre 2012 y 2014 se tienen autorizados y parte de ellos en construcción 1072 MW. Adicionalmente existen en nuestro país alrededor de 500,000 usuarios domésticos de alto consumo de energía eléctrica, este nicho de mercado representa una cifra potencial de aproximadamente 750 MW. En lo referente al mercado fototérmico, los programas gubernamentales de integración de eco-tecnologías a las viviendas nuevas, han demostrado la viabilidad del calentador solar como parte integral de la vivienda. Aunado al consabido gran potencial de energía solar que posee nuestro país estas cifras nos proporcionan un marco optimista para el desarrollo del mercado solar. Sin embargo necesitamos crear un marco de certidumbre y viabilidad para ese mercado mediante el fortalecimiento de la normatividad, capacitación y gestión de estos sistemas, áreas en las que la Asociación Nacional de Energía Solar participa activa y extensamente en colaboración con los principales agentes del Sistema Energético Mexicano. En esta plática se expondrán las etapas del crecimiento del mercado solar, su potencialidad y las actividades necesarias para fortalecerlo.

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*Author for all correspondence; alberto.valdes@anes.org

POTENTIAL OF USING ORGANIC SOLID WASTES FOR CO_2 CAPTURE AND PRODUCTION OF BIOFUELS

Gerardo Saucedo-Castañeda* (1)

(1) Metropolitan Autonomous University, Iztapalapa Campus, Biotechnology Dept., Mexico City, México

Inadequate disposal of the organic fraction of municipal solid wastes (OFMSW) has enlarged with the increasing of population; causing problems related to human heath and environment. The municipal waste generation in Mexico in 2011 was 40 000 million tons with a contribution of 12 % from the Valley of Mexico City. In this area OFMSW is about 44 % of total waste. We are leading a research in order to produce bioethanol and biogas from OFMSW, and to define a process for rapid mineralization of OFMSW for the capture of CO_2 and further cultivation of microalgae for biodiesel production. On this paper we are presenting some of the advances on this research.

Composting can be describe as the aerobic mineralization of organic matter carried out by microorganisms forming CO_2, H_2O and compost as the final product. It could be a good alternative for the stabilization of OFMSW. Nevertheless, under uncontrolled conditions the process is slow and heat and CO_2 are usually not used.

We studied mineralization of OFMSW under controlled conditions as a function of process variables as pH, temperature, C/N ratio and microbial consortium addition. The maximum first order constant rate of mineralization obtained was 5.28 d^{-1} at 35 °C and pH 6, is at least 3.5 times higher to that reported in literature (1.5 d^{-1}). As conclusion, this first step of treatment of OFMSW, under controlled conditions, can be achieved on a short period of time (3-4 days). This fact can reduce the size of bioreactors and increase the capacity of composting plants, after that stabilization of organic solid can be realized under conventional conditions. During process of rapid mineralization of OFMSW capture of CO_2 can be carried out for further microalgae cultivation.

On other kind of studies, this process of rapid mineralization is been studied in order to enhance production of hydrolytic enzyme activities (pectinases, cellulases and xylanase) with a potential of degradation of biopolymer in an advanced stage of the process.

Also we are analyzing the microbial communities, of rapid mineralization of OFMSW, using molecular biology techniques such as PCR of 16S rDNA, internal transcribed spacer (ITS) and denaturing gradient gel electrophoresis (DGGE).

The evidence found in this research indicated the potential of utilization of the organic fraction of municipal solid wastes for the production of biofuels, even when the individual process yields could not be very high; the overall approach of biorefinery is very convenient for the treatment of these residues.

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*Author for all correspondence

Chapter 2.
Sustainability and Environmental System Analysis

ESTRATEGIA INTEGRAL SUSTENTABLE PARA LA TOMA DE DECISIONES DIRIGIDAS HACIA UN ADECUADO USO DEL AGUA EN EL TECNOLÓGICO DE ESTUDIOS SUPERIORES DE ECATEPEC (TESE)

Katulli C. Corral-Mirand*(1)***; Karla I. Téllez-López****(1)**; Martha Ledesma-Flores (1); Fernando Jesús de Lorenz-Santos (1).

(1) TESE, Div. de Cont. y Admón.; Posgrado en Gestión Administrativa, Edo. De Méx, México

El agua siendo más que un recurso limitado, es el elemento principal de vida de cualquier especie en el planeta. México no está exento de un problema de importancia mundial como lo es el manejo inadecuado de los recursos hídricos, lo que genera diversos problemas, que van desde aquellos que repercuten en la salud, como son: enfermedades por la falta de agua potable o uso de agua contaminada, hasta la imposibilidad de garantizar el abasto a futuras generaciones, por el agotamiento.

La tendencia mundial en años recientes destaca la necesidad de promover la gestión del agua de manera integral para la sociedad con un enfoque holístico y de largo plazo; por lo que el objetivo de éste trabajo es evaluar la viabilidad de una estrategia integral sustentable que cubra los procesos económicos, administrativos y técnicos para la toma de decisiones sobre el manejo del agua, considerando aspectos de conservación sustentables. Para ello se toma como referente empírico el TESE que, siendo una institución educativa con proyección al desarrollo sustentable, sirve como modelo académico, de investigación y de influencia en la educación del uso eficiente del agua y la calidad de vida en el Estado de México. El trabajo se lleva acabo con un equipo multidisciplinario en el que también participan diversos grupos de interés del TESE.

El desarrollo del trabajo identificó áreas de oportunidad en materia del agua en el TESE, respecto al manejo de agua potable, pluvial y residual; se estudian principalmente el volumen de agua utilizada, el costo y los sistemas de gestión (económica, administrativa y técnica).Los indicadores del estudio son: comunidad TESE, servicios, pérdida de agua, costos asociados con la prestación de servicios, la Infraestructura e inversión para el suministro de agua potable, residual y pluvial, calidad del agua, volumen del agua y mantenimiento. La metodología se conforma en primer lugar por trabajo de campo en donde se hizo una planeación y la observación directa considerando los componentes para la elaboración de un diagnóstico (UNESCO, 2014) la cual consta en parte de entrevistas personalizadas y encuestas; en segundo lugar un trabajo de gabinete conformado por el desarrollo de las estrategias particulares: administrativas, sociales, ambientales, tecnológicas, técnicas, económicas de salud y de difusión; así como el desarrollo del modelo integral de entradas y salidas que será la fase medular de la estrategia integral sustentable que cubrirá los requerimientos y compromisos que la CONAGUA tiene en materia de cultura del agua.

Como resultado se desarrolló un modelo de estrategia integral sustentable; la cual se conforma por estrategias particulares: administrativas, sociales, ambientales, tecnológicas, técnicas, económicas, de salud y de difusión; para que sirvan como modelo en la toma de decisión técnica y administrativa en otras instituciones educativas del país cuyos misión, visión y objetivo prioricen la sustentabilidad,con el fin de generar herramientas para la toma de decisión que permitan beneficios en el TESE y disminuyan los impactos ambientales.

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*Author for all correspondence

REDUCCIÓN DE LA GENERACIÓN DE RESIDUOS SÓLIDOS PELIGROSOS Y USO EFICIENTE DEL AGUA EN UN PROCESO DE RECUPERACIÓN DE BATERÍAS INDUSTRIALES AL APLICAR LA METODOLOGÍA DE PRODUCCIÓN MÁS LIMPIA

Paulina Mendoza-Alcántara(1);Gabriel Pineda-Flores (1);
Carmen Monterrubio-Badillo*(1)

(1) Mexican Cleaner Production Centre-IPN, México DF, México

El presente trabajo se centra en la aplicación de la Metodología de Producción más Limpia en una empresa reparadora de baterías ácido-plomo ubicada en el Distrito Federal, México. El objetivo fue generar oportunidades de producción más limpia a fin de prevenir la producción de residuos sólidos peligrosos en el proceso de reparación de las baterías y fomentar el uso eficiente del agua en la empresa. Con estas oportunidades se fomentan beneficios económicos en la empresa y se disminuyen los riesgos al ser humano y al ambiente. El trabajo incluyó el levantamiento de datos, mediciones en campo y propuesta de oportunidades y recomendaciones de producción más limpia que producen los beneficios económicos y ambientales.

El proceso de reparación y mantenimiento de baterías genera plomo como residuo que termina mezclándose con otro tipo de residuos convirtiéndolos en residuos peligroso, mismos que son entregados al camión recolector como residuos sólidos urbanos. Esta fuga de material representa una pérdida económica y un aspecto ambiental negativo por la alta toxicidad del metal. Otro problema importante es el derrame constante de la solución ácida de las baterías por el mal manejo que realizan los clientes y el personal de la planta, esta actividad compromete la seguridad física de los trabajadores y genera pérdidas económicas.

En el diagnóstico se identificaron cuatro oportunidades de producción más limpia referentes a la prevención y gestión de los residuos así como al uso eficiente del agua. Al implantarse las cuatro oportunidades de producción más limpia, se tendrá un beneficio económico global de $ 5,926.49 pesos mx, requiriendo una inversión de $5,128.89 pesos mx. Esta inversión será recuperada en un periodo no mayor a 0.087 años. Si bien los beneficios económicos no son muy relevantes, los beneficios ambientales que se obtendrán por la implantación de las oportunidades encontradas son muy relevantes, entre los principales se encuentran la disminución de 0.064 T de CO_{2eq}, la disminución de 6.2 Kg de Pb que llega al flujo de residuos sólidos por tonelada de Pb fundido, disminución de los daños al suelo y en la contaminación del agua por evitar el derrame de 7.068 L/año de solución ácida.

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*Author for all correspondence

ANÁLISIS Y PROPUESTA DEL RECICLAJE DE PET COMO ALTERNATIVA PARA MEJORAR LA GESTIÓN DE RESIDUOS SÓLIDOS INORGÁNICOS EN LA LOCALIDAD DE MIAHUATLÁN DE PORFIRIO DÍAZ, OAXACA 2010-2011

Martha Márquez-Ojeda* (1)

(1) Universidad de la Sierra Sur, Oaxaca

La generación de residuos sólidos, resultado de las actividades humanas, ha provocado una serie de distintos impactos en los recursos naturales; por ejemplo, la gestión o la disposición final inadecuada de los residuos sólidos urbanos pueden contribuir a la contaminación del agua, el aire o la tierra, sobre todo si se toma en cuenta que la basura es un residuo colocado y tratado de la forma equivocada, dada la responsabilidad que le corresponde a los municipios de dar disposición final a los residuos sólidos municipales. De aquí se desprende la necesidad de realizar acciones encaminadas al manejo adecuado de los residuos sólidos urbanos y donde el reciclaje se constituye como una estrategia fundamental en beneficio del medio ambiente, y en consecuencia en beneficio de la sociedad, En la década pasada se inicio el uso masivo de una nueva resina plástica, el PET, la cual posee la propiedad distintiva de la barrera de gases[1], otra propiedad su bajo peso con respecto al producto contenido y finalmente la más importante desde el punto de vista ambiental es que esta resina presenta mayores aptitudes para el reciclado.

El presente trabajo presenta un diagnóstico a nivel localidad sobre el manejo de los residuos sólidos municipales para la Localidad de Miahuatlán de Porfirio Díaz, Oaxaca para el año 2011 lo cual nos permite proponer y definir la situación actual de la misma y que a su vez nos permita diseñar una propuesta para el establecimiento de una planta recicladora de PET y con ello mejorar la eficiencia en la gestión de los residuos sólidos inorgánicos generados en la Localidad de Miahuatlán de Porfirio Díaz, Oaxaca.

El procedimiento con el que se realizó éste trabajo fue bajo rigor metodológico tomando como base normas oficiales Mexicanas 15, 22, 19 y 61 la muestra fue definida con el propósito de conocer la cantidad de generación de residuos sólidos a nivel domiciliario, derivándose la cantidad de residuos sólidos generados por habitante al día y la cantidad de residuos sólidos por tipo (metal, vidrio, plástico (PET) entre otros. Lo cual nos permite aplicar la misma metodología para otra Localidad, Municipio o incluso a nivel Distrital. De un total de 9598 viviendas la muestra fue de 95 viviendas en las cuales se recolectaron los residuos sólidos por una semana y posteriormente se aplicaron las normas antes mencionadas.

Finalmente para la Localidad de Miahuatlán de Porfirio Díaz, Oaxaca es viable la instalación de una planta tratadora de residuos sólidos y al mismo tiempo considerar otros aspectos prioritarios como son la creación de una conciencia de reciclaje, iniciando desde la separación de los residuos en el lugar de origen a modo de obtener envases de PET lo más limpios posibles, a fin de favorecer la etapa de reciclaje.

[1] La barrera de gases es la propiedad que posee el PET y que impide la salida y permite el almacenamiento de gas sobre todo en las bebidas o productos comestibles.

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*Author for all correspondence

DIAGNÓSTICO DE LOS RESIDUOS GENERADOS POR LA INDUSTRIA PETROLERA EN EL ESTADO DE COAHUILA

Micheline Gutiérrez-Prieto (1); Myriam A. Amezcua-Allieri* (1);
Juan A. Zermeño-Eguia Lis (1)

(1) IMP, México DF, México.

Los procesos industriales sin exceptuar los de la industria petrolera, tienen que adaptarse a marcos normativos y técnicas que controlen y reduzcan los residuos que generan sus actividades. Atendiendo a esta problemática en México, la Ley General para la Prevención y Gestión Integral de los Residuos (LGPGIR), sustentada en el Plan Nacional, establece y fomenta la gestión integral de los residuos mediante la aplicación de herramientas, estrategias y acciones contempladas en los Programas Estatales de Prevención y Gestión Integral de Residuos (PEPGIR). Con base a un cuestionario elaborado como apoyo al PEPGIR de Coahuila, el trabajo presentado identifica las áreas de oportunidad en cuanto al manejo y disposición final de los residuos generados por las actividades de la industria petrolera en el estado, siendo éste el que opera el proyecto más importante en el país de gas no asociado.

El análisis de la información se realizó durante el período 2008-2010 y 2012, identificando las instalaciones de las tres subsidiarias presentes en el estado: Pemex Refinación (PR), Pemex Gas y Petroquímica Básica (PGPB) y Pemex Exploración y Producción (PEP), con una breve descripción de las actividades que desarrollan, para posteriormente comparar la generación de los residuos durante el período de estudio, describiendo las características principales de cada uno de los residuos con la finalidad de identificar algunas opciones de valorización y manejo acorde con sus características, finalmente se realiza un análisis de los tratamientos que fueron aplicados de acuerdo a lo reportado.

En los resultados, destaca que los lodos aceitosos o sedimentos de hidrocarburos es el residuo mayoritario derivado de las actividades de mantenimiento, los cuales constituyen más del 55% de los residuos que genera la Industria petrolera en el estado, considerando la factibilidad técnica en el estado, dentro de las sugerencias realizadas se encuentra la valorización energética para este residuo. La propuesta realizada es orientada a la gestión responsable de los residuos, donde se proponen una serie de alternativas de disposición para los residuos identificados acorde con la infraestructura autorizada por la SEMARNAT y disponible en el estado para el manejo de los residuos acorde con su composición y a lo establecido por el marco legal en materia.

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*Autor para toda correspondencia

STOCHASTIC APROACH TO ECONOMICAL ANALYSIS OF BIOMASS POWER PLANTS

Renata Varfolomejeva* (1); Marija Zima-Bočkarjova (2); Antans Sauhats (1); Evgenijs Kucajevs (1); Nauris Jankovskis (1,3)

(1) RTU, Institute of Power Engineering, Riga, Latvia;
(2) ABB Corporate Research, Switzerland.
(3) JSC Latvenergo, Riga, Latvia;

The global community is motivated to move towards renewable energy supply and market based operation in power systems. In the core of this change is an aspiration of the reduced the influence of power generation on the environment and the increased power supply efficiency, which is achieved through market conditions and free competition within generation and sales of energy. These new conditions define significant changes in the design and planning of the operation of the biomass power plants (this kind of renewable generation is developing rapidly in the northern Europe).

First step in the power plant development project is pre-feasibility study. As a result of such study, a developer would choose a place of construction, type of fuel, the connection scheme to the power grid, would estimate the necessary capital expenses, the energy to be produced, production costs and profits from the energy sales. Economic analysis is an important part of the pre-feasibility study of the power plant. Usually, power plant design is evaluated in terms of the profit maximization and the following economic criteria, e.g. NPV, IRR, payback time.

The introduction of the markets in power systems substantially changes the approach to economic analysis of the power plant profitability. Free market and competition implies uncertainty. Consequently, the energy prices and revenues of power producers are subject to significant fluctuations. The criteria for profit maximization for the stakeholders shall be reformulated. The uncertain and random parameters, such as the ambient temperature, thermal energy consumption, price of energy has to be taken into account. The task assumes a stochastic form. Additional input information is needed in a large volume. As a result, the problem becomes much more complicated and requires new algorithms and software tools to solve it.

This paper proposes a procedure to estimate the average value of these criteria taking into account random nature of the variables and using the historic values of parameters. Besides, we use neural network based algorithm to predict processes in future from the historical data. We capture information on various input parameters, such as temperatures prices etc from the internet. A case study is conducted on a realistic project of the cogeneration biomass power plant (4 MW) and demonstrates the advantages of the stochastic approach. It is concluded that the proposed approach to profitability analysis, developed algorithms, the data capture from Internet, enhanced user friendly interface shall support and enable improved decision making.

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*Author for all correspondence

RECONSIDERATION OF SUPPORTING SCHEME FOR RENEWABLE ENERGY PRODUCERS

Renata Varfolomejeva* (1); Marija Zima-Bočkarjova (2); Antans Sauhats (1); Inga Umbrasko (1); Hasan Coban (1)

(1) RTU, Riga Technical University, Riga, Latvia;
(2) ABB Corporate Research, Switzerland.

Striving to prevent climate changes, global community has established favourable national feed-in support schemes for the renewable electricity. Renewable energy is promoted through these schemes in the whole world and, particularly, in European countries. Frequently, the support is provided by a guaranteed purchase price, which is significantly higher than the market price of electricity.

In Latvia, an Electricity Market Law prescribes that an electricity producer, which operates renewable energy sources, specifically in co-generation mode, may acquire the right to sell all the produced electricity within the framework of the mandatory procurement at a guaranteed purchase price. As a result all the energy, which is produced from renewable energy sources, is sold to the public trader at a guaranteed purchase price, but the public trader sells and buys energy at the market prices, which vary significantly over the time. All electricity consumers pay the difference. The main drawback of the described support scheme is related to the absence of the coordination of power plant (PP) operation with the electricity market price or indirectly, electricity demand.

This paper demonstrate that cooperation of the public trader (PT) and PP operator in the market conditions could minimize additional cost of the support for the consumers and/or provide an additional income to PT and the PP owner. For this purpose, the coalition between PT and PP will be formed (creation of such coalition does not contradict the norms of law). Then, using scenario approach, planning problem of the day-ahead PP operation will be formulated as maximization of the expected total profit over the day taking into account profits of both PT and PP. The production planning can be made using the game theory and Bayes-Laplace criteria. Co-operative game theory and the Shapley value can be applied for the fair additional profit allocation. We demonstrate the advantages of the collaboration by the case study of a small hydro PP, the PT and the co-generation PP. The modification of the schedule of the co-generation power plant to adjust to the electricity market prices and, thus, the electricity demand creates an additional income for the PT.

It is concluded that the support principles of PP have to be reviewed. Proposed stochastic approach, formulation of the objective function and Shapley allocation for the additional revenues can increase the effectiveness of the operation of any kind of PP, provided that the daily power production is controllable, but water or fuel resources are constrained.

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*Author for all correspondence

WATER SAVING POTENTIAL IN A RURAL COMMUNITY IN MEXICO TO REDUCE THE POTABLE WATER CONSUMPTION

G. Gómez-Millán* (1); S. Deegener (1); R. Otterpohl (1)

(1) University of Technology Hamburg-Harburg, Institute of Wastewater Management and Water Protection, Hamburg, Germany.

In order to reach a sustainable future, society must move towards a goal where efficient and appropriate water use is the rule, and not the exception. There are abundant resources for water supply that are available, and not used to their full potential. To contribute and enhance the use of sustainable methods, this project studies the practices related to water and sanitation of a small community in rural Mexico.

The case study takes place in the mountains of Jalisco, Mexico. The rural community, of 180 inhabitants and 100% indigenous (Huichol), subsists under the UN minimum limits of life dignity. The community has latrines that serve the role of public toilets, which are not being used. The inhabitants prefer going into the woods to relief themselves, which causes groundwater pollution; it contaminates agricultural produce, and helps the spread of diseases such as diarrhea and cholera. They have to walk more than two hours each day to the closest stream to get water, and they lack a proper domestic wastewater treatment system.

Different technologies to reduce freshwater consumption have been analyzed. Three technologies have been identified and have been further investigated: harvesting rainwater, reusing greywater, and using Terra Preta Sanitation system, as well as promoting an integrated system with the purpose to enhance and live under a sustainable way with less impact on the environment. These three alternatives technologies are compared next to the traditional practices and the integrated system performance in a cost-benefit analysis. From geological and meteorological data, the rainwater harvesting potential has been determined; greywater reuse, from studying the traditional practices of the inhabitants and the best suitable technology for the users; Terra Preta sanitation, as a method of containment and sanitization of human excreta serves an important role of preventing the spread of diseases, and protecting both environmental and human health.

Interviews with the main identified stakeholders (NGO, government, independent sponsors) took place during a visit to Mexico. The benefits were ranked according to the three pillars of sustainable development (environmental, social and economical pillar).

The system with the greatest beneficial outcomes is offered by the integrated system. The construction cost of an integrated system is around 1220 € per household. This resulted with the community receiving a huge amount of benefits from it.

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*Author for all correspondence

ANTICIPATING THE WORST TROPICAL WEED WATER HYACINTH INVASION BY ASSESSING POPULATION CONTROL AND ECONOMICALLY VIABLE BIOMASS REUSE: A KEY STUDY FOR TRANSFORMING A SCOURGE INTO AN OPPORTUNITY

***Isabelle Perraud-Gaime* *** (1); Ernesto Favela-Torres (2)

(1) UMR IMBE IRD237 CNRS7263; Aix Marseille University, CNRS, IRD, Avignon University, St Jerome Faculty, Av Escadrille Normandie Niemen, case 421, 13397 Marseille, France
(2) Universidad Autónoma Metropolitana Iztapalapa, Biotechnology Department, Av. San Rafael Atlixco 186, CP 09340 México DF.

Water hyacinth *(Eichhornia crassipes)* is a free-floating plant widely recognized as the world's worst aquatic weed. It has become a scourge in many parts of the world, choking waterways, leading to serious problems in navigation, irrigation, recreation and power generation. Eradication is difficult and expensive. Water hyacinth biomass can be used for several purposes. In general, an individual approach is not enough for a sustainable use of this plant. Fresh water hyacinth has been used to remove heavy metals from water bodies and the dried plant is currently used to absorb pollutants from water and soil. An international and multidisciplinary research group integrated by different public and private organizations participated in a research project (LIRIASA), founded by the FONCICyT (UE-Conacyt) to evaluate different strategies of utilization of water hyacinth based on the distribution, composition and age of the plant. Applications related to bioethanol and oligosaccharides production were technically and economically evaluated. The obtained results suggest the development of a biorefinery based on the integral utilization of water hyacinth.

The process consists of controlling the proliferation moving the plant into certain parts of the aquatic environment, in particular, nearby anthropogenic discharges to make full-use of the depurative action of the plant. The proliferation of the biomass is controlled mechanically with floating barriers and frequent harvests. The harvested biomass is separated between roots and aerial parts. Aerial parts are used to produce enzymes and high value-added molecules (patent-pending). They can also be used to produce bioethanol instead of having an energetic supply on site. The rooted part is biochemically not suitable for these applications. Depending on its metal content, it can serve for biogas production, compost production or it can be chemically processed for metal recovery. Due to the high added value of the molecules produced in pharmacology, this whole process might be economically viable. Moreover, the biorefinery can be set up nearby the invaded water bodies being energetically independent and reducing transport and storage costs. Therefore, sustainable use of water hyacinth biomass can represent a valuable solution to face the environmental problems caused by the fast growth and invasion of water hyacinth.

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*Author for all correspondence

OPTIMIZING MEXICO´S POWER GENERATION MIX TO 2050 CONSIDERING EXTERNALITIES AND LIFE CYCLE ASSESSMENT

Edgar R. Sandoval-García* (1); L.E. Urbán-Rivero (2)

(1) CINVESTAV- IPN, México DF, México;
(2) UAM-Azcapotzalco, México DF, México;

Since its beginning, the Mexican electricity sector has been one of the pillars that have supported the domestic economic growth. Today under the global necessity of tracing new routes for sustainable growth and remedying the causes of climate change, the transition from a mix of power generation based on fossil fuels to an enhanced balanced mix becomes extremely important, including conventional and alternate sources of energy. This would provide a mix that can effectively meet the energy requirements of the country in the long term, taking into account the externalities that rebound in the future of resources for future generations.

Considering carbon emissions, use and contamination of water, and energy payback rate for technology type, as well as natural and law restrictions of maximum participation by technology, an optimal electricity generation portfolio was modeling using the Simplex method with weighted objective function.

It is imminent that to fulfill the environmental commitments that the country has in a global way, the government acts in accordance with national energy laws and the society outline routes of sustainable growth, the share increase in the optimal mix of alternative energy sources (such as solar, biomass and geothermal energy), will play a fundamental role. According to the possible combinations obtained from the optimal energy mix, alternative energy sources participation can reach levels of up to 40% in power generation.

The obtained data allows clarity and expands the decision making criteria (beyond the simple criterion of the lowest generation cost) when defining the optimal electricity generation mix. Although it is difficult to prioritize the externalities considered in the study, it is feasible to adjust the mix according to the needs and conditions of the country according to the path towards sustainable development.

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*Author for all correspondence

AVALIAÇÃO DE CICLO DE VIDA NA GESTÃO DE RESÍDUOS SÓLIDOS: ESTUDO DE CASO DA COLETA SELETIVA REALIZADA NO MUNICÍPIO DE SAPUCAIA NO ESTADO DE RIO DE JANEIRO - BRASIL

Rosa Maria L F. Robertson (1) *; Claudio Fernando Mahler (2);
Marcelo Guimarães Araujo (3);

(1) Mestranda, PEC – COPPE – UFRJ, Rio de Janeiro RJ, Brasil;
(2) Professor, PEC – COPPE – UFRJ, Rio de Janeiro RJ, Brasil;
(3) Colaborador, PEC – COPPE – UFRJ, Rio de Janeiro RJ, Brasil;

A dinâmica da urbanização crescente somada à maior facilidade de acesso aos bens de consumo resulta no aumento da quantidade de resíduos sólidos produzidos, trazendo novos desafios para a gestão e gerenciamento destes. Outra característica é que o maior desenvolvimento tecnológico torna a composição dos resíduos mais complexa, devido à grande variedade de substâncias que passam a ser utilizadas e descartadas. Tais dados implicam em novas variáveis para o correto gerenciamento dos resíduos sólidos urbanos.

A Lei 12.305 de 2010, que instituiu a Política Nacional de Resíduos Sólidos veio com a determinação de encerramento de locais de disposição incorreta até agosto de 2014, e estabelecendo a hierarquia para os resíduos sólidos: passando pela prevenção com a redução da produção destes, para a reutilização e reciclagem e por último a disposição em aterro apenas do que for rejeito, cobrando dos municípios a adequação as exigências. A gestão de resíduos sólidos em municípios, em especial os de pequeno porte, ainda não possui uma metodologia definida que possa ser utilizada como diretriz.

O uso da ferramenta de ACV permite que o inventário dos resíduos possa ser realizado e associado o estudo de impactos ambientais para alternativas distintas de tratamento de resíduos possibilitando a comparação entre elas. Seguindo está linha de raciocínio e dentro do contexto descrito a opção foi um programa de *software* que permitisse tal comparação e sua base para a avaliação de impactos é o método reconhecido em que os impactos para o ar, água e solo fossem representados.

O município de Sapucaia está localizado no estado do Rio de Janeiro e tem população de 17.504 hab (2010), com a densidade demográfica de 32,39 hab/km², com produção per capita de resíduos de 0,73 Kg/dia. Os dados primários referentes às coletas foram conseguidos no município. A separação dos resíduos acontece nas residências entre secos e úmidos com sua coleta em dias alternados. Os resíduos da coleta seletiva são direcionados a área de triagem que fica no município e inclui os catadores cadastrados. Os resíduos restantes seguem para o aterro sanitário do município, que é de pequeno porte e possui o Índice de Qualidade do Aterro (IQA) como adequado, estando localizado próximo ao centro da sede do município. Os cenários propostos incluíram o aumento da quantidade de recicláveis para a coleta seletiva e o tratamento biológico do resíduo orgânico antes da disposição em aterro. Os resultados obtidos para os impactos totais foram comparados demonstrando que a eficiência da coleta influi positivamente na redução do impacto seja pela substituição de matéria-prima como pela menor geração de emissões no aterro.

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* Author for all correspondence.

A LOW COST MEMBRANE FITTED IN A SINGLE CHAMBER MICROBIAL FUEL CELL

Giovanni Hernández-Flores (1); Juvencio Galíndez-Mayer (2); Omar Solorza-Feria (1); Elvira Ríos-Leal (1); María T. Ponce-Noyola (1); Fernando J. Esparza-García (1); Tatiana Romero-Castañón (3); Héctor M. Poggi-Varaldo*(1)

(1) CINVESTAV - IPN, México DF, México;
(2) ENCB-IPN, México DF, México;
(3) IIE, Cuernavaca, Morelos, México.

The perfluorosulfonic acid membranes, such as Nafion®, are the typical membrane used in microbial fuel cell (*MFC*) to separate the anode from cathode. Nafion is considered as a proton exchange membrane (*PEM*) and their use in *MFC*s determines the performance and the total cost of the device.

Thereby, the objectives of this research were (*i*) to evaluate the potential of a new organic membrane (*NOM*) based on agar, on a single chamber *MFC* (*SCH-MFC*) and (*ii*) to estimate its cost and compare to that Nafion® 117 (*NF-117*).

The *SCH-MFCs* employed were two horizontal cylinder built in Plexiglas 80 mm long and 57 mm internal diameter. Graphite flakes were used as anodic material and the air-cathode was in direct contact with the winger. The *SCH-MFC* was loaded with a model extract found in the biological hydrogen production from the organic fraction of the municipal solid wastes. A sulfate-reducing inoculum was seeded in the *SCH-MFC* and the membranes tested were *NOM* and *NF-117*. Before to fit to the *SCH-MFC*, the membranes were hydrated. The *SCH-MFC* performance was determined using the polarization curve method, by varying the external resistances and recording both the voltage and the current intensity.

The maximum volumetric powers ($P_{V,max}$) were 2120 and 14,181 mW/m^3 for *NOM* and *NF*-117, respectively, whereas the internal resistances (R_{int}) were 192 and 110 Ω using *NOM* and *NF-117*, respectively. The $P_{V,max}$ delivered using *NOM* was ~15% of that with *NF-117*, and although the R_{int} value was twice the value of the *NF-117*, this relatively low value, was encouraging. On the other hand, analyzing the *SCH-MFC* regarding the costs, the cost ratio *NOM*/*NF-117* was quite low, (US$ 14/m^2)/(US$ 1733/m^2) ~ 1/120 ~ 1%. According to this attractive point of view, it is possible to sacrificing some power output of the *SCH-MFC* (85%) but achieving outstanding savings on membrane costs (99%).

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*Author for all correspondence

ECONOMIC EVALUATION OF A BIOREFINERY FOR BIOFUELS PRODUCTION FROM WHEAT STRAW WITH A CONSOLIDATED BIOPROCESS APPROACH

Carlos E. Molina-Guerrero (1); Arturo Sánchez (2); Analí Soto (2); ***Idania Valdez Vazquez****(1)

(1) Depto. de Ing. Amb., DICIVA, Universidad de Guanajuato, Irapuato, México.
*e-mail:valdez_idania@yahoo.com
(2) CINVESTAV- IPN, Unidad de Ingeniería Avanzada, Guadalajara, Jal. México.

A biorefinery is defined as a facility that integrates different biomass conversion processes and equipment to produce biofuels, biomaterials, heat and/or power. In a lignocellulosic biorefineries, the carbohydrate fraction (hemicellulose and cellulose) is separated and fermented into useful products such as gaseous and/or liquid biofuels. Conventional biochemical biorefineries use saccharification to perform the cellulose hydrolysis, and then fermentation from the obtained simple sugars. This biorefinery configuration has high operating costs and relative low yields (based on the feedstock processed). As an alternative, the consolidated bioprocess (CBP) integrates the enzyme production, saccharification and fermentation into a single unit which could decrease the operating cost and improve the yield. The objective of this work was to design the plant flow sheet of non-conventional biorefinery using wheat straw as feedstock to obtain butanol, hydrogen and methane using microbial consortia with a CBP approach. The conceptual design, mass and energy balances were performed using SuperPro Designer v8.5. The feedstock composition was (in dry basis) of 44.8% of cellulose, 26.6% of hemicellulose and 6.8% of lignin. The plant capacity was defined in 1000 ton dry basis/day. The main processing stages were (*i*) fibers hydration, (*ii*) hydrogen production from xylan, (*iii*) butanol and acetone production from cellulose, (*iv*) methane production from the organic fatty acids coming from the H_2-producing reactor, (v) and downstream processes. The operating condition for each reactor (temperature, solid load, dilution rate and inoculum) was based on experimental results obtained in our group and literature. The butanol production plant had a capacity of 23 X 10^3 Ton/yr. In addition, hydrogen (5.5 X 10^3 Ton/yr), methane (25.5 X 10^3 Ton/yr) and acetone (11.5 X 10^3 Ton/yr) were also produced. The total capital cost for this plant was \$168.09 X 10^6. The butanol production cost was estimated in \$2.12/kg which is competitive with the synthetic butanol cost. The payback time was estimated in 4.20 years, and the net operating cost was estimated in 44,187,753 \$/yr. The simulation showed that the production cost was very sensitive to the biological reactor size (H_2- and butanol-producing reactors), therefore, the selection of the microbial consortia to be used is critical to further reduce the production cost since these should be very efficient to degrade the lignocellulosic substrate and ferment it in a short period time.

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*Author for all correspondence

LIFE CYCLE ASSESSMENT (LCA) OF A FOUR-STAGE BIOREFINERY MODEL

Carlos Escamilla-Alvarado (1); Héctor M. Poggi-Varaldo*(1); Teresa Ponce-Noyola (1); Elvira Ríos-Leal (1); Fernando Esparza-García (1); Josefina Barrera-Cortés (1); Jaime García-Mena (1); Ireri Robles-González (2); Noemi Rinderknecht-Seijas (3)

(1) CINVESTAV- IPN, México DF, México;
(2) Nova Universitas, Oaxaca, México;
(3) ESIQIE-IPN, México DF, México.

A biorefinery model was investigated under a life cycle assessment (LCA) approach. The goal and scope were to perform the LCA of a novel four stages biorefinery model H-M-Z-S that converts the organic fraction of municipal solid waste (OFMSW) into bioenergy (hydrogen and methane) and bioproducts (holocellulolytic enzymes and hydrolysates).The functional unit was 1 ton OFMSW 20%TS fed to hydrogen producing stage (H-stage).Production data and series-parallel configuration of the biorefinery were obtained from lab-scale experiments with extrapolations and assumptions typical of scale-up of industrial processes. Inventory data and processes were acquired and modified from SimaPro v7.1.8 libraries. The characterization of the impacts was effected using method CML 2001.

Three iterative steps were followed: mass balance using extrapolated experimental data, energy balance based on libraries information and own calculations, and impact assessment of 6 categories. Due to the easier digestibility of the fermented solids (FS) produced in H-stage, these were distributed as substrate to the other stages: 40% to methane producing stage (M-stage), 40% to enzyme production stage (Z-stage), 20% to saccharification stage (S-stage).The energy balance showed that H-stage provided a small amount of clean electricity through PEM fuel cell, whereas M-stage provided a greater amount of heat and power through a combined cycle plant. In terms of power the biorefinery was self-sustainable, requiring 73% of the electricity produced. However, there was a need of energy for heating several biorefinery processes: up to 45% of heating requirements had to be supplied by burning natural gas in a heat boiler. Overall, the net energetic balance was near zero.

Concerning environmental analysis, the CO_2 balance was negative mainly due to the fixation of CO_2 from air into the biomass, meaning that the biorefinery saved GHG from environment. This was reflected in the global warming potential category, which had savings of -130 kg $CO_{2,eq}$. The main impact on abiotic resource depletion was ascribed to natural gas used to supply the heat deficit in Z and S stages, whereas the ozone layer depletion was affected principally by the transport of the natural gas in pipeline. Regarding acidification, eutrophication and photochemical oxidation, the major impacts were observed due to the biogas and energy production in M-stage. The final use of the bioproducts is as replacement of similar commodities produced by conventional technologies.

More data need to be compiled in order to get more realistic results in the Mexican context, yet these results are a benchmark that will help to improve the biorefinery model.

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*Author for all correspondence

PRELIMINARY METHODOLOGY OF ENVIRONMENTAL EVALUATION, BASED ON WATER CYCLE, APPLIED IN SMALL TOWNS IN THE NORTH OF THE STATE OF PARANÁ

Renata Romagnolli (1); Fernando Fernandes (2); ***Deize Dias Lopes**** (2)

(1) Dep. Arquitetura/CTU/UEL,Londrina- PR, Brasil;
(2) Dep. Construção Civil/CTU/UEL,Londrina- PR, Brasil;

Although there's a diversity of methodological procedures for the execution of urban planning and directives, such procedures show little information about the criteria considered to evaluate the environment. The use of formal strategy to evaluate the area where one's planning to build may create objective conditions for the exchange of experiences and assist the planners and decision makers. Based on such considerations, this study had as its main objective to propose a methodology to aid in environmental evaluation with emphasis on the cycle of water and basic sanitation. The materials utilized in the elaboration of this methodology were extracted from studies, researches and bibliographies about the proposed theme and also from the consultation of experts on sanitation. The study was divided into eight thematic axes: socio-economic aspects, area rural and urban, physical aspects of the source of supply, water supply and sewage systems, drainage urban system and solid waste. As a result of this methodology, environmental evaluation tables were created and were applied in case studies in small towns in the north of the state of Paraná. These towns were chosen due to their lack of financial resources and qualified labor for the implementation of techniques that aim at preserving and conserving their environment. Data related to the application of the material were obtained from city halls, technicians responsible for the water supply system in the towns studied in this work, local companies and through in situ visits. This work was able to evidence the tendencies towards the sustainability of sanitation systems, to point out the sectors that are in need of courses of actions and investments concerning the preservation of the environment and above all it proved capable of aiding in the elaboration of environmental studies and evaluations in an easy, practical and efficient manner.

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*Author for all correspondence

SUSTENTABILIDAD DEL PLANETA Y DESARROLLO SOSTENIBLE EN LA CONSERVACION DEL MEDIO AMBIENTE

Germán Domínguez-Bocanegra* (1)

(1) Tecnológico de Estudios Superiores de Ecatepec, División de Contabilidad.

Desde el siglo pasado el mundo se encuentra preocupado por la situación del calentamiento global, provocado por la elevada contaminación del planeta, detonado principalmente por la industria, por tal motivo y después de la segunda guerra mundial en 1946 en Ginebra Suiza nace la Organización Internacional de Normalización (ISO), la cual busca la estandarización a nivel internacional, por lo que se convirtió en el organismo internacional para el establecimiento de normas, trabajando con cuerpos nacionales de normalización, departamentos de gobierno y representantes de la industria principalmente corporaciones transnacionales; todas las normas desarrolladas por ISO son voluntarias, por consenso y del sector privado, al ser una institución no gubernamental, no tiene autoridad para imponer sus normas en ningún país u organización (Martha Blanco Cordero, 2004).

El principal objetivo de las normas ISO es que buscan la sustentabilidad del planeta ya que el desarrollo sostenible se ha convertido en el principal concepto de investigación y estudio relacionado con la conservación del medio ambiente. La Producción más Limpia es una estrategia ambiental preventiva integrada que se aplica a los procesos, productos y servicios a fin de aumentar la eficiencia y reducir los riesgos para los seres humanos y el ambiente.

En la actualidad el término sustentabilidad se ha convertido en un nuevo paradigma, para la administración y dirección de una empresa, tanto en el sector público y privada, desde hace décadas los empresarios buscan implementar a través de un grupo de profesionales conformados por administradores, ingenieros, contadores y expertos en mercadotecnia, nuevas estrategias que le permitan aumentar sus ganancias y en muchos de los casos subsistir en un mercado cada vez más globalizado y competitivo, por tal motivo es importante que los empresarios y los dirigentes de las empresas consideren una serie de elementos que permitan una nueva forma de gestión y ejecutar negocios, en la cual la empresa, establezca las operaciones que le permitan lograr sustentabilidad de los productos y servicios que ofrecen.

En el presente estudio se analizaron los diferentes aspectos organizacionales para obtener una mayor sustentabilidad del planeta y lograr el desarrollo sostenible en beneficio del medio ambiente y a la vez contar con ventajas competitivas para el consumidor al momento de comercializar productos y servicios en un mercado, analizando los principales productos funcionales, económicos y nobles para el medio ambiente y evitar así una mayor contaminación.

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*Author for all correspondence

INCUBADORA DE NEGOCIOS EN EL TESE COMO CATALIZADOR EN LA TRANSFERENCIA EN LAS PYMES DEL MUNICIPIO DE ECATEPEC

Germán Domínguez-Bocanegra* (1); Daniel Morales-Chong (1);
Katulli C.Corral-Miranda (1); Karla I. Tellez-Lopéz(1); Fernando Cázares-Padrón (1).

(1) TESE División de Contabilidad.Edo. deMéx,

En el presente estudio se analizó la importancia que tiene la incubadora de negocios del Tecnológico de Estudios Superiores de Ecatepec (TESE), en el proceso de transferencia de tecnología, la cual puede ser la encargada de asesorar y promover el desarrollo de nuevas tecnologías a través de estudiantes, académicos y empresas establecidas en el Estado de México y por ende del país.

El principal objetivo de esta investigación fue realizar un análisis de los componentes con los que cuenta la incubadora de negocios del TESE, para la implementación de un Centro de Transferencia de Tecnología, que permita brindar de manera óptima la asesoría y el apoyo a estudiantes, académicos y empresas del Estado de México en la obtención de registros y patentes que permitiría fomentar y aprovechar al máximo el desarrollo de nuevas tecnologías.

En la actualidad podemos comprobar la importancia que tiene la creación de las incubadoras de negocios dentro de las universidades ya que se han convertido en un motor de suma importancia para el Estado de México y a nivel nacional; no solo porque puede ofrecer consultorías para la creación de nuevos negocios y asesoría en rubros administrativos y técnicos para las empresas ya establecidas para la generación de nuevos empleos en el país. Con el transcurso del tiempo y la experiencia adquirida dentro de la incubadora del TESE los funcionarios, consultores y docentes de distintas áreas del conocimiento, analizaron que la Incubadora puede participar como un catalizador en la transferencia de tecnología.

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*Author for all correspondence

NANOTECNOLOGÍA SUSTENTABLE A TRAVÉS DEL ANÁLISIS DE CICLO DE VIDA

Elsa Gabriela Alvarado Díaz* (1)

(1) Ciclo de Vida en el Centro de Análisis de Ciclo de Vida y Diseño Sustentable (CADIS)

Las propiedades únicas y novedosas de los nanomateriales han sido empleadas por la ciencia y la industria en el desarrollo de nuevos productos y tecnologías. En los últimos diez años, el gobierno mexicano ha considerado la nanotecnología como un sector estratégico dentro de los últimos Planes Nacionales de Desarrollo, que ha promovido la implementación de políticas públicas para la transferencia de las nanotecnologías en los laboratorios hacia plantas de producción industrial y finalmente al mercado. Esto también ha promovido la colaboración entre instituciones públicas y privadas como centros de investigación, universidades y empresas. Sin embargo, las capacidades únicas de los nanomateriales se acompañan de incertidumbres respecto a sus impactos potenciales al ser humano y medio ambiente, que son difíciles de cuantificar debido a la baja comprensión de su destino y exposición. El Análisis de Ciclo de Vida (ACV) es una metodología para cuantificar los impactos ambientales de los productos y compararlos con otras alternativas.

Este caso de estudio se centra en la evaluación ambiental de la producción de aditivos de plástico con nanopartículas de Óxido de Zinc (ZnO) incorporadas como material de relleno. Además, el estudio refleja, desde la perspectiva mexicana, cómo el ACV es útil para la toma de decisiones en la gestión ambiental de las empresas y en el diseño de productos en las primeras etapas de desarrollo.

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*Author for all correspondence

ANÁLISIS DE CICLO DE VIDA (ACV) DE BIOFUNGICIDAS

Monserrat Coatl Popoca* (1)

(1) Ciclo de Vida en el Centro de Análisis de Ciclo de Vida y Diseño Sustentable (CADIS)

De acuerdo con datos de las Naciones Unidas cerca de 800 millones de personas alrededor del mundo no disponen de alimentos suficientes para una vida sana y activa. Desde hace varios años, una forma de incrementar el rendimiento en la producción agrícola ha sido mediante el uso de pesticidas, los cuales tiene repercusiones negativas en la salud del ser humano y en los ecosistemas. En México, la creciente demanda de alimentos y las exigencias del mercado global entorno a la producción sustentable hacen necesaria la adopción de nuevas técnicas para el control de plagas.

Por ello, el objetivo de este trabajo fue realizar un Análisis de Ciclo de Vida (ACV) de biofungicidas elaborados a base de *Bacillus Subtilis*. Se analizaron dos formulaciones: polvo humectable y suspensión acuosa. Se evaluaron cuatro escenarios considerando el uso de los productos para el control de plagas en cultivos de vid y chile. Mediante el ACV fue posible determinar las etapas del ciclo de vida que tienen mayor contribución en el desempeño ambiental de los biofungicidas. Se concluyó que el biofungicida en suspensión acuosa presenta un menor impacto ambiental en comparación con la formulación en polvo, a pesar de requerirse en promedio un 57% más de producto para tratar la misma extensión de cultivo. Finalmente, se exponen las acciones propuestas para minimizar el impacto ambiental en las áreas de oportunidad detectadas.

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*Author for all correspondence

LIFE CYCLE ASSESSMENT: A TECHNIQUE PROVIDING A NEW WEALTH OF INFORMATION TO SUPPORT THE SUSTAINABILITY-RELATED DECISION MAKING (SOCIAL DIMENSION APPROACH)

Alejandro de Jesús Padilla* (1); Juan Morgan-Sagastume (1);
Adalberto Noyola-Robles (1); L. Patricia Güereca-Hernández (1)

(1) Instituto de Ingeniería, Universidad Nacional Autónoma de México, México DF, México;

Being sustainability a global concept, that covers moreover present and future generations, inevitably calls for a system-wide analysis. Such a system perspective is at the core of the life cycle approach, which can provide a valuable support in sustainability evaluations and decision making. In fact, the main argument for a life cycle view is to prevent false optimization and wrong choices, like the burden shifting within or between each scope: environment, economy and society; or to the future. However assessment are required in order to quantified the achievements in sustainability.

Despite the great number of methods and approaches developed, the broad notion of sustainability is still not sufficiently reflected in the existing assessment frameworks and tools. In fact, each of them addresses only specific aspects of sustainability, and thus fails in describing its main characteristics, i.e., complexity (multi-disciplinary knowledge, multi-spatial and time scales), uncertainty (many variables, poor information, and data availability), and urgency (urgency of processes, such as climate change). Keeping the life cycle approach and method as a reference, an integration framework for sustainability has been proposed, namely Life Cycle Sustainability Analysis (LCSA).

With respect to LCA, it deepens the scope of mechanisms, by means of including also physical, social, economic, cultural, institutional and political ones. Moreover, it broadens the scope of indicators and the object of the analysis, shifting from individual product systems to sectors, basket of commodities, markets, or whole economies. This work present one dimension of LCSA, it describes an overview of social LCA historical development from the 1990s, discusses why S-LCA is of interest to businesses and society, describes the methodology, and contrasts it with other social assessment techniques. A presentation of advancement in case studies development will help convey the actual state of the art of S-LCA.

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*Author for all correspondence

Chapter 3.
Risk Assessment and Environmental Impact

EFECTO DE LA EXPLOTACIÓN AGROPECUARIA SOBRE LAS AGUAS RESIDUALES DEL VALLE DEL YAQUI

Karina Coronado-Apodaca (1); Fernanda Navarro-Aguirre (1); Edna Meza-Escalante (1); Pablo Gortáres-Moroyoqui (2) Gabriela Ulloa-Mercado; ***Denisse Serrano-Palacios**** (1).

(1) ITSON, Ciencias del Agua y Medio Ambiente, Ciudad Obregón, México;
(2) ITSON, Biotecnología y Ciencias Alimentarias, Ciudad Obregón, México.

Las aguas residuales generadas por los diferentes sectores productivos, cuentan con características propias de la fuente de origen, las cuales determinan el nivel de afectación a los cuerpos receptores. El objetivo de este trabajo fue realizar muestreos y caracterización de las aguas residuales producidas en el Valle del Yaqui. El estudio se llevó a cabo al sur de Cd. Obregón, Sonora, donde podemos ubicar al Valle del Yaqui y limitándose a un área de estudio de 288 km^2. El área fue dividida en tres zonas, la primera se centró en muestras de agua residual mixtas, es decir, donde convergen aguas residuales agrícolas y pecuarias, la segunda contenía aguas residuales agrícolas y la tercera aguas residuales de fuentes pecuarias, esto con la finalidad de comparar la influencia de cada sector en la concentración de las aguas residuales producidas y descargas en bienes nacionales. Se tomaron 8 muestras de cada zona, 4 de ellas antes de entrar a la actividad productiva, las cuales correspondían al agua proveniente de los canales de distribución y las otras 4 muestras, se tomaron en puntos determinados después de que las aguas son explotadas en los diferentes sectores. Cabe mencionar, que cada muestra se realiza de forma integrada con la finalidad de realizar un muestreo más representativo. Los análisis evaluados fueron la DQO, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$ $P\text{-}PO_4^{3-}$, $N\text{-}NH_4^+$, Sólidos Totales (ST) y Sólidos Volátiles Totales (SVT).

Los resultados obtenidos en este estudio, muestran que el agua residual más contaminada, es la que tiene origen en el sector pecuario debido a presencia de granjas (porcinas y avícolas), ya que al ser comparadas las concentraciones obtenidas con la norma de descarga en bienes nacionales NOOM-001 SEMARNAT-1996, sobrepasa los límites máximos permisibles en tres de los parámetros analizados: 450 mg DQO/L, 30 mg $P\text{-}PO_4^{3-}$/L y 4 g ST/L. Por lo cual, concluimos que el efecto de estas aguas sobre las aguas residuales agrícolas, pueden ser representativas. Cabe señalar, que existe la posibilidad de que las concentraciones de algunos de los parámetros analizados presenten un efecto de dilución al irse mezclado en su recorrido con otras fuentes de descargas de aguas, o incluso una biodegradación durante su largo recorrido hasta la zona de descarga en el mar de Cortez. Se espera en trabajos posteriores, evaluar los parámetros en el punto final de descarga, pudiendo inferir a ciencia cierta si ocurre o no una auto purificación de las aguas residuales.

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*Author for all correspondence

PHYSICOCHEMICAL EVALUATION OF SOILS AND SEDIMENTS NEAR MINING OPERATIONS IN ASIENTOS, AGUASCALIENTES

Kerry N. Mitchell (1); Magdalena S. Ramos-Gómez (1); Alma L. Guerrero-Barrera (1); Laura Yamamoto-Flores (1); Frederic Thalasso-Siret (2);
Francisco J. Avelar-González**(*1)

(1) UNIVERSIDAD AUTÓNOMA DE AGUASCALIENTES, Aguascalientes, México;
(2) CINVESTAV- IPN, México DF, México;

Soils and sediments near mining activities are frequently direct receptors of mining waste and as such, represent a potential environmental risk. The study of this potential risk should include not only total metal quantification, but also the study of physicochemical characteristics that play an important role in their solubility, mobility and toxicity in affected sites. Thus, the aim of this study was to evaluate total metal concentrations and the physicochemical characteristics that may affect metal mobility in nearby soils and sediments of the Piedras Negras River in Asientos, Aguascalientes; an area recently affected by a substantial increase in mining activity. Soil and sediment sampling were carried out once during the dry season and once during the rainy season. Soil moisture and organic matter were initially determined. After pretreatment which consisted in drying and sieving through a 2mm mesh, cation exchange capacity and total metal content were determined. The pH, redox potential, soluble salts were determined in a 1:10 dilution soil:water.

Sediments from all sites were classified as *very polluted* according to guidelines established by the USEPA, and above the maximum permitted limits for commercial, residential and farm use according to the NOM-147-SEMARNAT/SSA1-2004 in at least 2 metals, in select cases, more than 200 times guideline values. 70% of soils from the 10 sample sites were classified in the same way. Though neutral conditions were predominant, 20% of samples from both substrates were moderately acidic in nature (<6.5). 80% of soil and sediment samples presented highly reductive conditions (-100 to -300mv). Both substrates had medium to low (<25 Cmol/kg) cation exchange capacities. Salinity measured as conductivity in µS/cm in both substrates were found to be normal, though values obtained in sediment samples were twice as high as those obtained in soil samples. Differences between soil and sediment samples were found in values obtained for soluble salts. Nitrate concentrations were 1.5 times higher in sediments. Sulfides and sulfates had 7 and 15 times higher concentrations respectively in sediments.

It was concluded that the sample area was affected by nearby mining activities. High concentration of metals and soluble salts in sediment samples indicate that the river is a direct receptor of mine drainage; whilst soils appear to be affected by wind carried mine tailings. Current physicochemical properties may favor mobility, and thus represent an environmental toxicity risk.

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*Author for all correspondence

ETHANOL CONTAMINATION IN SOME WATER BODIES IN NORTHERN QUINTANA ROO AND ITS POSSIBLE RELATION TO ALCOHOL CONSUMPTION BY HUMANS

Rodolfo Reyna-Velarde* (1); Rosa E. Pérez-Hernández (2); Zenayda A. Poot-Luján (2); Elvira Ríos-Leal (3); Diana S. Acosta-Ramírez (3)

(1) Universidad Mexiquense del Bicentenario, Unidad de Estudios Superiores Tultitlán, Tultitlán Méx.
(2) Universidad Politécnica de Quintana Roo, Cancún QR, México
(3) CINVESTAV-IPN, México D.F. México

From the beginning of history, human society has consumed alcohol to celebrate or to perform religious ceremonies, pleasure or recreation. Also, from time immemorial are known adverse effects of consumption of such beverages. In recent years the alcohol consumption has increased exponentially, largely due to socio-cultural and economic conditions. The National Health and Nutrition Survey 2012 noted that Mexico´s alcohol users increased by 34.6 % between 2006 and 2012; lately, in almost all national statistics show that alcohol is the most commonly used drug by young people, including children. Since Quintana Roo is a largely-preferred touristic zone, with 14,000,000 visitors each year, there is the possibility that water bodies could be contaminated with ethanol coming from human activities, even touristic or local ones. Ethanol could be introduced to water bodies like sinkholes (called *cenotes*), channels and lagoons, by mean of visitors urine, excrement, sweat or breathe, and by mean of spill residues of alcoholic beverages.

The aim of this work was to evaluate pollution due to ethanol in some water bodies of northern Mexican state of Quintana Roo, including Cancun hotel zone, and to explore possible relationship with human alcohol consumption in two different seasons.

Two samplings were performed in five sinkholes and five points bordering Nichupté Lagoon-system along Cancun´s hotel zone. The samples were taken in two different touristic seasons: January (low-season) and March-April (high-season) 2013. Ethanol concentration in samples was evaluated by Flame Injection Detector-Gas Chromatography (FID-GC). At the same time, an AUDIT-modified questionnaire was applied to visitors and people near the examined water bodies, to have some knowledge about the alcohol-consumption and dispose manners.

Ethanol was detected in three sinkholes and all-five points Cancun´s hotel zone, particularly in high-season samples, with concentration values in range of 0.0014 to 4.03 mg L^{-1}. On the other hand, questionnaire results show that about 80 % of the respondents answered that just spill the alcoholic-beverages residues near to the water bodies, and about 70 % answered that had consumed alcohol before entering the water body. There are no law-restrictions about alcohol consumption near or into the water bodies in Mexico.

Our results show that ethanol contamination in some Quintana Roo water bodies could be due to human alcoholic-beverages consumption, and this is potentially dangerous to native organisms and regional environmental balance.

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*Author for all correspondence

TOLERANCE TO STRESS OF *Vallisneria americana* BY ENRICHMENT WITH IONIC SPECIES OF NITROGEN

V. Ruiz-Carrera*** (1); A.J. Sánchez (1); E. Ríos-Leal, E. (2); R. Rodríguez-Vázquez (2)

(1) UJAT-CICART, Villahermosa, Tabasco. México.
(2) Departamento de Biotecnología y Bioingeniería, CINVESTAV-IPN, D.F., México.

Vallisneria Americana is the emblematic submerged macrophyte in wetlands of North America with populations that tend to decline or disappear by enrichment of N. To differentiate tolerance markers associated with that produced by the increase of the total N concentration and N ionic in the aqueous medium; their effects were evaluated on plants *in vitro* culture. The plants developed from seed, in artificial N-free medium hard water and two-phase autotrophic condition.

The experiment was randomized with bifactorial arrangement of four concentrations of total N (500 to 2000 $\mu g\ L^{-1}$) and three sources of ion N (NH_4, NO_3^- and NH_4^+: NO_3^-), five replicas per treatment and a control of natural water (174 $\mu g\ L^{-1}$ of N). Total biomass (% dry weight), and NH_4 (GC), NO_2 and NO_3 (HPLC) contents were determined in tissue of leaves and roots lyophilized. The NH_4 was not detected in any tissue.

Stress tolerance was estimated based on symptoms of senescence, and the endogenous contents ($\mu g\ g^{-1}$) and accumulation ($\mu mol\ g^{-1}$) of N. Both parameters showed significant variations by the supply of N, N concentration, their interaction and the control. *Vallisneria americana* accumulated NO_3-N and NH_4:NO_3 in roots and leaves at all concentrations of total N ($p < 0.05$). Accumulation exhibited a greater proportion of high significances ($p < 0.0001$) compared to the content.

In vitro approach, the tolerance of *V. Americana* N ions stress was determined by its ability to assimilate NH_4 and accumulate NO_2 and NO_3.

*Author for all correspondence

ANALYSIS OF SOIL CONTAMINATION AT THE FORMER WOOD TREATING PLANT

Roman Marecik * *(1)*; Agnieszka Wolniewicz (2); Agnieszka Piotrowska-Cyplik (3); Jakub Czarny (4); Łukasz Chrzanowski (5); Zuzanna Szczepaniak (2); Justyna Staninska (1); Paweł Cyplik (1)

(1) Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences, Wojska Polskiego 48, 60-627 Poznań, Poland;
(2) PROTE Technologies for our Environment, Ltd. Dziadoszańska 10, 61-248 Poznań Poland.
(3) Institute of Food Technology of Plant Origin, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland;
(4) Institute of Forensic Genetics, Al. Mickiewicza 3/4, 85-071 Bydgoszcz, Poland
(5) Institute of Chemical Technology and Engineering, Poznań University of Technology, Pl. M. Skłodowskiej-Curie 2, 60-965 Poznań, Poland;

For the wood impregnation creosote was used, i.e. an oily mixture of liquid and volatile organic compounds mainly from gas and coking plants where creosote components such as benzene, ethylbenzene, toluene, xylenes, phenol, pyridine, and polycyclic aromatic hydrocarbons were by-products of the anaerobic process of distillation coal. Such a mixture of those hydrocarbons was supplemented by aliphatic hydrocarbons and coal derivatives in the form of oil and waste oils to increase liquidity of creosote. Aliphatic hydrocarbons and coal derivatives in the form of oil and waste oils were added to a hydrocarbons mixture to increase the liquidity of creosote. At the time of a maximum development of the plant about 150 people were hired there and the volume of production reached 1 million sleepers annually (more than 100 m3 of wood). In 2009 we started the land rehabilitation after the plant was closed. Most of the analyzed soil samples showed a high content of organic substances. The following indicators were examined: the content of volatile aromatic hydrocarbons BTEX, the sum of the chlorophenols, the sum of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), the content of mineral oils (hydrocarbons C12 - C35). All the indicators in the case of most of the samples reported a significant excess of accepted values BTEX concentration ranged from 0.85 to 76.60 0 mg/kg dm. The identified content of chlorophenols varied from 3.0 to 9156.5 mg/kg dm The content of mineral oils (hydrocarbons C12 - C35) in the tested samples ranged from 4.1 to 42754 mg/kg dm. Polycyclic aromatic hydrocarbons (PAHs) were determined in a wide range from 2.0 to 231,0 mg / kg dm. The content of chlorinated hydrocarbons ranged from 0.02 to 28.5 mg / kg dm.

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*Author for all correspondence

PRELIMINARY METHODOLOGY OF ENVIRONMENTAL EVALUATION, BASED ON WATER CYCLE, APPLIED IN SMALL TOWNS IN THE NORTH OF THE STATE OF PARANÁ

Renata Romagnolli (1); Fernando Fernandes (2); ***Deize Dias Lopes**** (2);

(1) Dep. Arquitetura/CTU/UEL,Londrina- PR, Brasil;
(2) Dep. Construção Civil/CTU/UEL,Londrina- PR, Brasil;

Although there's a diversity of methodological procedures for the execution of urban planning and directives, such procedures show little information about the criteria considered to evaluate the environment. The use of formal strategy to evaluate the area where one's planning to build may create objective conditions for the exchange of experiences and assist the planners and decision makers.

Based on such considerations, this study had as its main objective to propose a methodology to aid in environmental evaluation with emphasis on the cycle of water and basic sanitation. The materials utilized in the elaboration of this methodology were extracted from studies, researches and bibliographies about the proposed theme and also from the consultation of experts on sanitation. The study was divided into eight thematic axes: socio-economic aspects, area rural and urban, physical aspects of the source of supply, water supply and sewage systems, drainage urban system and solid waste.

As a result of this methodology, environmental evaluation tables were created and were applied in case studies in small towns in the north of the state of Paraná. These towns were chosen due to their lack of financial resources and qualified labor for the implementation of techniques that aim at preserving and conserving their environment. Data related to the application of the material were obtained from city halls, technicians responsible for the water supply system in the towns studied in this work, local companies and through in situ visits. This work was able to evidence the tendencies towards the sustainability of sanitation systems, to point out the sectors that are in need of courses of actions and investments concerning the preservation of the environment and above all it proved capable of aiding in the elaboration of environmental studies and evaluations in an easy, practical and efficient manner.

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*Author for all correspondence

EVALUACIÓN DE DAÑO GENOTÓXICO Y NEUROTÓXICO EN POBLACIÓN EXPUESTA A FLUORURO Y ARSÉNICO A TRAVÉS DE AGUA DE CONSUMO

Jessica Cordero-Arreola(1); Diana Rocha-Amador(2); Yolanda Alcaraz-Contreras(2);Raúl Morales-Villegas(3);Melissa Bocanegra-Salazar(4); Dania López-Guzmán*(1)

(1) Facultad de Ciencias Químicas, Universidad de Juárez del Estado de Durango. Durango, México.
(2) Departamento de Farmacia. División de Ciencias Naturales y Exactas. Universidad de Guanajuato. México.
(3) CIACYT-Medicina. Universidad Autónoma de San Luis Potosí. México.
(4)Facultad de Ciencias Forestales. Universidad Juárez del Estado de Durango. Durango. México.

La presencia natural de contaminantes como el fluoruro (F^-) y el arsénico (As) en agua de consumo, cuyo origen es principalmente subterráneo es de los principales problemas de salud pública a nivel mundial, llegando a afectar incluso varios estados de la República Mexicana, entre ellos Durango y Guanajuato. Debido a los efectos en salud que se han asociado a estos contaminantes, el objetivo del presente estudio pre-experimental de tipo transversal analítico fue evaluar algunos de los efectos en salud enresidentes de los estados de Durango y Guanajuato expuestos a F^- y As mediante agua de consumo humano. Se determinaron concentraciones de F^- mediante potenciometría con electrodo ion selectivo y el análisis de As se realizó por espectrometría de absorción atómica mediante generador de hidruros (EAA-GH), posteriormente se evaluó coeficiente intelectual total (CI) en los participantes mediante el uso de la prueba Beta III y se evaluó el daño genotóxico mediante la técnica de micronúcleos (MN) en células epiteliales de mucosa oral.

Nuestro trabajo arrojo como resultado una importante exposición ambiental, ya que se encontró que la población de Durango está expuesta a concentraciones de 4.11 mg F^-/L (3.44-4.67 mgF^-/L) y 56 µgAs/L (27.9-68.5 µgAs/L), mientras que la población de Guanajuato está expuesta a concentraciones de 5.81 mg F^-/L (4.71-6.65 mg F^-/L) y 26.35 µgAs/L (19.4-40.9 µgAs/L). De la misma manera en cuanto a la evaluación de CI de los participantes se obtuvo como resultado que el 100% de los individuos evaluados obtuvieron valores de CI total por debajo del puntaje promedio. En cuanto a la presencia de MN, estos se observaron con una media de 3.75 en la población de Durango y 2.35 en la población de Guanajuato.

Estos resultados evidencian que pese a que existen instituciones tanto nacionales como internacionales (Secretaria de Salud y Organización Mundial de la Salud, respectivamente) que sugieren concentraciones optimas tanto para F^- como para As en agua de consumo, estas se encuentran por encima de lo recomendado, lo cual puede representan futuras afectaciones a la salud en poblaciones expuestas de manera constante, sin embargo se considera importante seguir investigando el tema e informar a las autoridades, para evitar que la gravedad del daño sea mayor, pudiendo relacionarse incluso como el origen de enfermedades como cáncer.

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*Autor para correspondencia

RISK ASESSMENT FOR *Cryptosporidium* AND *Giardia* IN WATER, AIR, SOIL AND VEGETABLES FROM A NATIVE COMMUNITY IN MEXICO

Ana P. Balderrama-Carmona (1); Pablo Gortáres-Moroyoqui* (1);
Cristóbal Chaidez- Quiroz (2); Luis H. Álvarez-Valencia (1,3)

(1) ITSON, Cd. Obregón, Sonora, México;
(2) CIAD, Culiacán, Sinaloa, México;
(3) UANL, San Nicolás de los Garza, NL, México.

Cryptosporidium oocysts and *Giardia* cysts are ubiquitous parasites in the environment that produces gastrointestinal illness. Quantitative microbial risk assessment (QMRA) is an approach to determine the risks for pathogenic microorganism. Developed countries stated a risk for infective parasites in drinking water of $1x10^{-4}$ per year. Population in Yaqui native territories in Sonora, México have water of uncertain quality. In Pótam the water drawn from the local well is used for drinking and does all the domestic activities this implicates an indirect pollution of food and soil. The goal for this study was to perform risk assessment analysis for *Cryptosporidium* and *Giardia* (oo) cysts in well water, soil, air dust, and vegetables in Pótam, Sonora, México.

Environmental samples of water, soil, air, and vegetables were collected during 18 months (September 2009 through March 2011) in Pótam, Sonora, México. *Cryptosporidium* and *Giardia* (oo) cysts concentrations in all environmental samples were identified by fluorescent antibody procedure. The QMRA assumptions considered to state people exposure and an exponential dose-response model approach.

In well water all the samples were positives and in the rest matrixes were detected (oo) cysts with different occurrences. For all the matrixes the exposure increases linearly with increasing consumption. The minimal risks of giardiasis due to well water drinking (8,400:10000) were higher than established (1:10000). High risks of cryptosporidiosis and giardiasis were detected from samples of soil, especially for children. The (oo) cyst concentration in air dust is not related to the concentration of total suspended particles detected. Serrano chili and saladette tomatoes were the vegetables tested where their (oo) cysts pollution is due to possible indirect pollution with water, soil, and potential contamination by host when choosing vegetables. The total annual risks infection for both parasites is 1:1.

The concentrations of (oo) cysts in the environmental samples produced high risks (1:1) compared with guidelines in other countries (one infection for 10000 individuals) but this research adds risks in all sampled sources. The results from the present study represent useful information that could be taken in generate different programs focus on (water treatment, sanitation) decreasing parasites infection in indigenous communities.

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*Author for all correspondence

Chapter 4.
Air Pollution and Climate Change

HIGH HYDROGENE SULFIDE CONCENTRATION ELIMINATION BY COLORLESS SULFUR AEROBIC BACTERIA ISOLATED FROM AN ANAEROBIC MUNICIPAL SLUDGE DIGESTER

ELhouari AbdelAziz (1); Fejrioui Assia (1); Lafar Sara (1); Bennisse Rhizlane (1); Qatibi Abdel-Illah* (1)

(1) Anaerobic Microbiology Team, Cadi Ayyad University, Morocco

Hydrogen sulphide (H_2S) is a major problem associated with anaerobic treatment of sulfate, sulfite and thiosulfate containing wastewater where it can be found in the treated water and the biogas, causing problems of corrosion and a strong unpleasant smell. Physicochemical methods for it removal from gas emissions in use today have relatively high energy requirements or high chemical and disposal cost. Biological treatment using bio filters have been proposed as a convenient alternative for treating gaseous emissions containing H_2S and reduced sulphur compounds. *Thiobacillus* species seems to be adequate because of smaller food conditions and in particular *T thioparus* and *T thioxidans* that have the capacity to develop by using H_2S like source of energy. The inoculation of bio filtration systems with pure cultures of those chemolitoautotrophic bacteria from the genus *Thiobacillus* has allowed the decrease or even the elimination of the initial lag phase and the increase in the efficiency of removal of H_2S and organ sulfur compound, maintaining a stable operation. Other species evaluated for sulfur reduced compounds removal are *T. denitrificans*, *T. ferrooxidans* and *T. novellus.*

In the course of our study on microbiological interactions between colorless sulfur bacteria and sulfate-reducing bacteria in an anaerobic municipal sludge digester, we have isolated nine aerobic sulfur bacteria. These new isolates were very motile, gram-negative, non-spore-forming and ovoid with internal reserves of sulfur. Occurred singly, or in pairs and as a chain. At this stage of our work, these bacteria are not taxonomically identified yet but seems differed, by several aspects of cellular and cultural morphology, to other colorless sulfur bacteria.

In pure culture in aerobic condition, isolates are able to growth in a mineral medium on sulfides as energy source in the presence of sodium bicarbonate as carbon source. A better growth and elimination of sulfides were observed in the presence of acetate. Moreover, in the presence of acetate, cells aggregate into a community in which cells attach to one another.

Preliminary tests of coculture in microaerophilic condition between the new isolated strains and a *Desulfovibrio* sp strain DZ, isolated from the same digester, *D. marrakechensis* (DSM 19337T) *D. alcoholivorans* (DSM 5433T) and *D. fructosivorans* (DSM 3604T) are able to eliminate sulfides produced by the reduction of sulfate by the four sulfate-reducing bacteria in the presence of lactate as energy source.

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*Author for all correspondence

OXIDATION OF METHANE IN BIOTRICKLING FILTERS INOCULATED WITH METHANOTROPHIC BACTERIA

Manuel Cáceres (1); Juan Carlos-Gentina (1); Germán Aroca-Arcaya* (1)

(1) School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

The oxidation of methane (CH_4) in biofilters has been proposed as an alternative to mitigate greenhouse gas emissions with low concentration of CH_4. Organic packing materials, like compost or soils, are the most used in the bio-oxidation of CH_4 due its high availability and it can be used as nutrient supply, however it can have a short lifetime, and problems associated with high pressure drop and channelling can appear during the operation. Inorganic packing materials present several advantages as good mechanical resistance, low pressure drop, defined physical properties and provide a more stable behavior in long-term operation. The aim of this research was to characterize the oxidation of CH_4 in biotrickling filters (BTFs) packed with polyethylene rings supporting a biofilm of methanotrophic bacteria.

Two identical BTFs were set up with columns of clear PVC (height: 1.2 m; i.d.: 0.15 m) and packed with 0.020 m^3 of polyethylene rings (1333 $m^2 \cdot m^{-3}$, 77% free volume) and then inoculated with a mixture of methanotrophic bacteria *Methylomicrobium album* and *Methylocystis* sp. Both BTFs were operated at the same conditions. The inlet stream was a gas mixture of air and CH_4 at different concentrations. The CH_4 elimination capacity was measured once the steady-state was reached.

At all conditions tested both BTFs showed a similar behaviour. The maximum CH_4 elimination capacity was 5.2 $g_{CH4} \cdot m^{-3} \cdot h^{-1}$ at an inlet methane concentration of 3.92% (v/v) and EBRT of 67 minutes. The CH_4 removal efficiency decreased rapidly when the gas flow rate increased. Global mass balance indicates that by each mole of oxidized CH_4, 2 moles of O_2 were consumed and 1 mol of CO_2 was produced. The maximum reduction of CO_2 equivalents was 98.5 $g_{CO2} m^{-3} h^{-1}$ achieved at load of 23.2 $g_{CH4} \cdot m^{-3} \cdot h^{-1}$. Due the high free volume of polyethylene rings no pressure drop or clogging problems were detected after one year of operation, however the available specific area for biofilm formation is low.

It is concluded that at low concentrations of CH_4 in gas phase, the oxidation of CH_4 in BTFs inoculated with methanotrophic bacteria is limited by transport phenomena. It is possible to reduce the global warming potencial of gas emissions with low concentration of CH_4 using a BTF inoculated with methanotrophic bacteria.

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*Author for all correspondence

BIO-OXIDATION OF METHANE IN A MEMBRANE REACTOR WITH A BIOFILM OF *Methylomicrobium album*

Germán Aroca-Arcaya* (1,2); María José Cárdenas (1); Alberto Aguirre (2); Matías Gómez (1); Sebastián Madrid (1); Juan Carlos Gentina (1)

(1) Biochemical Engineering School- Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile;
(2) Doctoral Program in Biotechnology. Programa Conjunto Pontificia Universidad Católica de Valparaíso–Universidad Técnica Federico Santa María, Chile.

Methane (CH_4) is an organic gas emitted from several industrial processes. It represents approximately 23% of total emissions of greenhouse gases worldwide. According to the latest report of the IPCC, methane has a global warming potential 34 times greater than CO_2 on a time scale of 100 years. Common methods for eliminating methane in industry are combustion to generate energy, or just burn it in flares. However 55% of anthropogenic methane emissions cannot be thermally oxidised. Technologies based in the action of microorganisms with methane oxidation capability have been proposed and tested in configurations like biofilters and biotrickling filters. The membrane biofilm reactors (MBfRs) represent an alternative for eliminating pollutants in air. They are characterized by high gas utilization efficiency and high specific surface area for biofilm accumulation. MBfRs have been described previously for TCE removal, reduction of oxidized contaminants such as perchlorate, nitrate, bromate, chromate and selenate, treating wastewater, but there are no reports related with methane gas bio-oxidation. The aim of this work was to evaluate the performance of an MBfRs for bio-oxidizingCH_4.

The system was operated at 30°C and pH 6.8 under different CH_4 specific loading rates using *Methylomicrobium album*as inoculum for forming a biofilm over a polydimethylsiloxane (PDMS) hydrophobic membrane with enhanced properties for separating methane.

The results showed that an increase in CH_4 specific loading rate from 51 to 169 [g CH_4/m^2h] resulted in an increase in specific elimination capacity of CH_4 from 10 to 36,5 [g CH_4/m^2h], however the CH_4 removal efficiency at CH_4 loads tested ranged between 19 and 23%. This behavior showed that an increase in the CH_4 availability in the gas phase of the reactor jointly with a high activity of the biofilm that was growing on the membrane were important for the improvement of CH_4 transfer rate along the membrane, and thus for the improvement of CH_4 elimination capacity of the system.

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*Author for all correspondence

SIMULTANEOUS TREATMENT OF DIMETHYL DISULFIDE AND HYDROGEN SULFIDE VAPORS IN AN ALKALINE BIOTRICKLING FILTER

Luis Arellano-García (1); Sergio Revah* (2)

(1) IPH-UAM Iztapalapa, México DF, México;
(2) DPT-UAM Cuajimalpa, México DF, México;

The exposure to odors caused by sulfides is a cause of concern due to its noxious effects and toxicity. Moreover the World Health Organization recently categorized the persistence of odorants as a type of air pollution. The treatment of sulfide-polluted air in biotrickling filters (BTF) has been proved to be a cheap and efficient process to remove the pollutants from air streams. Lately alkalinity has been utilized in BTFs as novel characteristic to foster the removal of acid sulfides as hydrogen sulfide (H_2S), ethanethiol, carbon disulfide etc. Nonetheless a mixture composes the majority of sulfides emissions where other non-polar compounds as dimethyl disulfide (DMDS) are likely to be present. The aim of this work was to evaluate the elimination capacity of an alkaline BTF during the treatment of a DMDS-H_2S mixture, with loads between 5 and 60 $g_{pollutant}$ $m^{-3}_{reactor}$ h^{-1}. The BTF was inoculated with an alkaliphilic sulfooxidizing bacterial consortium (ASBC) obtained from a soda lake (Texcoco, Mexico) and adapted to sulfides consumption. The experiments were carried out using a lab-scale BTF, packed with polyurethane foam colonized with the ASBC and a 40s gas residence time. The continuous BTF operation was maintained for 150 days increasing periodically the sulfides load feeding. Additional biodegradation experiments were executed in gas-liquid batch cultures, measuring sulfides consumption through the analysis of the gas phase.

Maximum elimination capacities of 19 and 56 g m^{-3} h^{-1} were obtained for H_2S and DMDS respectively, with removal efficiencies higher than 90%. These results are comparable to other studies reported in the literature at neutral pH. From the BTF operation results, at the concentration levels assayed, no interference of H_2S over the DMDS degradation was registered. However, the presence of degradation intermediaries was observed.

In conclusion the alkaline BTF showed to be an efficient process to treat a mixture of organic/inorganic gaseous sulfides. One advantage of the process is the interaction of acid sulfides with the alkaline liquid in the BTF, which improve the treatment of polluted air. Also the use of slow-growing alkaliphilic bacteria, compared to commonly utilize neutrophilic bacteria, may extend the operational lifespan of the BTF with satisfactory treatment efficiencies.

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*Author for all correspondence

IMPACTO DE LAS CARACTERÍSTICAS FISICOQUÍMICAS Y DINÁMICA DE CARBONO EN SUELO, DESPUÉS DE NUEVE AÑOS DE LA REINTRODUCCIÓN CON VEGETACION NATIVA EN UN SUELO PERTURBADO

Martha V. Almanza-Estrada (1)***;*** Blanca G. Elías-Balandrán (2); María M. Vargas-Zúñiga (1); Yazmín A. Sánchez-Ruan (1); Alejandra Gutiérrez-Vázquez (1); Juan A. Mejía-Gómez (1); Jaquelina González-Castañeda* (1)

(1) DICIVA, CIS, Universidad de Guanajuato, Irapuato, Gto., México;
(2) Universidad Politécnica Bicentenario, Silao, Gto., México;

El CO_2 es considerado un gas de efecto invernadero (GEI). Su captura en el suelo contempla el recambio de materia orgánica y se propone como una alternativa para la mitigación de sus efectos nocivos. El objetivo de este trabajo fue evaluar el impacto de las características fisicoquímicas y la dinámica de carbono en suelo, después de nueve años de la reintroducción con vegetación nativa: huizache (*Acacia schaffneri*) y gatuño (*Mimosa monancistra*), en un suelo perturbado, en el estado de Guanajuato.

Se realizaron muestreos a una profundidad de 0 a 20 cm bajo el dosel de las plantas en época de sequía (mayo) y después de las lluvias (noviembre), en el año 2010. La caracterización fisicoquímica se hizo mediante la Norma Oficial Mexicana NOM-021-SEMARNAT-2000. La dinámica de carbono a nivel laboratorio, se realizó mediante la técnica reportada por Jenkinson y Powlson (1976), la cual permite cuantificar el contenido de carbono en el suelo (expresado como producción de CO_2). El análisis estadístico se hizo mediante la Prueba de Tukey a $p < 0.05$, de 3 repeticiones por tratamiento, por triplicado.

Todos los tratamientos mostraron diferencia significativa con respecto al Testigo (T), para pH tanto con agua como con $CaCl_2$, mostrando valores entre 7.87 y 6.90 (mayo y noviembre respectivamente), pudiendo clasificarse de medianamente alcalinos a neutros. Para la CE se observaron valores entre 0.27 y 0.42 dS m^{-1}, mostrando un efecto poco significativo sobre la salinidad. La densidad aparente correspondió a suelos arcillosos (1.00 y 1.11 g cm^{-3}). La capacidad de saturación fue mayor para los tratamientos con respecto a T. La Materia Orgánica fue mayor en noviembre, como era de esperarse, clasificándose los suelos como bajo para mayo (1.03 a 1.024%), en tanto que para noviembre como medio (1.51 a 1.87%). La dinámica de carbono mostró menor producción de CO_2 en T que en los tratamientos, en mayo HCM a los 28 días de incubación, mostró los valores más altos (953 $x10^3$ mg C-CO_2kgss^{-1}), en tanto que GSM en noviembre para el mismo periodo de incubación produjo 2058 $x10^3$ mg C-CO_2kgss^{-1}, de manera global se observó de 1.84 a 2.4 veces la producción de CO_2, entre mayo y noviembre para los tratamientos, en tanto que para los testigos la producción se mantuvo. Se concluye que la reintroducción de vegetación nativa, como era de esperarse, no modificó significativamente las características fisicoquímicas del suelo. Sin embargo la captura de carbono se incrementó, siendo una alternativa para mitigar los efectos de los GEI.

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*Author for all correspondence

NANO- SIZED Na_2ZrO_3 ABSORBENT PARTICLES FOR CO_2 CAPTURE AT HIGH TEMPERATURE

Virginia Collins Martínez (1); Diana Barraza Jiménez (2); Vanessa Guzmán Velderrain; Leonor Cortés-Palacios (3); Alberto Díaz Díaz (1), Alejandro López Ortiz*(1)

(1) CIMAV, Chihuahua, Chihuahua, México.
(2) CIAD, Cd. Delicias, Chihuahua. México.
(3) UACH, Chihuahua, Chihuahua, México.

Today, natural gas reforming is the dominant technology for large scale hydrogen production. In order to enhance this process (improve its efficiency), CO_2 is captured in situ at high temperatures (T > 600 °C) by means of a solid synthetic absorbent. Sodium zirconate (Na_2ZrO_3) has been established as a CO_2 absorbent, which presents the highest absorption kinetics among other synthetic based materials such as Li_2ZrO_3. Due to its good absorbent properties, it is important to study its absorption kinetics and to determine those material properties that need to be modified to improve its absorption performance, for example, its size at the nanoscale.

In the first part of the research, CO_2 absorption kinetics for micron-size sodium zirconate (Na_2ZrO_3) were studied and their parameters; reaction order, rate constant, apparent, intrinsic and diffusional activation energies were established through TGA experiments as a function of CO_2 concentration and temperature. A global reaction rate of first order in CO_2 concentration and a strong dependence in temperature was found. The approximate solution to the shrinking core model was used to fit the data. Modeling results indicated the surface reaction as the main resistance to the reaction rate, controlling reaction kinetics in combination with a small contribution of the product layer diffusion resistance toward the end of the reaction. The temperature dependence of the diffusion coefficient Dg, was described by an Arrhenius-type, which is characteristic of a nonporous solid. This diffusional kinetic limitation was increased especially towards the end of the reaction.

In the second part of the study it was decided to overcome Na_2ZrO_3 diffusional kinetic limitations by reducing the size of the absorbent from micron-size to nanosize particles. This was accomplished through a spray pyrolysis synthesis process, which employed thermal decomposition of sodium acetate (CH_3COONa) and Zirconium (IV) acetylacetonate ($Zr(C_5H_7O_2)_4$) solution precursors in ethanol. The desired product formation was characterized by XRD, while the gaseous by-products of the reaction were analyzed using FTIR spectroscopy. A TGA kinetic reaction study was made to determine the activation energy, pre-exponential factor and the reaction order. It was found that the during the nano-Na_2ZrO_3 formation mechanism, reagents thermal decomposition rates were higher than sodium zirconate formation velocity. However, at higher temperatures, both rates are increased, thus reducing the sodium zirconate formation time.

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*Author for all correspondence

Chapter 5.
Aquifer Remediation

PRODUCTION OF A PHOTOSYNTHETIC MICROBIAL CONSORTIUM IN A FLAT-PANEL AIRLIFT PHOTOBIOREACTOR IN BATCH AND SEMICONTINUOUS CULTURE OUTDOORS

Dulce J. Hernández-Melchor (1); Eliseo Cristiani-Urbina (2);
José Fernando Esparza-García (1), Rosa Olivia Cañizares-Villanueva* (1)

(1) CINVESTAV- IPN, México DF, México;
(2) ENCB-IPN, México DF, México;

Cyanobacteria are oxygenic phototrophic organisms, distributed in aquatic and terrestrial environments. They have the capability to produce high value compounds useful in different areas of biotechnological interest. One strategy that has been used to improve the productivity of cyanobacteria and algae in photobioreactors to scale up its production in semicontinuous culture, which offers advantages such as to obtain a high and constant inoculum, high growth rates, in addition long operation time of reactors. In our research group a photosynthetic microbial consortium (MC) with nitrogen fixation capability, was studied. It consists primarily of cyanobacteria, so its potential use as a biofertilizer has been proposed. The aim of this study was to produce the MC in a flat-panel airlift photobioreactor of 150 L capacity (PBR) in batch and semicontinuous culture outdoors, and to evaluate its productivity and nitrogen fixation capability.

The MC production to large scale was realized using a 150 L capacity flat-panel airlift photobioreactor in batch culture for 14 d, and semicontinuous culture in the same PBR for 19 d. The MC growth was determined by dry weight biomass each 24 h by the method proposed by Tredici *et al*. and the nitrogenase activity using the method proposed by Venkataraman.

The growth of the MC in the PBR in batch culture started with 197 mg L^{-1} of dry weight biomass, the maximum value of the biomass was obtained at 14 day with 600 mg L^{-1}. The productivity at the end of the batch culture was of 28.6 mg L^{-1} d^{-1}. The maximum value of the nitrogenase activity was obtained at 14 day with 4814 nmol ethene g^{-1} dry weight h^{-1}. In the second experiment was realized the growth of the MC during three growth cycles in semicontinuous mode, using a dilution rate of 50 %. The duration of each cycle was of 7, 7 and 5 d. The productivity obtained at the end of each cycle was 43.7, 33.4 y 67.7 mg L^{-1} d^{-1} respectively. The maximum values of the nitrogenase activity was obtained at the end of each cycle and were 4963, 5366 y 5689 nmol ethene g^{-1} dry weight h^{-1} respectively. The values of the nitrogenase activity obtained in this study for the MC in a PBR in batch and semicontinuous culture were approximately two and three times higher that reported for *Anabaena variabilis* Sao (2678 nmol eteno g^{-1} dry weight h^{-1}) and *Nostoc muscorum* DOH (2237 nmol eteno g^{-1} dry weight h^{-1}).

In conclusion was possible to produce the MC in a PBR in batch and semicontinuous mode outdoors, without affecting the nitrogen fixation capability. This strategy can be used to produce the MC in a large scale.

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*Author for all correspondence

DECREASE OF INHIBITORY EFFECT OF 2-CHLOROPHENOL ON THE NITRIFICATION IN A SEQUENCING BATCH REACTOR (SBR)

Miguel Martínez-Jardines (1); Anne-Claire Texier (1); Emmanuel Pérez-Alfaro (1); Flor Cuervo-López* (1)

(1) UAM-Iztapalapa, México DF, México

One of the consequences of pollution in aquatic ecosystems is eutrophication which is mainly caused by ammonium (NH_4^+). Another pollutant which is contaminating soils and aquifers is 2-chlorophenol (2-CP), as this compound is widespread used as a structural part of wood preservatives and pesticides. Nitrification, an aerobic respiratory process where the ammonium is oxidized to nitrate, is an alternative for removing nitrogen compounds from wastewaters. Nevertheless, the presence of organic matter can result in important diminishes in the performance of the nitrifying process. Sludge acclimation and SBR systems seem to be an alternative for 2-CP removal. The aim of this study was to evaluate the effect of 2-CP on a nitrifying sludge previously exposed to phenolic compounds by means of specific rates of substrates consumption ($q_{N\text{-}NH_4^+}$, $q_{2\text{-}CP}$) and product generation ($q_{N\text{-}NO_3^-}$) in a SBR.

Two sequencing batch reactors (SBR1 and SBR2) were set up. SBR1 was inoculated with a nitrifying sludge previously exposed to 2-CP. SBR2 was inoculated with a nitrifying sludge which had been fed with NH_4^+ and *p*-cresol. The experiments were conducted in four stages. First, abiotic assays were conducted in order to establish the possible loss of 2-CP in SBR systems. In a second stage, a nitrifying steady state was reached in the SBR reactors. In a third stage, control nitrifying cultures were carried out in both SBR reactors. The reactors were fed with 100 mg $N\text{-}NH_4^+$/l and 250 mg $C\text{-}NaHCO_3$/l. Finally, 20 mg C-2-CP/l were added in each reactor in order to evaluate the effect of 2-CP on the nitrification. Kinetics assays were performed along the cycles (fill = 30 min, reaction time = 30 d; sedimentation = 30 min and drain = 2 h) for determining $q_{N\text{-}NH_4^+}$, $q_{2\text{-}CP}$ and $q_{N\text{-}NO_3^-}$ throughout the cycles. Product yields (Y) and substrate consumption efficiencies ($E_{N\text{-}NH4+}$, $E_{C\text{-}2\text{-}CP}$) were also determined.

Once nitrifying steady state was reached, the addition of 20 mg of C-2-CP/l in both SBR reactors provoked a negative effect on the nitrifying process; regardless the sludge had been previously exposed to phenolic compounds. Nevertheless, after 6 operational cycles, a significant increase in $E_{N\text{-}NH4+}$ (99.74%), $Y_{N\text{-}NO3-}$ (0.99) and $E_{C\text{-}2\text{-}CP}$ (100%) was achieved with sludge previously exposed to 2-CP (SBR1), whereas $E_{N\text{-}NH4+}$ of 99.76%, $Y_{N\text{-}NO3-}$ of 0.23 and $E_{C\text{-}2\text{-}CP}$ of 100% were achieved in the SBR2. Likewise, the inhibitory effect of 2-CP also was diminished since $q_{N\text{-}NH_4^+}$and $q_{N\text{-}NO_3^-}$ values in both reactors were increased along operational cycles. Therefore, the use of SBR resulted in a decrease of the inhibitory effect of 2-CP on the nitrification process, achieving a better performance with the sludge previously exposed to 2-CP.

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*Author for all correspondence

INDUCCIÓN DE EMBRIONES SOMÁTICOS EN *Typhadomingensis*

G. Hernandez-Piedra (1); ***V. Ruiz-Carrera**** (1)

(1) UJAT-CICART, Villahermosa, Tabasco. México.

La producción de embriones somáticos como método de propagación para la macrófita acuática emergente *Typhadomingensis* fue investigado en la fase de inducción. El experimento fue bifactorial aleatorizado (2 x 3) usando cultivos de plantas *in vitro* en lote agitado. El efecto del ambiente de cultivo fue analizado en luz y obscuridad y el de 2, 4-D en concentraciones de 0.5, 1.0 y 2.0 $mg \cdot L^{-1}$ en MS 0.5 con 3% de sacarosa. La evolución de características celulares embriogénicasy no embriogénicas fue evaluada durante dos meses. La inducción embriogénica detonó en el ápice caulinar. En la tercera y cuarta semana de cultivo fueron formadas las masas de células embriogénicas en nódulos y no-embriogénicas friables. Las células nodulares adquirieron cierto grado de competencia embriogénica con 2, 4-D indistinto al ambiente de cultivo. Sin embargo, la mayor productividad de células embriogénicas se presentó en 0.5 y 1.0 $mg \cdot L^{-1}$ de 2, 4-D ($p > 0.05$). El 50% de los tratamientos registraron embriones somáticos en la sexta semana de cultivo.

El embrión somático presentó suspensor unido al explante y protuberancia en el extremo distal. Los nódulos celulares embriogénicos y un tercio de los embriones somáticos mostraron oscurecimiento progresivo en el tiempo. El potencial de expresión y mapa de destino de embriones somáticos de *Typhadomingensis*se presentan como avance biotecnológico para la repoblación sustentable de humedales ante los escenarios de cambio en la biodiversidad y los servicios ecosistemáticos asociados.

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*Autor para toda correspondência

EVALUACIÓN DE LA ACTIVIDAD EMULSIFICANTE Y SURFACTANTE DE UN CONSORCIO MICROBIANO DEGRADOR DE HIDROCARBUROS NATIVO DE UN CENOTE

Manuel Alejandro Lizardi-Jiménez* (1); Cesar Hernández-Flores (2); Stefano Decler (2); A. Jiménez - González (1); S.A. Medina - Moreno (1)

(1) Universidad Politécnica de Pachuca, Ingeniería en Biotecnología, Pachuca, México.
(2) Universidad Politécnica de Quintana Roo, Ingeniería en biotecnología, Cancún, Quintana Roo

Los derrames de petróleo en suelo, y sus componentes principales son susceptibles de biodegradación mediante consorcios microbianos. Este trabajo tiene como objetivo evaluar la producción de biosurfactantes (BS) y/o bioemulsificantes a partir de un consorcio microbiano degradador de hidrocarburos. Se determinó el efecto de la relación C/N y pH en la producción de consorcio microbiano degradador de hidrocarburos. Se muestreó un cenote con ubicación en Cancún Quintana Roo, se identificó como contaminado por los hidrocarburos Fenantreno y Naftaleno que corresponden a hidrocarburos del grupo Aromáticos Policíclicos bajo el cumplimiento de la NMX-AA-117-SCFI-2001. Se cultivó el consorcio nativo del cenote en bioreactores de columna de burbuja de 1 litro, con medio mineral a pH inicial de 6, 7 y 8 (Lizardi-Jimenez y col, 2014). Se emplearon como fuentes de carbono el Petróleo Crudo Maya (CM) y el diésel para la producción de BS. Para ambos sustratos se empleó 3 relaciones carbono/nitrógeno (C/N): 3, 6 y 9 g C (g N)$^{-1}$.

Se ensayó en 18 minibiorreactores con diferentes condiciones. Se llevó una cinética de producción de BS a cada biorreactor por 4 días. El medio de cultivo se centrifugó a 12000 rpm durante 30 min y 4°C. La tensión superficial de los cultivos se determinó con un tensiómetro de anillo de Platino-Iridio a una temperatura constante de 25°C, y utilizando como fluidos de referencia agua destilada y diésel. Se llevó la cinética de producción de BS de aquellos microorganismos productores de tensoactivos durante 4 días de los 18 biorreactores. Una vez obtenida la cinética de producción de BS, se llevó la cinética de la AE (actividad emulsificante) de aquellos microorganismos productores de tensoactivos durante 4 días. En conclusión, la producción de biosurfactantes y bioemulsifictantes cambia con la variación del pH y relación C/N, la tensión superficial y la actividad emulsificante también se ven afectadas. La relación C/N de 6:1 de diésel y crudo maya, en conjunto con un pH de 6, generan las mejores tasas de producción de BS.

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*Autor para toda correspondência

BIOSTIMULATION AS AN EFFICIENT STRATEGY FOR THE RECUPERATION OF THE SOIL AND AN AQUIFER POLLUTED WITH Cr(VI) IN GUANAJUATO, MÉXICO

Paloma Lara (1); Italia Moreno (1); Bianca Flores (1); Enrique Morett (1); Katy Juárez* (1)

(1) Instituto de Biotecnología, UNAM, Chamilpa, Cuernavaca, Morelos.

The uncontrolled Cr (VI) industrial wastes and their improper disposal has resulted in an anthropogenic pollution of various environments including soils and aquifers. The selective pressure in these environments have led to developed of several strategies to resist high concentrations of Cr(VI) in microorganism exposed to this metal, mainly through chromate efflux and Cr(VI) reduction to Cr(III). The microbial reduction of Cr(VI) can be potentially promoted by addition of electron donors and a carbon source as a safe and cost-effective technology alternative to the expensive traditional physicochemical methods. The present study had two aims: the first was the evaluation of the efficiency of different electron donors to promote the Cr(VI) reduction in samples of polluted soil from a deposit of industrial wastes located in Guanajuato, México. The second aim was the isolation and characterization of bacteria Cr(VI) resistant and reducers from the aquifer located near the zone of the waste residues.

The methodology was: Soil samples were taken at 30 to 40 centimeters deep. The samples were sieved and Cr(VI) concentration were determined. The biostimulation assays were performed in batch under aerobic, microaerobic and/or anaerobic conditions. Moreover samples of the local aquifer highly contaminated with chromates were taken at 30 meters deep. From these, an anaerobic enrichment culture with Cr(VI) resistance and reduction ability was followed up. Then was performed the microbial isolation on solid medium with Cr(VI). The analysis of 16S rDNA allowed us to identify the genera and species of some isolates. The Cr(VI) resistance and reduction were assayed under aerobic and anaerobic conditions. The results were: Acetate, molasses and the mixture of molasses with lactate were all efficient to promote the microbial reduction of Cr(VI). Fifteen different facultative anaerobe microorganisms belonging to the phyla proteobacteria, firmicutes and actinobacteria showed high levels of resistance, with a minimum inhibitory concentration of about 1195 mg/l de Cr(VI) in aerobiosis. One of them is closely related to *Klebsiella pneumonie* KCTC 2242, it is able to perform Cr(VI) and Fe(III) reduction under anaerobic conditions. In the conclusion the assays of soil biostimulation in batch suggest that this technology offers a potential solution to the serious pollution problem on this site.

In the other hand, our selection strategy permitted isolation of different native bacterial strains, which could be used for treatments of wastes with Cr(VI). Furthermore show that in the aquifer are present microorganisms potentially capable to be biostimulated to clean this aquifer.

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*Autor para toda correspondência

COMBINING ABIOTIC AND BIOTIC PROCESSES FOR AGGRESSIVE TREATMENT OF CHLORINATED ETHENES

Daniel Leigh* (1)

(1) Peroxychem LLC, Walnut Creek, CA, USA

Soil and groundwater at IR Site 29 Concord Naval Weapons Station in Concord, CA (Site) has been affected by a discharge of trichloroethene (TCE). The Navy intends divest the property under the Base Realignment and Closure (BRAC) program. To expedite the Navy's goals, an aggressive treatment approach combining biological and abiotic processes is being applied to remediate the TCE.

A TCE plume extends approximately 700 feet down gradient from the source area and up to 100 feet below ground surface. The aquifer consists of unconsolidated silt, sands and clays. Groundwater in the treatment area is highly aerobic. A previously conducted pilot test demonstrated that application of enhanced reductive dechlorination (ERD) resulted in complete degradation the CE concentration from approximately 5,000 microgram per liter (µg/L) to less than 1 µg/L in approximately 500 days.

A subsequent pilot test was conducted at the Site to compare biologically mediated ERD to in situ chemical reduction (ISCR). The ERD pilot test was conducted by distributing emulsified vegetable oil and a dechlorinating microbial culture (SDC-9™). The ISCR test enhanced biotic reductive dechlorination by distributing Emulsified Lecithin Substrate® (PeroxyChem Environmental Solutions) and SDC-9™. Abiotic processes in the ISCR approach were applied by distribution of zero valent iron (ZVI).

ZVI effectively buffered the groundwater. Notably, arsenic persisted at over 4 times the MCL in the ERD test; whereas arsenic never exceeded the MCL in the ISCR test. TCE and DCE were degraded to below the maximum contaminant levels (MCL) in both pilot tests; however, vinyl chloride was only degraded to below the MCL in the ISCR pilot test. The ISCR approach achieved the remedial goals in approximately 1/3 the time required by ERD. This reduction of treatment time is attributed to the rapid abiotic degradation of DCE by ISCR.

Conclusion: ISCR was demonstrated to be a much more aggressive approach to remediate chlorinated ethenes than ERD. ELS® is an effective organic substrate for reductive dechlorination. The ISCR process was selected for full-scale treatment to more aggressively treat the contaminants, reduce the potential for generation of toxic degradation products, provide long lasting substrates that reduce the potential for rebound of the contaminants and to achieve the Navy's goal of rapid divestiture of the Site.

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*Autor para toda correspondência

BIODEGRADACIÓN DE LA MEZCLA COMERCIAL DE LOS HERBICIDAS ATRAZINA Y S-METOLACLOR EN UN REACTOR EMPACADO DE CANALES MULTIPLES

Alberto Cabrera-Orozco* (1); Nora Ruiz-Ordaz (1); Juvencio Galíndez-Mayer (1); F. Fernando Martínez Jeronimo (1)

(1) ENCB-IPN, México DF, México.

La atrazina y el s-metolaclor son dos de los herbicidas más utilizados a nivel mundial. Su amplia aplicación ha provocado que se detecten en cuerpos de agua subterránea y superficial. Los estudios de biodegradación reportan una gran diversidad de microorganismos con la capacidad de biotransformar y mineralizar la atrazina; en el caso de s-metolaclor no se ha tenido mucho éxito. En este contexto, el objetivo de esta investigación plantea la degradación de la mezcla comercial de los herbicidas atrazina y s-metolaclor (Primagram Gold), en un reactor trifásico prototipo.

El aislamiento de la comunidad microbiana se realizó a partir de muestras de suelo agrícola mediante transferencias sucesivas en medio mínimo mineral con el herbicida Primagram Gold. La comunidad microbiana aislada se le denominó cultivo T. Se realizó una réplica de este cultivo, el cual se mantuvo con las mismas condiciones que el cultivo original; a dicha réplica se le enriqueció con un cultivo axénico de la cepa de la levadura *Candida tropicalis.* La comunidad microbiana obtenida se le denominó cultivo TC. Los cultivos se operaron en lote repetido y se comparó su eficiencia de remoción.

Ambos cultivos removieron la atrazina en un 100%; el cultivo TC presentó una mayor velocidad de remoción. Este mismo cultivo presentó una mayor eficiencia de remoción de s-metolaclor con un 67%, contra un 38% obtenido por el cultivo T. Se seleccionó la comunidad enriquecida (TC) y se inoculó un reactor empacado multicanal prototipo. El sistema se operó en régimen continuo variando flujos de alimentación y concentración de herbicida. En todas las condiciones probadas se alcanzó una remoción de atrazina por encima del 99%, resultado similar a lo obtenido en el sistema por lote. En el caso de s-metolaclor la máxima eficiencia de remoción obtenida fue de 47%; eficiencia menor a la obtenida en los cultivos por lote. El análisis de las distintas condiciones de corrida demostró una correlación inversa (r^2=0.91), entre el aumento del flujo de alimentación y la eficiencia de remoción de s-metolaclor. La evaluación toxicológica demostró que el proceso de depuración disminuyó la toxicidad del medio líquido, sin embargo, el efluente aún presentó una toxicidad importante en los organismos acuáticos *Daphnia magna* ($LC_{50\ Entrada}$=18.42%; $_{Salida}$=63.55%), *Ceriodaphnia dubia* ($LC_{50\ Entrada}$= 12.57%; $_{Salida}$= 41.55%), y *Pseudochineriella subcapitata* ($IC_{50\ Entrada}$= 0.000029%; $_{Salida}$= 0.002%). En conclusión, los factores evaluados, tanto el sistema de operación, ya sea en lote o contínuo, la velocidad de flujo y la concentración no afectaron la eficiencia de remoción de atrazina, pero si la de s-metolaclor.

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*Author for all correspondence

BIODEGRADACIÓN DE UN COLORANTE MODELO ADSORBIDO SOBRE COMPLEJOS DE BIOMASA-ARCILLA

Lucas M. Guz (1); Melisa S. Olivelli (1); Roberto Candal (1);
Rosa M. Torres-Sánchez (2); Gustavo Curutchet* (1)

(1) Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, Argentina.
(2) Centro de Tecnología de Recursos Minerales y Cerámica, CONICET.

Los procesos de separación de los adsorbentes de la solución luego de procesos de biosorción son de suma importancia, siendo la inmovilización de biomasa microbiana sobre sustratos de bajo costo una de las estrategias que facilitan este paso de separación. Matrices biomasa-arcilla fueron desarrolladas previamente en nuestro laboratorio y resultaron eficientes tanto en la biosorción como en la separación posterior. Sin embargo estos procesos tienen por principal inconveniente el formar lodos cargados con el contaminante, siendo necesario desarrollar técnicas de degradación de los contaminantes orgánicos recalcitrantes adsorbidos. A su vez, la presencia de biomasa asociada a la fracción mineral de suelos y sedimentos en ambientes naturales, cumple un rol importante en la adsorción y destino de este tipo de contaminantes en el ambiente. En estudios previos se aisló una bacteria (*Actinobacterium* sp.) autóctona de un afluente del río Reconquista (Buenos Aires, Argentina) que se encuentra contaminado con efluentes industriales. Esta cepa presentó un potencial de degradación de soluciones del colorante modelo, cristal violeta, de más del 90% en menos de 24 horas además de una adsorción del mismo de 330 mg de colorante/g de biomasa. El objetivo de este trabajo es evaluar la degradación biológica de un colorante retenido en complejos biomasa-arcilla generados por crecimiento de dicha bacteria sobre una montmorillonita. Este proceso es de interés tanto en la descontaminación de lodos generados en procesos de tratamiento de efluentes por biosorción como en la comprensión de procesos de atenuación que ocurren en ambientes naturales

Se generó un complejo arcilla-biomasa, haciendo crecer la cepa de *Actinobacterium* sp. sobre una montmorillonita. Los complejos fueron utilizados para adsorber una solución de cristal violeta. Se estudio la cinética de degradación del colorante adsorbido, en experiencias de resuspensión y en fase sólida. Se utilizó espectrofotometría UV-visible para la determinación de colorante y respirometría para estimar los parámetros metabólicos. El complejo biomasa-arcilla presentó una degradación entre 55 y 33 % del colorante, estando el proceso ligado al metabolisimo oxidativo. No se observó actividad de degradación sobre montmorillonita pura con colorante adsorbido. Este estudio muestra la posibilidad de biodegradar contaminantes adsorbidos y su importancia en la remediación de suelos y sedimentos contaminados.

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*Author for all correspondence

Chapter 6.
Soil and Sediment Remediation

SUELO SALINO- SÓDICO: MEJORANDO SUS CARACTERÍSTICAS CON EL USO DE BIOSÓLIDOS

Eustacio Ramírez-Fuentes (1); Ma. Nieves Trujillo-Tapia (2); Zenyase R. Pérez-Buceta (1); Carlos Estrada-Vázquez* (2)

(1) Universidad del Mar, Instituto de Recursos, Puerto Ángel, Oaxaca, México
(2) Universidad del Mar, Instituto de Ecología, Puerto Ángel, Oaxaca, México

Las plantas de tratamiento de agua residual (PTAR) generan diariamente toneladas de lodos, los cuales pueden ser estabilizados (biosólidos) y utilizados como mejorador de las características físicas y químicas del suelo. La salinidad es el principal factor limitante para el crecimiento y productividad de las plantas y la fertilidad del suelo. El objetivo del trabajo fue: Evaluar mediante bioensayos el efecto de los biosólidos en las propiedades físico químicas del suelo salino-sódico. La metodología constó de tres etapas: 1) el monitoreo de un reactor biológico de lodos activados y la caracterización de agua residual tratada; 2) la caracterización del suelo y el biosólido, y 3) la realización de bioensayos.

De los resultados obtenidos se encontró que el reactor de lodos activados operado como lote secuencial (SBR, siglas en inglés), se mantuvo en estado estable con valores promedio de eficiencia de remoción de materia orgánica de 60.1%; índice volumétrico de lodos (IVL) de 80.34 $mL*g^{-1}$; sólidos suspendidos volátiles (SSV) de 3340 $mg*L^{-1}$; el pH se mantuvo muy cerca de la neutralidad (6.7) y la conductividad eléctrica tuvo en promedio 0.151 $dS*m^{-1}$. Por otra parte, el agua residual tratada y el biosólido cumplen con la NOM-003-SEMARNAT-1997, y NOM-004-ECOL-2002 respectivamente, y pueden ser utilizados para riego y uso agrícola. Con la aplicación de 400 kg ha^{-1} de biosólido se incrementó el contenido de N total, carbono orgánico, materia orgánica y el potencial de hidrógeno en el suelo; por otra parte, la conductividad eléctrica y el porcentaje de sodio intercambiable en el suelo disminuyeron en 48.5 y 71.6% respectivamente. En el bioensayo se utilizaron plantas de *Ocinum basilicum* e *Hibiscus sabdariffa*, se registró un 0% de sobrevivencia en el suelo salino-sódico, mientras que con la adición del biosólido se obtuvo 100% de sobrevivencia en ambas especies.

En conclusión, el uso del biosólido mejoró las propiedades físico-químicas del suelo y permitió el establecimiento y crecimiento de *Ocinum basilicum* e *Hibiscus sabdariffa,* especies poco tolerantes a la salinidad.

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*Autor para correspondencia

ESTUDIO DE LA DEGRADACIÓN MICROBIANA DE HIDROCARBUROS PRESENTES EN LODOS RESIDUALES DE UN SERVICIO DE UN AUTOLAVADO CON BACTERIA FIJADORA DE NITRÓGENO DE VIDA LIBRE AISLADA DE SUELOS CONTAMINADOS DE HIDROCARBUROS

Verónica Lavadores-González* (1); Sergio Esteban Vigueras-Carmona (1); Graciano Calva-Calva (2); Josefina Pérez-Vargas (1)

(1) TESE, Div. Ing. Qca. y Bqca., Edo. de Méx, México.
(2) CINVESTAV- IPN, México DF, México.

El desarrollo de pequeñas industrias como los servicios de autolavado, en nuestros días genera miles de metros cúbicos de agua contaminada por gasolina, aceite automotriz, limpiadores y lubricantes entre otros, como resultado del lavado de carrocerías y motores. Siendo el agua un recurso natural no renovable muchas de estas pequeñas empresas han tomado conciencia de la necesidad de reutilizar el agua y en mucho de los casos es un requisito usar agua tratada en estos servicios de autolavado, por lo que varias investigaciones se han enfocado en el tratamiento de estas aguas residuales.

Existen procesos físicos, químicos y biológicos para el tratamiento de estas aguas, las cuales deben de cumplir ciertas características para poder descargarla a la red de alcantarillado municipal de acuerdo a la norma 02 de SEMARNAT, en donde generalmente se obtienen lodos residuales.

Los lodos residuales obtenidos por un tratamiento de coagulación y floculación del agua contiene sólidos entre ellos hidrocarburos totales, los cuales en muchas ocasiones son tirados o vertidos al drenaje sin ningún tratamiento previo, originando una fuente de contaminación.

La biorremediación es utilizada para disminuir la contaminación de aguas residuales industriales y contaminantes derivados del petróleo por microorganismos, ya sean bacterias del género de *pseudomonas* o bacterias fijadoras de nitrógeno de vida libre que tienen la capacidad de degradar diferentes derivados del petróleo entre ellos el queroseno y naftaleno.

En el presente trabajo se evaluó la eficiencia de eliminación de los hidrocarburos totales presente en los lodos residuales de esta agua tratada con la bacteria fijadora de nitrógeno de vida libre (BFNA). Se realizaron cuatro experimentos tomando como fuente de carbono estos lodos residuales, determinando el crecimiento de la BFNA, por absorbancia, concentración de proteínas, unidades formadoras de colonias, sólidos totales y la degradación de estos contaminantes por cromatografía de gases.

Este estudio sobre la degradación microbiana de hidrocarburos presentes en lodos residuales de un servicio de un autolavado con BFNA, nos permitirá conocer si esta bacteria es eficiente en la remoción de estos contaminantes.

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*Author for all correspondence

PENTACHLOROPHENOL MECHANISMS OF REMOVAL BY *Rhizopusoryzae* ENHE

H. Hugo León-Santiesteban (1); Kazimierz Wrobel (2); Sergio Revah (3);
Araceli Tomasini* (1)

(1) Department of Biotechnology.Universidad Autónoma Metropolitana-Iztapalapa, Mexico, D.F. Mexico.
(2) Department of Chemistry. Universidad de Guanajuato, Guanajuato, Mexico.
(3) Departament of Processes and Technology. Universidad Autónoma Metropolitana-Cuajimalpa, Mexico, D.F. Mexico;

Pentachlorophenol (PCP) was used as biocide, now the use is restricted, but the contamination of soils and groundwater persists. PCP is very toxic so it is important to study its removal. Filamentous fungi, principally *Basidiomycetes*, have been studied to remove PCP. It has been showed that *Rhizopusoryzae* ENHE, a zygomycete isolated from PCP soil contaminated, is able to tolerate and remove the toxic. Information is missing about the different physical, chemical, or biological mechanisms that could explain removal of PCP by zygomycetes. The aim of this study was to determine the effect of carbon source in the response of *R. oryzae* ENHE to the PCP toxicity and to explain the mechanisms involved in the PCP removal.

R. oryzae ENHE, registered number CDBB-H-1877, was used (1). PCP degradation was studied as a function of the carbon sources. Glucose (Glc) and glutamic acid (Glu) were used as carbon sources and ammonium sulfate (AS) and sodium nitrate (SN) as nitrogen sources (2). Sorption isotherms of PCP were determined using metabolically inactive *R. oryzae* CDBB-H-1877 wet biomass, at pH 3.3 and 7.5 Analytical analyses were carried out by gas and liquid chromatographic or colorimetric determination.

Results showed that biosorption was the main mechanism of PCP removal. Wet biomass of *R. oryzae* sorbed between 80-90% of initial PCP in less than 10 min, and maximal sorption capacity was 37 mg PCP g^{-1} biomass at pH 3.3 regardless of the carbon source. Degradation was another mechanism observed, in this case depends on carbon source used. In both culture, with glucose or glutamic acid, fungus presents methylation and dechloration (oxidative and reductive) as metabolic mechanisms. In cultures with glucose was determined 0.116 µg pentachloroanisol, 0.034 µg of tetrachlorhydroquinone and 0.0205 µg of 3, 4, 5-trichlorphenol, besides 6 non-identified compounds. In cultures with glutamic acid was determined 4.017 µg of pentachloroanisol, 0.152 µg tetrachlorhydorquinone, 2.73 µg 3,4,5-trichlorophenol and 3 non-identified compounds. The degradation mechanism of PCP included methylation and dechlorination reactions. In this study, 9 metabolites were found from PCP degradation with glucose and 6 with glutamic acid, indicating that biomass growth in glucose is more active that biomass growth in glutamic acid media.

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*Author for all correspondence

RESPONSE OF BARLEY PLANTS IN AN ARSENIC-POLLUTED SOIL TREATED WITH NANOSCALE ZERO VALENT IRON (nZVI)

S. Diez-Pascual (1); M.C. Lobo (1); A. González (1); J. Alonso (1); E. Rodríguez-Valdés (2); M. Gil-Díaz*(1)

(1) IMIDRA, Finca "El Encín", Alcalá de Henares 28800 (Madrid) Spain;
(2) Universidad de Oviedo, Mieres 33600 (Asturias), Spain;

The use of nanoscale zero valent iron (nZVI) as a new tool for the treatment of polluted soils (nanoremediation) has received considerable attention in recent years due to its high reactivity, in-situ application and cost-effectiveness. The nanoremediation processes with Fe (0) have resulted effective to degrade and/or immobilize a vast range of environmental pollutants in soil and water, such as chlorinated organic compounds, metals, metalloids and inorganic anions.

In the present study, brownfield soil highly polluted with As was used. In previous experiments performed by this research group we concluded that the application of commercial nZVI (NANOFER 25S, NANOIRON) significantly decreased the As availability in this soil. In addition, the lower availability of As in soil led to a decrease in the soil phytotoxicity to vetch (*Vicia sativa* L.*)* and barley (*Hordeum vulgare* L.*)* germination. In view of these results, an experiment in growth chamber in controlled conditions was performed to determine the stability of the As immobilization with nZVI and the influence on barley growth. Two doses of nZVI were assayed, 1% and 10% of the commercial suspension, four pots per condition and two plants per pot. After a month the barley plants were harvested. Then, As, Fe and macronutrients distribution in root and shoot were analysed. Physico-chemical soil parameters were evaluated to determine the impact of this nanoremediation strategy on soil properties. Also, As and Fe concentration in the different soil fractions was determined according to a sequential extraction procedure.

The As immobilization was more effective and stable at 10% of nZVI. The barley plants growth in the soil treated at 10% of nZVI presented a significantly higher yield than the ones from the soil treated at 1%, and from the untreated soil. The accumulation of As in plant was significantly lower in plants from the soil treated at 10%. In conclusion, the application of nZVI to soils is a promising strategy of As immobilization to remediate polluted sites. Long-term monitoring studies are necessary to evaluate the stability of the As immobilization with nZVI and its influence on soil properties and organisms.

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*Author for all correspondence

MICROORGANISMOS FIJADORES DE NITROGENO DE VIDA LIBRE CON CAPACIDAD DE DEGRADACIÓN DE CLORPIRIFOS

D. A. Moreno-Medina (1); J. Delgadillo-Martínez * (1); R. M. López-Romero (1); D. Espinosa-Victoria (1); M. R. Mendoza-López (2); O. García-Barradas (2).

(1) CP. Campus Montecillo, Edo. De México, México;
(2) SARA-UV, Veracruz, México.

El clorpirifos (O, O -dietil O-(3, 5, 6-tricloro-2-pyridinil)-fosforotioato) es un plaguicida organofosforado no sistémico que afecta el sistema nervioso de insectos blanco mediante la inhibición de su actividad acetilcolinesterasa por fosforilación. La acumulación en el suelo y cuerpos de agua, como consecuencia del uso indiscriminado, causa problemas potenciales al ambiente y a la salud pública.

El objetivo principal de este trabajo fue el aislamiento y caracterización de microorganismos capaces de degradar este tipo de compuestos para contar con herramientas para restaurar ambientes contaminados y tratar los residuos para su disposición final.

Los microorganismos se aislaron de suelos de cultivos de crisantemo con un amplio historial de aplicación de clorpirifos. Primeramente se aislaron nueve cepas capaces de crecer en concentraciones de hasta 350 mg L^{-1} de plaguicida en un medio de cultivo rico en nutrientes. Posteriormente se sometieron a crecer en medios de cultivo mínimos, en donde se seleccionaron solo cuatro cepas que crecieron en estas condiciones y en presencia del plaguicida como única fuente de carbono. Estas cuatro cepas también tuvieron la capacidad de fijar nitrógeno, los resultados con un nivel de significancia de $p < 0.05$ mostraron tasas de reducción de acetileno de 15.5, 23.9, 28.3 y 30.1 nmol h^{-1} mL^{-1}, para las cepas CP30, CP37, CP46 y CP47, respectivamente.

Es importante mencionar que para la cepa CP30, el crecimiento fue mayor en presencia del plaguicida (50 mg L^{-1}) comparado con el crecimiento en medio sin plaguicida en mediciones hechas durante 96 horas. El crecimiento máximo se presentó a las 72 ($9.2x10^9$ UFC mL^{-1}) y 48 ($6.7x10^8$ UFC mL^{-1}) horas, para la cepa cultivada con y sin plaguicida, respectivamente.

Complementariamente y tomando en cuenta que los microorganismos capaces de degradar estos compuestos lo hacen gracias a sus enzimas, se realizó un análisis filogenético de genes que codifican para las enzimas que degradan compuestos organofosforados, y que posiblemente se encuentren dentro de las cepas aisladas. La identificación molecular de las cepas y la evaluación de la remoción de clorpirifos está en curso.

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*Author for all correspondence

PAH REMOVAL IN A LONG-TERM BIOREMEDIATION EXPERIMENT OF ESTUARINE SEDIMENTS CONTAMINATED WITH DIESEL OIL

Mitsue Maia Nakazawa (1); Sávia Gavazza (2); Mario T. Kato (1); Lourdinha Florencio*(1)

(1) DECivil-UFPE, Recife, PE, Brazil;
(2) CAA-UFPE, Caruaru, PE, Brazil.

Mangrove ecosystems are one of the most sensitive and vulnerable to oil spills. The oil in mangrove soils can persist for years, making cleanup techniques limited. The aim of this research was to evaluate the bioremediation of estuarine sediments contaminated with diesel oil. Two experiments were performed: natural attenuation (NA) and natural attenuation stimulated (NAS) using a rhamnolipid biosurfactant. Sediment samples (30 cm) were collected at an uncontaminated area of Ilha da Cocaia, near SUAPE Port and Industrial Complex - Brazil. The samples were accommodated into 35 cm glass columns and the diesel oil (39,5 g diesel kg^{-1} sediment) was spilled on the surface of the columns. For the NAS the rhamnolipid was also added at the top of the columns at the beginning of the experiment. The essay was performed in triplicate inside of aquarium tanks filled with seawater, which was replaced each 12 hours/day. At determined time interval one column from each triplicate was removed and the concentrations of the 16 PAHs prioritized by EPA were monitored for 349 days. For analysis, the column profiles were separated into surface, middle, and bottom portions.

The composition of the microbial community was also evaluated through PCR-DGGE technics, aiming to contribute to the knowledge about the diversity of microorganisms involved. The analysis of PAHs with 3-4 rings (phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene) shows increasing concentrations in the middle and bottom portions particularly between days 111-338 and especially for NAS experiment. This may be related to the solubilization of diesel caused by biosurfactant used. 5-rings PAHs (benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a, h]anthracene) and 6-rings PAH (benzo[g,h,i]perylene) were also detected in deeper portions of the columns, suggesting the percolation of these PAHs with high molecular weight. Average total organic carbon concentrations were reduced by about 91% in NA samples and 89% in NAS samples with no statistical significant difference ($p > 0.05$) between treatments. It is concluded that the use of biosurfactant showed no significant difference in the bioremediation of estuarine sediment compared to NA experiment. Most PAHs analyzed showed fluctuations in their concentrations, possibly due to adsorption and desorption processes, making it difficult to obtain the removal efficiency. The analysis of DGGE gels indicated a slight shift in the microbial community structure over time. The occurrence of some intense bands suggests the presence of adapted organisms that may have played an important role in degradation of diesel oil.

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*Author for all correspondence

FEASIBILITY OF A BIOREMEDIATION PROCESS COUPLED TO CO_2 FIXATION USING GREEN MICROALGAE FOR HYDROCARBON CONTAMINATED SOIL

Abdeldjalil ABID **(1)**; Yasmine Souissi (2); Faten Saïdane Bchir (1); Fatma Ezzahraâ Ben Ammar (1) and Moktar Hamdi (1)

(1) Laboratory of Microbial Ecology and Technology, Department of Biological and Chemical Engineering, National Institute of Applied Science and Technology (INSAT), Tunis, University of Carthage, Tunisia;
(2) Department of Biological Engineering, Polytechnic Institute 30, Road Khereddine Pacha 1002 Tunis, Private University of Tunis (ULT), Tunisia;

Petroleum industries' activities, such as oil exploration, production transportation and refining raise serious threats on human health and environment. The hydrocarbon spreads on the ground water surface and can also be conducted to ground water as well as on soil particles surfaces. Bioremediation consists in a process that uses microorganisms in order to mineralize hydrocarbon contaminant. In the other hand this mineralization it is important raison for greenhouse effect through the CO_2 that result.

In the current study, biodegradation activity of hydrocabonoclastic microflora and biostimulation with inorganic nutrient (NPK) were investigated through the determination of optimal conditions which can improve bioremediation process. It has been noticed that after 56 days, about 98% of the total petroleum hydrocarbon's (TPH) removal rate in the soil treated by microcosm's technique was observed relative to approximately 2.22×10^7CFU/g soil of bacterial number. This performance was achieved with microbial metabolism is concomitant with hydrocarbons biodegradation. Moreover, hydrocarbon fractions including alkanes and aromatic compounds were degraded faster than the other complex fractions according to GC-MS and FT-IR analysis.

In order to fix the CO_2 resulting from mineralization and to reduce the greenhouse effect, a new strategy based on microalgae growth in the microflora involved in the contaminated hydrocarbon was established. The result showed a good growth of microalgae associated with a high removal rate of hydrocarbon and minerals amount in treated soil.

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*Author for all correspondence

ISOLATION AND CHARACTERIZATION OF ARSENIC-RESISTANT ENDOPHYTIC BACTERIA ASSOCIATED WITH PLANT GROWING ON MINE TAILINGS

Brenda Román-Ponce* (1); Salvador Embarcadero-Jiménez (1); Ma. Soledad Vásquez-Murrieta (1); En Tao Wang-Hu (1)

(1) ENCB-IPN, México DF, México;

Mexico is one of the most important mining countries in the world; therefore huge mine spoils can be found throughout the country. Some endemic plant species that tolerate high concentration to heavy metals have been found in the mine spoils. These plants may harbor microbial communities with efficient capacity to detoxify metals due transforming them into insoluble salts or relatively oxidation states non-toxics or some of them can utilize arsenic in their metabolism, either by means of generating energy through chemoautotrophic arsenite oxidation by using arsenate as a terminal electron acceptor. Until now, there are no reports of endophytic bacteria associated with plant resistant to heavy metals in mine tailings from Villa de la Paz, San Luis Potosí, so the aim of this work was isolation, characterization and evaluate the capacity to arsenic transforming of these bacterial.

A total of 52 morphologically different colonies were isolated from the root samples of *Prosopis* sp. and *Spharealcea angustifolia***.** The colony morphology of the strains was circular, convex and entire type for 80% the strains, which had irregular colonial margins; the remaining 20% had irregular colonial margins. Cellular shapes of 75% the strains were found to be Gram positive bacilli, 23% of them were Gram positive cocci and only 2% were Gram negative bacilli. Other parameters evaluated were the ability of microorganisms to grow at various pH values and various salt concentrations of the test isolates were obtained which grew at pH 11 and 15% salinity. Almost half of the isolates showed enzymatic activities such as amylase, pectinase and xylanase; while some isolates showed other enzymatic activities. The endophytic bacterial showed a high degree of resistance to heavy metals, especially to arsenic, zinc, and copper. More than 40 % isolates showed heavy metals resistance in liquid MES buffered minimal medium (MBMM) containing heavy metal ions at 1.5 mM for Cu^{2+}, Zn^{2+} and Pb^{2+}, 100 mM for $As^{5+}$and 20 mM As^{3+}, respectively. The order of the toxicity of the metals to strains were found to be Pb>Cu>Zn As^{3+}>As^{5+}. Arsenic transformation capacity of 26 isolates was tested on CDM supplemented with 2 mM of As^{3+} and As^{5+}. Fifty-eight percent of the tested isolates were capable of transforming arsenate to arsenite based on the formation of red-brownish precipitate in the CDM medium extract. While forty-two percent of the endophytic bacteria oxidized arsenite.

Our results demonstrated that the endophytic bacteria isolated had the innate capability of expressing multiple heavy metal resistance, plant growth-promoting characteristics, and several enzyme activities facilitating the plant infection. In addition, some of them could transform arsenic which is a feature with potential in the remediation of sites contaminated with this heavy metal.

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*Author for all correspondence

ACUMULACIÓN DE Hg Y As POR *Brassica rappa L.* INDUCIDA POR AGENTES QUELANTES Y TRATAMIENTOS NUTRICIONALES CULTIVADA EN RESIDUOS MINEROS

Juan A. Flores-de la Torre* (1); Rubí E. Luna-Sandoval (1);
Guillermo Rodríguez-Hernández (2); Eduardo Manzanares-Acuña (3)

(1) Universidad Autónoma de Zacatecas, Unidad Académica de Ciencias Químicas, Zacatecas, México,
(2) Universidad Autónoma de Zacatecas, Unidad Académica de Ciencias Agronómicas, Zacatecas, México.
(3) Universidad Autónoma de Zacatecas, Unidad Académica de Estudios Nucleares, Zacatecas, México.

La minería genera una gran cantidad de residuos ricos en metales pesados que son depositados en la superficie del entorno minero, ocasionando que dichos metales puedan incorporarse a la cadena trófica, además de provocar un impacto ambiental. Entre las estrategias que se han empleado con éxito para inmovilizar o extraer a los metales pesados en los ecosistemas se encuentra la fitoremediación, donde además se puede ver favorecida por la acción de agentes quelantes que puedan coordinar simultáneamente a un ión metálico y tratamientos calcáreos, orgánicos y nutritivos en los suelos contaminados.

Se obtuvieron jales mineros de la mina “El Bote”, Zacatecas (22º46´35´´N, 102º26´21´´W), en un muestreo de tipo exploratorio basado en la Norma NMX-AA-132-SCFI-2006, logrando obtener muestras representativas del área, las cuales se tamizaron, se les determinó el pH y cuantificó mercurio y arsénico, por medio de espectroscopía de fluorescencia de rayos X (EDXRF) para posteriormente realizar cultivos en dichas muestras, con 6 tratamientos de mezclas diferentes de soluciones nutritivas, así como con 4 agentes quelantes (Tiocianato de amonio, ácido cítrico, ácido Arscórbico y EDTA), usados con el propósito de inducir la hiperacumulación de metales. Una vez cosechadas las plantas se cuantificó arsénico (As) y mercurio (Hg) en las diferentes secciones de la planta (hoja, tallo, raíz) y en las rizósferas donde fueron cultivadas.

Se encontraron concentraciones de As de hasta 2500 ppm y 430 ppm de Hg en los suelos y valores de pH desde 7 hasta 7.83, lo que pudiese provocar un comportamiento distinto en la bioquímica de la absorción de los metales por las plantas. Referente a la aplicación de materia orgánica en combinación con soluciones nutritivas incrementaron la biomasa y en combinación con agentes quelantes como el EDTA y el ácido ascórbico, se triplicó la concentración de As en la planta (100 ppm) y la concentración de Hg absorbida por la planta se incrementó de 45 ppm a 70 ppm.

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*Author for all correspondence

RESPONSE OF RHIZOSPHERE MICROBIAL COMMUNITIES OF *Hordeum vulgare* TO DIFFERENT DOSES OF CR (VI) CONTAMINATION

Maite Rozas, Águeda González, Mar Gil-Díaz, Araceli Pérez-Sanz, Mª Carmen Lobo, Pilar García-Gonzalo*

IMIDRA. Finca "El Encin" A-2, Km 38,2 28800 Alcalá de Henares (Madrid), Spain.

Rhizosphere, as an important interface of soil and plant, plays an important role in phytoremediation of contaminated soil by heavy metals. Plants and bacteria can form specific associations and the adaptation capabilities of both partners are of importance in minimizing the detrimental effect of heavy-metal pollution. Many rhizobacteria, tolerant to heavy metals, are known to affect heavy metal mobility and availability to the plant and therefore, have potential to enhance phytoremediation processes.

The aim of this study was to analyze the rhizosphere microbial structure and diversity, and microbial biomass, from two genotypes of *Hordeum vulgare* L. grown on spiked soils treated with two different doses of Cr (VI). A pot experiment was performed in triplicate with cultivar Pedrezuela and breeding line "502" grown on agricultural soil spiked with two different chromium doses (20mg.kg^{-1} and 60mg.kg^{-1} soil).

Denaturing gradient gel electrophoresis (DGGE) of 16S rDNA fragments has been used to profile a structure of the bacterial community in 9 soil samples including rhizosphere and non-rhizosphere control soils. A hierarchical cluster analysis was calculated from the DGGE banding patterns generated from the universal bacterial primers 341F and 907 R.

The UPGMA clustering analysis revealed the differences among soil samples. The bacterial profiles were separated into two major clusters with a similarity about 53%, indicating that major differences were related to chromium effect. Soil effect was observed with a similarity coefficients of 0,57% and 0.66% and finally, a genotipic effect was found among DGGE profiles. Bacterial diversity was compared through Richness and Shannon-Weaver indices and data revealed a hormetic response to low doses of chromium. This stimulatory effect was also observed on microbial biomass.

Possible mechanisms responsible for the low toxicity of Cr (VI) to bacteria may include the blockading of chromate transport, chromate-active efflux and the activation of the Cr (VI) decreasing mechanisms. However hormetic response is suggested as an advantage in the area of phytoremediation because the stimulation of root growth and, enhanced bacterial growth and diversity, could improve the uptake of toxic metals from soils.

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*Author for all correspondence

EFECTO DE HIDROCARBUROS SOBRE LA GERMINACIÓN Y PERFIL DE COMPUESTOS FENOLICOS DE *Cyperus laxus* CRECIDAS *IN VITRO*

***Sarahí I. Morales-Navarro* (1);** Noemí A. Rivera-Casado (2);
Mónica S. Salazar-Plata (1); Octavio Gómez-Guzmán (2); Josefina Pérez-Vargas (3);
Graciano Calva-Calva*(2)

(1) UVM, Ciencia de la Salud, México DF, México;
(2) CINVESTAV- IPN, Biotehcnology and Bioengineenig Department, México DF, México;
(3) TESE Div. IBQ, Edo. México, México.

Cyperus laxus es una planta que crece en la zona tropical del sur de México y es una de las especies pioneras de sitios del estado de Tabasco intemperizados después de haber sido impactados por derrames de petróleo crudo. En estudios anteriores de fitorremediación de suelos de esos sitios se encontró que esta planta puede remover grandes cantidades de hidrocarburos, incluyendo poliaromáticos (HPA). Sin embargo se desconoce el mecanismo bioquímico que usa la planta para la remoción de estos compuestos, especialmente en ausencia de microorganismos, así como el efecto de los hidrocarburos sobre su germinación y fisiología. El objetivo de este estudio fue evaluar el efecto del antraceno (ANT), fenantreno (FNN) y queroseno (K) sobre la germinación, crecimiento, cambios fisiológicos y perfil de compuestos fenólicos de esta planta en condiciones *in vitro* de cultivo.

Se monitoreó el desarrollo y cambios fisiológicos semanalmente por 35 d, después de los cuales se evaluó la remoción de hidrocarburos y el perfil de compuestos fenólicos previamente reportados como potenciales mediadores metabólicos en la fitoremoción de HPA.

Los resultados demuestran que de los hidrocarburos sólo el K concentraciones mayores a 3mM afectó drásticamente la frecuencia de geminación y el crecimiento. Sin embargo, a la tercera semana el FNN produjo un crecimiento ageotrópico paralelo al medio de cultivo con un claro incremento del grosor de las hojas con formación de caireles de hasta 180°. Después de 35 d de cultivo se observó una clara remoción de los hidrocarburos a partir del medio de cultivo. Por otro lado, para analizar el perfil de compuestos fenólicos estos se extrajeron por separado de las hojas y de raíz-bulbo. Los extractos se fraccionaron en la parte acuosa y la orgánica para su análisis por HPLC UV. En general se detectó la presencia de un gran número de fenilpropanoides y flavonoides libres, sin embargo en los tratamientos con hidrocarburos se observaron derivados de los mismos compuestos pero conjugados con los hidrocarburos. En la fase orgánica, se detectó ANT y FNN libres y algunos de sus metabolitos de oxidación, principalmente el *trans*-4,3-dihidrodiol-fenantreno y el 9-fenantrilglucopiranósido. En conclusión la germinación *in vitro* se inhibió drásticamente con concentraciones arriba de 5 mM de K provocando cambios fisiológicos importantes en las plantas. La presencia de fenoles conjugados con los hidrocarburos exógenos, además de los metabolitos derivados de la trasformación de los hidrocarburos, soportan la hipótesis de que la conjugación de metabolitos vegetales con los HPA puede ser uno de los mecanismos de remoción de estos compuestos en los sistemas de fitorremediación.

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*Author for all correspondence

POTENTIAL CRUDE OIL DEGRADATION BY A MICROBIAL CONSORTIUM ISOLATED FROM THE GULF OF MEXICO

Rosa Canul-Puc (1); ***Ulises García-Cruz*** (2); Juan Sánchez-Àvila (2); Gerardo Gold-Bouchot (2); M. Leopoldina Aguirre-Macedo *(2)

(1) ITESCAM, Campeche, México;
(2) CINVESTAV- IPN, Yucatan, México;

Crude oil can be deliberately or accidentally released into the environment leading to serious pollution problems resulting on disturbances of biotic and abiotic components of ecosystems. The bioaugmentation employs the biodegradative potentials of microorganisms that can be used to recover contaminated sites. No single microorganisms has the enzymatic ability to metabolize the different compounds of oil, nevertheless a consortium composed of many different bacterial species can degrade oil significantly. The aim of this study was to isolate a microbial consortium from marine sediments capable to biodegrade oil constituents. Marine sediment samples collected from different localities in the Gulf of Mexico were screened for microorganisms capability of oil degradation.

Three mixed aerobic hydrocarbon-degrading microbial communities were obtained (R1F, R3F and R2M) after serial subculturing using Bushnell-Hass medium amended with crude oil. After that the R1F and R3F consortium were tested in 1L flask using a C/N rate of 8 and 10 in mineral medium added with 379.0 mg/l and 461.0 mg/L of light crude oil respectively. The R2M consortium was tested under marine condition using real seawater and mineral medium at C/N=10 rate in 1 litter flask adding 862 mg/L light crude oil. All flasks were operated for 30 days at 24°C and 400 rpm. In the three consortium the production of biosurfactant was detected. Highest removal rate of total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbons (PAH´s) and biomarkers, was observed at C/N=10 in comparison with C/N=8 rate. In the case of TPH the highest consumption rate was observed in R2M (q_c=27.2 mg/L·d) > R3F (q_c=21.0 mg/L·d) > R1F (19.6 mg/L·d) achieving consumption efficiency of 92, 88 and 82% respectively. Similar values were obtained for PAH´s where all quantified aromatics compounds were degraded around 90%. The biomarkers were degraded above 90% in C/N rates 8 and 10. In all the consortiums hydrocarbonoclastic bacteria were detected and the increased in C/N rates favored the biomass concentration in the flasks. Bacteria of the genus *Serratia*, *Pantoea* and *Klebsiella* were identified.

It is concluded that, the R1F, R3F and R2M consortium were capable to degrade all oil constituents with high efficiency at the C/N=10 rate. Not effect between the consortiums tested with freshwater and seawater was observed. It was possible obtain different consortium from not polluted sites with high capacity to remove hydrocarbons, which can be applied to the remediation of different polluted environments.

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*Author for all correspondence

EVALUATION OF HEXADECANE'S DEGRADATION USING DIFFERENT MARINE SEDIMENTS FROM RIVERS AND LAGOONS OF THE GULF OF MEXICO

N. Ulises García-Cruz (1); Juan Sánchez-Ávila (1); David Valdés-Lozano (1); Gerardo Gold-Bouchot (1); M. Leopoldina Aguirre-Macedo*(1)

(1) CINVESTAV- IPN, Mérida, Yucatan.

The oil industry in the south of The Gulf of Mexico is extensive due to extraction and transport of this energy source. Therefore, the rivers and lagoons in this area are prone to contamination by petroleum spills. On another hand, one of the principal hydrocarbon present in the petroleum, is the hexadecane; which is commonly is used to evaluate the capacity of some microorganism for petroleum degradation in water and soil. Thus, the goal of this research was to evaluate the effect of environmental factors (hydrocarbonoclastic bacteria (HCB), PAH´s, organic matter, D.O., *E*° and turbidity) on the biodegradation of hexadecane (HXD).

Nine marine sediments were used: six from rivers (Gonzalez, Coatzacoalcos, Grijalva, San Pedro-San Pablo, Tonalá, and Papaloapan) and three from lagoons (Mecoacan, Ostion, and Champoton). All samples were taken from the river or lagoon delta. The experiment was carried out by duplicate in serum bottles (60 mL), sealed with PTFE lined rubber septa and aluminum crimp caps. Each serum bottle contained 15 mL of sludge, 35 mL of seawater, 0.06 mL/L of Tween-20 and 250 mgHXD/L. The flasks were incubated for 24 days at 24°C and serum bottles were sacrificed every 7 days. The hexadecane was measured with GC-FID whereas the polycyclic aromatic hydrocarbons (PAHs) by GC-MS. The Gompert´s model was used to calculate the hexadecane consumption rate (q_s). The heterotrophic and hydrocarbonoclastic bacteria were quantified and the pH, OD, alkalinity, *E*° were measured following the APHA methods.

The highest rates of biodegradation were observed in sediments of Gonzalez river (39.8 mgHXD/L·d) and Champoton lagoon (34.1 mgHXD/L·d) with an efficiency of 89 and 86% respectively and the lowest consumption rate was found in Coatzacoalcos river with 5 mg/L·d and an efficiency of 47%. In the case of Ostion lagoon and Grijalva river, a low consumption rate was observed (12.5 and 8.6 mgHXD/L·d), nevertheless both sediments reached an efficiency around 80%. In all evaluated sediments, microbial activity was detected. One factor that could possibly affect the consumption rate is the presence of PAH´s in the sediments, as was observed in the Coatzacoalcos river where was detected the highest concentration (0.98 µgPAHs/g). Another factor that affect the hexadecane consumption rate was the concentration of the HCB how was observed in Tonala river and San Pedro-San Pablo river with the low concentration of HCB and low q_s (13.6 and 16.1 mgHXD/L·d).

It is concluded that the consumption rate of HXD may be inhibited by the PAH´s concentration in the sediments. In other hand, the concentration of HCB did not favor the degradation of HXD, maybe the presence of other kind of bacteria could have participated in the consumption of HXD.

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*Author for all correspondence

VERMIREMEDIATION OF SOIL CONTAMINATED WITH CRUDE OIL

Luke Martinkosky*(1); Seana K. Davidson (1); Heidi L. Gough (1)

(1) University of Washington, Civil and Environmental Engineering, Seattle, USA

Soil contaminated with hydrocarbons originating in crude oil is a widespread problem and these compounds are difficult to remediate. Earthworms are common in soils and stimulate microbial activity and the rapid breakdown of organic materials through mechanical activity and by digestion of materials in their gut. Past research has indicated that earthworms can promote degradation of some petroleum hydrocarbons. Using the species *Eisenia fetida* and *Aporrectodea caliginosa*, our research examines the use of earthworms to degrade hydrocarbons in a defined laboratory made soil and in soil from an active remediation site.

Lab scale experiments were established to evaluate hydrocarbon degradation over time in the presence and absence of worms. Small scale toxicity tests for both species of earthworm were performed using the remediation site soil. The defined soils were contaminated with crude oil at a concentration of approximately 30,000 milligrams per kilogram (mg/kg) of Total Petroleum Hydrocarbons (TPH) and degradation tests were performed. The soil treatments were monitored over time for TPH using gas chromatography with a flame ionization detector (GC-FID). The treatments evaluated TPH loss due to volatilization (no feed, no worms), microbial activity (organic feed, no worms), and earthworm-stimulated degradation (organic feed with *E. fetida*). Each of these treatments was performed with and without an inoculum of remediation site soil to determine the effect of the existing microbial community on degradation rates. In addition, TPH degradation in remediation site soil was also evaluated in a similar manner. These treatments evaluated TPH loss due to volatilization (no feed, no worms), microbial activity (organic feed, no worms), *E. fetida*-stimulated degradation (organic feed with *E. fetida*), and *A. caliginosa*-stimulated degradation organic feed with *A. caliginosa*).

Earthworm toxicity levels were established for both species at up to 30,000 mg/kg TPH. In the defined soils with earthworms and remediation site inoculum TPH concentrations declined from 29,000 mg/kg to 15,000 mg/kg after 154 days, while defined soils with earthworms and no inoculum had TPH concentrations change from 29,000 mg/kg to 19,000 mg/kg after 154 days. Both of these reductions were significantly greater than TPH reductions observed in the volatilization and microbial activity controls. In the remediation site soils contained *E. fetida*, TPH declined from 25,500 mg/kg to 9,600 mg/kg in 70 days and TPH declined from 23,500 mg/kg to 7,000 mg/kg in the presence of *A. caliginosa* after 70 days. These reductions were more than twice what was observed in the volatilization and microbial activity controls. We conclude that the use of earthworms to degrade hydrocarbons is a viable remediation strategy.

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*Author for all correspondence

INTRACELLULAR ENZIMES OF *Pleurotus ostreatus* INCQS 40310 IN THE ATRAZINE DEGRADATION OU AINDA TRANSFORMATION

Aline Ramalho Brandão Pereira* (1,2); Alexandre França (2,3); Patrícia Maia Pereira (1,2); Manuela da Silva (4); Viridiana Ferreira-Leitão (1,2)

(1) UFRJ, Rio de Janeiro, Brasil;
(2) INT, Rio de Janeiro, Brasil;
(3) IFRJ, Rio de Janeiro, Brasil;
(4) FIOCRUZ, Rio de Janeiro, Brasil.

The atrazine herbicide has been increasingly used worldwide in different crops of economic relevance, including sugar cane, which is very important and traditional in Brazil due to sugar and ethanol production. The widespread use of this pesticide, already prohibited in some countries, can contaminate soil and water bodies. Besides the prevention of contamination, it is crucial to invest in decontamination technologies. Within this context, the aim of the present study is to evaluate the degradation of atrazine by *Pleurotus ostreatus* INCQS 40310, and relate it with the intracellular enzymes (cytochrome P450 enzymes) and extracellular enzymes (laccase, manganese peroxidase and lignin peroxidase) production.

For the experiments, atrazine (10 mg/L) was added to the culture medium previously optimized by a fractional factorial experimental design for atrazine degradation. Then the fungus was inoculated (three disks containing mycelium) in flasks and incubated at 30 °C, 150 rpm. After 10, 15 and 20 d, samples were analyzed and the concentrations of the reminiscent herbicides were measured by HPLC. 71% of atrazine degradation was measured after 10 days. In this condition, the extracellular activity of laccase, manganese peroxidase and lignin peroxidase was determined and only a low laccase activity was detected.

As the action of extracellular enzymes in the atrazine biotransformation was not observed, we started to evaluate the role of intracellular enzymes in this process. For the intracellular atrazine degradation test, a similar experiment was conducted with and without atrazine and after 20 days of incubation, the produced biomass was filtered and washed three times with sodium acetate buffer (pH 5.5) for the extracellular enzymes removal. Then, the biomass was re-suspended in sodium acetate buffer (pH 5.5) and incubated at 30°C, 150 rpm, with atrazine (10 mg/L) for 96 h. After this period, 9% of atrazine was degraded by biomass cultivated with atrazine and 44% by biomass cultivated without, probably due to intracellular enzymes activity, such as cytochrome P450. The low rate degradation occurred in the cultivation with atrazine may be explained by the atrazine toxicity. More experiments are needed to understand this effect of the atrazine in the fungus metabolism.

These results show an initial step for the elucidation of *P. ostreatus* INCQS 40310 enzymes role on atrazine degradation.

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*Author for all correspondence

BIOLIXIVIACIÓN DE METALES DE SEDIMENTOS ANAERÓBICOS DEL RÍO RECONQUISTA (ARGENTINA) COMO ESTRATEGIA POTENCIAL DE REMEDIACIÓN

Natalia Porzionato*(1); Roberto Candal (1,2); ***Gustavo Curutchet*** (1,2).

(1) 3iA-UNSAM; Buenos Aires, Argentina;
(2) CONICET, Argentina.

Los sedimentos anaeróbicos de cursos de agua sujetos a contaminación de origen industrial, acumulan contaminantes persistentes, fundamentalmente metales pesados. En condiciones oxidantes, generadas por dragado o por escorrentías de lluvias, el reservorio de metales acumulado puede solubilizarse en la columna de agua con el consiguiente impacto negativo al ecosistema. Los procesos biocatalizados de oxidación y reducción de compuestos del azufre en el sedimento son las reacciones clave, al estar involucrados en los procesos de movilización e inmovilización de metales pesados. Son estos mismos bioprocesos, los que en condiciones controladas podrían ser empleados para la remediación de los sedimentos contaminados.

El objetivo del presente trabajo fue evaluar la posibilidad de acidificación y lixiviación de metales pesados de sedimentos anaeróbicos de la Cuenca del Río Reconquista ante cambios en el potencial redox del sistema, y proponer a la biolixiviación en biopilas *(heap leaching)* como una estrategia de remediación. Dicha evaluación se llevó a cabo a través de ensayos estáticos y cinéticos. Los primeros, realizados según el método de Kersten & Förstner, en algunos casos no mostraron significativa tendencia a la acidificación. Aun así, las experiencias cinéticas de oxidación del sedimento en modo *batch*, demostraron la factibilidad de que procesos de acidificación ocurran en los sedimentos, con incrementos de sulfatos y metales en solución.

La potencialidad de remediación por biolixiviación fue demostrada a través de ensayos en biopilas conteniendo 100 gr de sedimento con 340 mg Kg^{-1} de zinc. Los sistemas se confeccionaron en columnas de 6 cm de alto y 21 cm de diámetro, acondicionando con perlita para mejorar del drenaje y agregando concentraciones crecientes de azufre elemental (1%, 2% 5% p/p) para incrementar el nivel de acidificación. Luego de 3 meses de acidificación generada por el riego periódico, se alcanzó una extracción del 70% del Zn inicial en el sistema con 5% p/p de azufre dispuesto de manera superficial sobre la biopila. Además se lograron enriquecer, aislar e identificar las especies bacterianas responsables de la catálisis de los procesos de movilización e inmovilización de los metales en el sedimento.

Se concluye que, dada la factibilidad que un drenaje ácido ocurra, generando un aumento en la disponibilidad y peligrosidad de metales en condiciones oxidantes, es recomendable no manipular indiscriminadamente los sedimentos de cursos de agua contaminados como el Río Reconquista, siendo posible llevar a cabo la remediación de estos por biolixiviación en biopilas de manera simple y económicamente viable.

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*Author for all correspondence

COMPARACIÓN ENTRE BIOESTIMULACIÓN Y BIOAUMENTACIÓN PARA LA BIORREMEDIACIÓN DE SUELOS CONTAMINADOS CON HIDROCARBUROS

Diana Cabañas-Vargas; Alma I. Corona-Cruz*; Rafael Rojas-Herrera.

FIQ-UADY, Mérida, Yuc, México;

Los microorganismos son el elemento catalizador en los procesos de biorremediación, debido a que son considerados los responsables del proceso de transformación de contaminantes como son, los hidrocarburos policíclicos aromáticos (hpa). Debido a las características particulares de todo microorganismo, el descubrimiento de nuevas especies, así como su caracterización, es de gran interés, a fin de llevar a cabo una implementación eficiente de este tipo de procesos evaluando todo bajo las condiciones locales. En este estudio se realizó la biorremediación de un suelo contaminado con hidrocarburos (petróleo) a escala de laboratorio, para evaluar la bioestimulación frente a la bioaumentación con elementos propios de la región.

Para la inoculación del tratamiento por bioaumentación se utilizó un consorcio microbiano autóctono con capacidad para degradar hidrocarburos. Se diseñaron 4 tratamientos: **1**. Blanco, que contenía Suelo (S-1Kg)+Petróleo crudo (P-40g), **2**. S+P+C (residuos putrescibles-200g), **3**. S+P+F (fertilizante-200g), **4**. S+P+ M (consorcio microbiano). Se empleó suelo tipo arcilloso con tamaños de partículas entre 2 y 2.36 mm, para facilitar el proceso aeróbio y lograr una distribución homogénea del contaminante, los experimentos se montaron en recipientes de cristal. El consorcio de microorganismos utilizado para estos tratamientos, fue aislado de suelo en las imediaciones de un taller mecánico (Martín M., 2010). Los nutrientes empleados fueron: Fertilizante (Vigoro ®) y Composta doméstica. Los tratamientos se muestrearon semanalmente durante un periodo de 5 semanas y fueron mezclados con regularidad. A las muestras tomadas se les analizó por un lado, de manera general la presencia de mesofílicos aerobios (en medio ACS), así como hongos y levaduras (PDA) y por otro el contenido de hidrocarburos mediante una técnica gravimétrica en la que se realiza la extracción (agitación-centrifugación) de los hidrocarburos del petróleo del suelo y que está basada en los métodos 3500B y 3540C de la EPA

La bioestimulación y la bioaumentación aumentaron la capacidad de degradación de petróleo y las tasas de reproducción de los microorganismos, observando que la bioaumentación reporto el mayor porcentaje de degradación (77%). No se encontró diferencia significativa entre la bioestimulación dada por fertilizante y el material putrescible (52%). Por otro lado los cálculos de UFC señalan que la bioestimulación claramente favoreció el crecimiento de los microorganismos.

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*Author for all correspondence

BIODEGRADACIÓN DE ATRAZINA EN SUELOS AGRÍCOLAS DE ESTADO DE MORELOS MÉXICO

Ana G. Ortega-Hernández; Carlos Díaz-Gómez; ***Rosa Angélica Guillén-Garcés****

Universidad Politécnica del Estado de Morelos, Ingeniería en Tecnología Ambiental, Morelos, México

La atrazina es el tercer herbicida más utilizado en México en la eliminación de maleza de hoja ancha en cultivos de sorgo, caña de azúcar, trigo y piña. Es un compuesto poco soluble en agua y posee una alta movilidad en el suelo, aumentando el riesgo de contaminación de los cuerpos de agua. El uso de Atrazina está prohibido en la Unión Europea y restringido en Estados Unidos. En México no existe una regulación para su uso. El estado de Morelos es productor de maíz, sorgo y caña de azúcar y este herbicida es ampliamente usado en el estado.

El objetivo general fue caracterizar la biodegradación de Atrazina en diferentes muestras de suelo agrícola del Estado de Morelos. Se hicieron muestreos en tres municipios del estado, Moyotepec, Yecapixtla, y Huautla, con base al grado de permeabilidad. El suelo fue caracterizado fisicoquímicamente y se realizó cinética de biodegradación en las diferentes muestras de suelo, aplicando una concentración similar a lo recomendado en campo. Se aplicó Atrazina al 100% y el producto comercial con 43% del ingrediente activo. Las condiciones experimentales fueron 50% de la capacidad de campo y se incubaron a 32 ±1°C durante 29 días.

Los resultados mostraron que las velocidades de biodegradación de la Atrazina comercial en los suelo de Moyotepec, Yecapixtla y Huautla fueron de $0.0117 d^{-1}$, $0.0245 d^{-1}$ y $0.0425\ d^{-1}$; con vidas medias de 59.24, 28.29 y 16.31 días y porcentajes de biodegradación de 20.5, 50.07 y 71.77%, respectivamente. En suelos donde hay mayor concentración de materia orgánica y una aplicación recurrente del herbicida las velocidades de degradación fueron mayores, debido probablemente a la presencia de microorganismos capaces de degradar el herbicida.

Se puede concluir con base en la velocidad de degradación de Atrazina al 100% y Atrazina comercial se pudo observar que la presencia de aditivos y coadyuvantes contenidos en el producto comercial disminuye la velocidad y porcentaje de biodegradación del herbicida, favoreciendo otros procesos de disipación

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*Author for all correspondence

EFECTO DEL 1,2,3-TRICLOROBENCENO EN LA BIODEGRADACIÓN DE UN SUELO CONTAMINADO CON HEXACLOROCICLOHEXANO EN UN REACTOR DE SUELOS ACTIVADOS CON AMBIENTE DESNITRIFICANTE

Gonzalo M. Escobedo-Acuña (1), Beni Camacho-Pérez (1), Elvira Ríos-Leal (1); M. T. Ponce-Noyola (1); J. Barrera-Cortés (1); Héctor M. Poggi-Varaldo***** (1), H. B. C. Poggi-Rinderknecht (2); N. Rinderknecht-Seijas (2)

(1) CINVESTAV- IPN, México DF, México;
(2) ESIQIE-IPN, México DF, México;

El γ-hexaclorociclohexano (lindano) es uno de los plaguicidas que más se han utilizado en el mundo. Es un compuesto altamente halogenado, tóxico y persistente en el ambiente. Durante la ruta de degradación anaeróbica del lindano, se producen diferentes metabolitos intermediarios, uno de ellos es el 1,2,3-Triclorobenceno (TCB), el cual ejerce un efecto sobre el desempeño del proceso de biorremediación de un suelo contaminado con lindano y obteniendo así una remoción pobre del lindano. Existe poca información referente al efecto que los metabolitos intermediarios en la ruta de degradación de un compuesto, puedan tener sobre la biodegradación del compuesto madre así como la falta de métodos para obtener dicha información.

Los objetivos de este trabajo fueron *(i)* determinar las velocidades iniciales de degradación del lindano a diferentes concentraciones, *(ii)* determinar las constantes cinéticas de degradación *(Q_i, K_s y K_i)* y *(iii)* determinar qué efecto ejerce el TCB en la biodegradación del lindano en un RSA con ambiente desnitrificante que trata un suelo rico en materia orgánica y contenido de arcilla (suelo pesado).

Para la implementación de la metodología de trabajo se realizó una analogía entre una cinética de degradación microbiana y una cinética enzimática, esto es posible debido a que ambas tienen la misma tendencia hiperbólica, además de que una cinética de degradación microbiana es conducida por enzimas predominantes. Se realizaron cinéticas microbianas de degradación de lindano a diferentes concentraciones y en presencia de distintas concentraciones de TCB, con el objetivo de obtener las velocidades iniciales de degradación del lindano y así una curva hiperbólica para realizar una transformación de tipo Lineweaver-Burk y determinar el efecto que ejerce el TCB.

Se obtuvieron las velocidades iniciales de degradación (Q_i) de una concentración inicial de 100 mg de lindano por Kg de suelo, a diferentes concentraciones de TCB (0, 32.5, 65, 120 mg/Kg) resultando en una disminución de Q_i al aumentar la concentración de TCB presente en la cinética de degradación. De igual manera se presentó para las cinéticas de degradación con una concentración de 50 y 150 mg de lindano por Kg de suelo pesado.

En base a lo anterior se puede concluir que el TCB tiene un efecto negativo sobre la biodegradación de lindano en un suelo pesado, el efecto que ejerce es análogo a una inhibición no competitiva, en donde el consorcio microbiano tiene igual afinidad por el sustrato madre que por el metabolito intermediario, sin embargo, en presencia del TCB, el consorcio microbiano debe degradar tanto el lindano como su metabolito intermediario, resultando en una disminución aparente de la velocidad inicial de degradación.

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*Author for all correspondence

COMPARISON OF METHODS FOR FITTING KINETIC DATA OF INHIBITION OF THE BIODEGRADATION OF A MAIN POLLUTANT FOR REMEDIATION ASSESSMENT

Gonzalo M. Escobedo-Acuña (1), B. Camacho-Pérez (1), E. Ríos-Leal (1); M. T. Ponce-Noyola (1); J. Barrera-Cortés (1); Héctor M. Poggi-Varaldo***** (1), H. B. C. Poggi-Rinderknecht (2); N. Rinderknecht-Seijas (2)

(1) CINVESTAV- IPN, México DF, México;
(2) ESIQIE-IPN, México DF, México;

The γ-hexachlorociclohexane (also called lindane) has been one of the most used pesticides worldwide. It is a highly halogenated compound, toxic and persistent in the environment. During its anaerobic biodegradation many intermediate metabolites are formed; some of them are more resistant to be degraded by the microbial population and can influence or inhibit the bioremediation process performance. So far, there is little information on the kinetic impact of intermediate metabolites on the degradation of the parent pesticide (likely inhibition) as well as scarce information on the likelihood and criteria selection of fitting methods for determining the kinetic coefficients.

Kinetic parameter values, in turn, could significantly influence the time frame of a bioremediation project. Therefore we assessed the effectiveness and accuracy of several methods for fitting kinetic data assuming a scenario of competitive inhibition with one inhibitor (intermediate metabolite) and a parent pesticide (substrate). We found that no single method showed the minimum bias in all kinetic parameters. Considering the scenario of only the dependent variable with error a first compromise solution is to choose the fitting based on Levenberg-Marquard (SP 'a'), whereas the second method of choice would be the weighed linear regression with linearized data Lineweaver-Burk (LB-WLR 'a'.) In order to improve the estimation of the 3 parameters, the use of two methods is recommended as a second trade-off. With the method SP 'a' good estimations of both q_{max} and K_s are obtained, whereas the fitting with method LB-WLR 'a' will give the best estimation of K_i. The batch method Batch LR-2Var, in spite of the advantage of smaller experimental work, leads to large biases of K_s and K_i. The weighed regression based on Batch was worse. This is unfortunate because from the experimental point of view the batch method required significant less experimental work than the other methods.

To the best of our knowledge, this is the first comprehensive, critical evaluation of fitting methods to microbial inhibition kinetics, based on a synthetic experiment (in silico). With caution due to that, our results reveal that some assumed advantages of weighed linear regression and less biased Levenberg-Marquardt based software were not confirmed, particularly when both variables were subject to error, because the fitting methods did not produce more accurate estimations of the kinetic parameters.

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*Author for all correspondence

BIOREMEDIATION OF A SOIL CONTAMINATED WITH LINDANE IN AN ELECTROBIOCHEMICAL SLURRY REACTOR: ROLE OF SURFACTANT AND CHARACTERIZATION OF MICROBIAL COMMUNITY

Beni Camacho-Pérez (1); Jaime García-Mena (2); Omar Solorza-Feria (3), Elvira Ríos-Leal (1); Selvasankar Murugesan (4); Alberto Piña Escobedo (2); Héctor Mario Poggi-Varaldo * (1)

(1) Dept. Biotechnology and Bioengineering, CINVESTAV – IPN
(2) Dept. of Genetics and Molecular Biology, CINVESTAV – IPN
(3) Dept. of Chemistry, CINVESTAV – IPN
(4) Dept. of Pharmacology, CINVESTAV – IPN

Lindane is an organochlorinated pesticide that has been extensively used worldwide for the control of agricultural and medical pests. Bioslurry reactors are the most common *ex-situ* reactor configuration for soil bioremediation because of several advantages. On the other hand, electrobiochemical slurry reactor (EBCR) constitutes a promising technology that could remove organic and recalcitrant pollutants in contaminated soils with simultaneous electricity output.

The aims of this research were *(i)* to evaluate the desorption of lindane from soil with Tween 80 at different concentrations; *(ii)* to determine the power output and removal of lindane from soil in presence of Tween 80 in the EBCR; and (*iii*) to characterize the microorganisms in the electrobiochemical slurry reactor using the Ion Torrent Personal Genome Machine.

In vitro adsorption and desorption tests were run with the model soil polluted with lindane and varying concentrations of Tween 80. The EBCR consisted of a Plexiglass cylinder approximately 6 cm in diameter and 8 cm in length, fitted with two anodes and two cathodes. Tween 80 was added at a dose of 2000 mg /L in the batch run of biotreatment of soil in the EBCR.

Approximately 10% of lindane was removed in the *in vitro* desorption experiments. The internal resistance of the EBCR determined by polarization curve was 820 Ω; a moderate volumetric power activity was recorded (374 mW/m^3) along with a potential of 600 mV when the two-electrode sets were connected in parallel. During the batch operation, the EBCR showed a 56% lindane removal ($\eta_{lindane}$) whereas the reduction in the abiotic control was only 3%. Unexpectedly the $\eta_{lindane}$ in the EBCR spiked with Tween 80 was lower than that in an EBCR operated without surfactant in a previous experiment. This could be ascribed to the increased degradable organic matter supply related to Tween in our EBCR that possibly shifted microbial metabolism from lindane degradation to degradable matter uptake. An average volumetric power of 685 mW/m^3 and average voltage of 420 mV were achieved.

The energy output obtained in our EBCR allows for the recovery of 20% of the power required for mixing, thus paving the way to sustainable bioremediation of soils. The bacterial community was analyzed in the slurry of EBCR the 0, 15, 22, and 30 days of operation. The most representative phyla were *Actinobacteria*, *Bacteroidetes, Firmicutes*, and *Proteobacteria*, that was consistent with findings reported in the open literature. The most dominant genera were *Arcobacter* (40.8%), *Thrichococcus* (41.6%) at 22 days of operation, when the EBCR exhibited the maximum voltage. *Arcobacter* was reported to harbor in electrodes of MFC, and rapidly generates a strong electronegative potential as a pure culture when it is supplied with acetate. *Thrichococcus* has been reported in autotrophic biocathodes of other bioelectrochemical systems.

Note: This abstract exceeds the one-page format because it is a report of a large project.

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*Author for all correspondence

ANAEROBIC DECHLORINATION OF ORGANOCHLORINE INSECTICIDES CHLORDECONE AND HEXACHLOROCYCLOHEXANE

Ronald Ranguin (1); Axelle. Durimel (1); Line Lomheim (2);
Laurent Laquitaine (1); Elizabeth A. Edwards (2); ***Sarra Gaspard**** (1).

(1) Laboratoire COVACHIMM2E, EA 30592 Université des Antilles et de la Guyane, BP 250, 97157 Pointe à Pitre, Cedex. Guadeloupe, French West-Indies, France.
(2) Laboratory BIOZONE, University of Toronto, Toronto, ON, Canada

Organochlorine insecticides, such as γ-hexachlorocyclohexane (γ-HCH; Lindane) were widely used until about 1997. Because of slow rates of degradation, they still persist in many subsurface environments, particularly in certain agricultural soils, and near industrial facilities where they were manufactured. These compounds have a health hazard owing to their toxicity and probably carcinogenicity. It is well established that γ-HCH can be degraded aerobically and also can be anaerobically reductively dechlorinated to chlorobenzene and benzene. Chlordecone ($C_{10}Cl_{10}O$) is another toxic and highly recalcitrant organochlorine insecticide formerly used on banana plantations in the Caribbean. In this study, degrade γ-HCH and chlordecone under anaerobic conditions using Zn (0) and vitamin B12 or bacterial microcosms soils prepared from Guadeloupe soils.

Anaerobic microcosms were prepared with soil and water from a variety of agricultural sites from Guadeloupe and spiked with γ-HCH, chlordecone, and acetone and ethanol as electron donors. Samples were analyzed by a variety of chromatographic methods to determine the changes in concentrations of halogenated compounds. DNA was extracted from microcosm samples to identify microbial populations after over 2 years of incubation. The overall microbial populations were assessed by pyrosequencing of amplified 16S rRNA genes in these DNA samples. Abiotic degradation of γ-HCH and chlordecone by Zn (0) alone and a mixture Zn (0) /vitamin B12 was also investigated.

Zn (0) alone could dechlorinate γ-HCH to produce chlorobenzene and benzene, while the chlordeconedechlorination by Zn (0) led only to the formation of $C_{10}HCl_9O$ and $C_{10}H_2Cl_8O$. In contrast, dechlorination of chlordecone by vitamin B12 reduced by Zn (0) resulted in the production of $C_{10}HCl_9O$, $C_{10}H_2Cl_8O$ and $C_{10}H_4Cl_6O$. Under anaerobic conditions dechlorination of γ-HCH was observed in soil microcosms. γ-HCH dechlorination resulted in the production of chlorobenzene and benzene at a ratio of about 3:1. The rate of dechlorination increased upon re-amending γ-HCH, suggesting that microbes are using γ-HCH or its dechlorination products for growth. $C_{10}HCl_9O$ and $C_{10}H_2Cl_8O$ intermediates were produced. Pyrotag analyses have revealed presence and possible enrichment of several putative organohalide-respiring bacteria in the soil including *Dehalobacter*, *Dehalococcoides* and *Geobacter*.

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*Author for all correspondence

HYDROCARBON BIODEGRADATION BIOPROCESS IDENTIFICATION AND CONTROL USING KALMAN FILTER RECURRENT NEURAL NETWORK

Ieroham S. Baruch* (1); Carlos R. Mariaca-Gaspar (2); Josefina Barrera-Cortes (3)

(1) DCA, CINVESTAV- IPN, México DF, México;
(2) ESIME-IPN, México DF, México;
(3) DBB, CINVESTAV- IPN, México DF, México

Biological treatment is attractive as a potentially low-cost technology, which converts toxic organic contaminants into CO_2 and biomass. Since the 70's, this technology has been applied for the hydrocarbon degradation, and today, it is considered as the best alternative to clean up polluted soils. For this bioprocess, one challenge is to provide enough O_2 and nutrients to enable rapid conversion of contaminants by either indigenous microorganisms or inoculated species.

Another challenge is to achieve efficient contact between the active micro-organisms and the contaminants, which may represented a problem with in-situ treatment. An attractive alternative to overcome this problem is to apply a biological treatment in slurry phase using Horizontal Rotating Drum (HRD). The HRD can effectively mix heterogeneous blends of a wide range of particle sizes, and high solid concentration (more than 60 %). The HRD operated with oxygen supply or aeration. Independently of the type of HRD operation (open or close), the insufficiency of water decreased the efficiency of hydrocarbon degradation in HRD favoring the formation of hydrocarbon balls. So one objective of the process control is to maintain the humidity at 60%, which is the maximal solid concentration determined as the best for hydrocarbon removal from polluted soils treated in open rotating slurry bioreactors. Nowadays, semi empirical models, based on the Monod equation, have been developed to predict micro-organism growth as a function of available contaminants concentration. However, as the application of such models requires experimental work for calculating the kinetics parameters involved, so an alternative modeling technique is required. The Kalman Filter Recurrent Neural Network Model (KF RNNM) offers many advantages as the possibility to approximate complex non linear high order multivariable processes, as the biodegradation process is.

The KF RNNM has been applied for measurement data filtering and parameters plus state estimation of hydrocarbons biodegradation process, contained in polluted slurry, treated in a rotating bioreactor. Then the KF RNNM is simplified and used to design a Sliding Mode Control (SMC) of two-input two-output high order nonlinear plant. The KF RNNM learning algorithm is the dynamic Backpropagation one (BP). The graphical simulation results of the system approximation, and indirect adaptive neural control, exhibited a good convergence, and precise reference tracking.

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*Author for all correspondence

INFLUENCE OF ORGANIC FERTILIZATION WITH COMPOST AT HIGH DOSES ON THE SOIL MICROBIAL ECOLOGY IN A LONG-TERM STUDY

Ana Ibáñez-Burgos (1), María J.Martínez-Iñigo (2), Gerardo López-López (1), José M. Rovira- Alós (3), María del C. Lobo-Bedmar (2), ***Isabel Sastre-Conde**** (3)

(1) Consejería de Agricultura, Medio Ambiente y Territorio de Islas Baleares. Spain
(2) IMIDRA, Madrid, Spain
(3) SEMILLA, Palma de Mallorca, Islas Baleares. Spain.

The use of composted sewage sludge as nitrogen source in crops dependent of this nutrient as the almond trees, involves adding high doses of compost increasing soil organic matter significantly. Overall, the overproduction of this type of residue are always a problem and it is even more complex when are generated in an Island as Majorca, because land surface is more limited. In this area, the calcareous lithography of soils promotes the leaching of both, nutrients and heavy metals content in the residues. Thereby, the aim of this research is focussed in analysing the effect of compost application at high dose on soil microbial ecology, in a long-term experiment (two year after application).

Four types of composted sewage sludge from diverse Majorca Treatment Plants (Calvia, Felanitx, Sa Pobla) and different stabilization degree were used. Compost were applied to a soil un-disturbed in 50 Kg-containers, wherein were transplanted trees sapling almond and maintained with irrigation during two years, under field conditions. Then, the compost dose was calculated based on the amount of the compost necessary for increasing the low soil nitrogen content to 0.6%. After two years, soils from the containers were sampled for physic-chemical (pH, EC, OM, N, P and exchangeable cations) and biological analysis (microbial biomass carbon, enzyme activities as β glucosidase, alkaline phosphatase, protease, urease and soil bacterial biodiversity using PCR-DGGE).

In general, treated soils showed a significant decrease in pH values, on the contrary, no significant differences were observed in the electric conductivity values in treated soils despite the applied dose. The values of macronutrients (C, N and P) in soils treated were doubled, especially in soils with compost from Sa Pobla, which correspond to those soils with lower values in pH and EC. Likewise, the exchangeable cations increased in all soils treated with compost, highlighting the values of exchangeable potassium.

The carbon percentages from microbial biomass in treated soil increased significantly in comparison to control. Both the alkaline phosphatase and the protease activity increased significantly in all treatments, whereas urease activity was hardly modified and β-glucosidase activity increased only in the soil treated with the compost with high degree of stabilization of organic matter. Changes promoted by compost on the soil nitrogen and phosphorus seem to be responsible of the effects observed in the enzymatic soil activities analyzed in this study. When these soil activities are expressed

per g of carbon of microbial biomass instead of per g of dry soil this resulted in a decrease of urease and β glucosidase activity in the treatments with the Calvia compost. This effect

could be due to the pollutants present in this material. The lack of significative effect in phosphatase and protease expressed per g of carbon of microbial biomass in comparison to the increase of these enzymes per g of dry soil, suggest that this increase should be due to extra-cellular enzymes. In the soil treated with Calvia compost, the bacterial community (PCR-DGGE profile) from bulk soil was also determined. Wherein, appeared a new band and disappear others in response to the high dose of the Calvia compost. It might be a response to a greater exposure to potential pollutants present in the compost.

In view of the results, organic fertilization from composted sewage sludge in almond crops could be a good alternative, since it increase the trees growth, and at a long term, they contribute positively on soil biological activity, where it is established a new equilibrium of bacterial populations.

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*Author for all correspondence

Chapter 7.
Wastewater Treatment

REMOCIÓN DE Cr (VI) Y MATERIA ORGÁNICA PRESENTES EN UN EFLUENTE DE UNA INDUSTRIA CURTIDORA POR UN TRATAMIENTO BIOLÓGICO-ELECTROQUÍMICO

Daniel Pacheco-González (1); Noé Trejo-Vela (1); Carmen Monterrubio-Badillo (1); Gabriel Pineda Flores*(1)

(1) Centro Mexicano para la Producción más Limpia-IPN, México DF, México

Las aguas residuales de la industria de la curtiduría se caracterizan por presentar un potencial contaminante alto, debido a que contienen materia orgánica y metales pesados disueltos. Para lograr la remoción de estas sustancias, es necesario aplicar procesos de tratamiento específicos para los contaminantes presentes.

El objetivo principal del trabajo estuvo enfocado en aplicar un sistema de tratamiento biológico-electroquímico a escala de laboratorio, a un efluente de una industria curtidora, para reducir la concentración de materia orgánica y cromo hexavalente presentes.

Se obtuvo una muestra de agua residual de una empresa curtidora, localizada en el estado de Guerrero México, siguiendo métodos normalizados. Para el tratamiento biológico se utilizaron reactores de 1.5 L, cada reactor contenía 800 mL de la muestra de agua residual por tratar más 200 ml de lodos activados como fuente de inóculo. Se trabajó con un gasto volumétrico de aire de 1 L/min y a temperatura ambiente. Para evaluar la remoción del contenido de materia orgánica se determinó la Demanda Química de Oxígeno (DQO) por el método colorimétrico de reflujo cerrado, desde el tiempo inicial del funcionamiento de los reactores y cada tercer día, durante 12 días.

Al aplicar el tratamiento biológico se obtuvo una remoción de la materia orgánica del 94.15%. El tratamiento electroquímico se aplicó al efluente tratado biológicamente, para lo cual se utilizaron dos celdas, una de vidrio pyrex de 78.5 ml y una de teflón de 125 ml, ambas con tapas desmontables con orificios para la colocación de tres electrodos (de trabajo, de referencia y contra electrodo). La celda de vidrio sirvió para caracterizar el comportamiento oxido-reducción de la solución usando las técnicas de voltametría cíclica y lineal; y en la de teflón se llevó a cabo el tratamiento electroquímico, que consistió en la aplicación de una corriente eléctrica de 0.4 mA/cm^2 durante diferentes tiempos (método cronopotenciométrico).

La mayor cantidad de cromo hexavalente fue eliminada después de 60 minutos, alcanzando una eficiencia de remoción de 80%. El cromo removido se depositó en lámina de acero inoxidable (23.76 cm^2) y fue caracterizado por Difracción de Rayos X y Microscopio Electrónico de Barrido.

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*Author for all correspondence

BIODEGRADATION OF OLIVE, CORN AND SOY OILS BY FREE AND IMMOBILIZED *Yarrowia lipolytica* YB-387

Henry Flores-Santiago *(1)*; Ma. Aurora Martínez-Trujillo (1); Mayola García-Rivero*(1)

(1) TESE, Division of Chemical and Biochemical Engineering, Edo. de México, México

The contamination from vegetable oil industry has many environmental problems because their manufacturing process requires 21 liters of water per liter of oil. Discharge of this wastewater into environment causes serious problems because the oil has a detrimental effect on microbial activity, among other issues. Because of this is important to improve the biodegradation of vegetable oil, for example by using immobilized cells, and to evaluate their capacity to degrade vegetal oils.

In this study, we evaluated the ability of *Y. lipolytica* YB-387 immobilized on polyurethane foam to degrade different vegetal oils concentrations. In addition, the capacity of immobilized cells was compared with that of suspended growth cultures.

The assays were carried out in Erlenmeyer flasks containing mineral medium and different oil concentration, and free or immobilized cells of *Y. lipolytica* YB-387. Samples were taken every 12 hours with three replications and one cero time. The cultivation conditions were 200 rpm, 30 ºC and pH 7. Please report aeration.

The biomass in suspended growth cultures exhibited a growth of 60 (please report the units) with corn oil and soybean oil. On the other hand, olive oil grew to 100 (please report the units). The immobilized cultivation grew to 10 (please report the units) corn oil and soy oil and olive oil grew to 35 (please report the units) much less than free cultivation.

Regarding the oil degradation in a suspended growth cultures, only olive oil was degraded by more than 50% nevertheless concentration of 216 g L^{-1} was the same as corn and soy oils. The immobilized cultivation, where corn oil and olive oils been able to degrade 75, 58 and 55% with initial concentration of 27, 54 and 108 g L^{-1} (They report 3 removals but only 2 oils, it seems that one oil is missing. However, at an initial oil concentration of 216 g L-1, oil removal was 35% for the 3 oils, much more than employ the free cultivation this appends only with 48 hours.

In conclusion, oil degradation by free cultivations has the most degraded, by using olive oil just in 27 and 54 g L^{-1} oil concentrations. Nevertheless, immobilized culture have the most degraded oil using the 3 vegetables oils, moreover this cultivation produce less biomass for this reason immobilized cultivation have more advantages by free cultivation.

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*Author for all correspondence

PRINCIPALES ALTERNATIVAS DE TRATAMIENTO DE AGUAS RESIDUALES URBANAS PARA LA GESTIÓN Y PROTECCIÓN RACIONAL Y SUSTENTABLE DE ECOSISTEMAS ACUÁTICOS: UNA REVISIÓN

***Chadli Amina** (1)**; Aguiar Ah (2); Hamel Laid (1)

(1) Laboratoire de recherche écodéveloppement des espaces, Sidi-Bel-Abbès, Algérie.
(2) Laboratoire de recherche en environnement et santé SBA.

El reuso y reciclaje de las aguas residuales es una alternativa en expansión principalmente cuando está asociado a la agricultura. Hay numerosas soluciones técnicas que permiten cumplir con las normas de reuso y reciclaje existentes, especialmente con las reglamentaciones de la OMS sobre irrigación restringida y sin restricciones

En aquellos países donde las reservas actuales de agua dulce han llegado o llegarán a su límite, el reciclaje de aguas residuales tratadas parece ser la técnica más recomendable, tanto a nivel económico (donde los tratamientos no intensivos son los más adecuados) como a nivel tecnológico, dado que el reuso y reciclaje agrícola e industrial de aguas residuales las más de las veces no requieren la calidad potable.

La selección de los métodos de tratamiento depende de muchos factores donde los más importantes son la calidad del efluente, el tipo de destino, las exigencias de calidad y tamaño de las instalaciones de tratamiento. La(s) tecnología(s) se seleccionarán, entonces, dependiendo también de las condiciones locales y de criterios técnico-económicos, y resultará en proyecctos basados en tecnologías intensivas o extensivas, estas últimas también conocidas como tecnologías apropiadas en los países subdesarrollados.

Estas últimas (lagunas, vertido sobre suelo basado en infiltración-percolación, y otras) se adaptan bien a las condiciones climáticas de las regiones tropicales y subtropicales. Por otro lado, su bajo costo de operación e inversión, así como la relativa simplicidad tecnológica les confiere ventajas indudables para su aplicación en países subdesarrollados.

Por otro lado, las tecnologías extensivas (tratamientos físico-químicos, biológicos, membranas, filtración, y otras, así como los procesos más avanzados de desinfección (con radiación de ultravioleta, ozonación) son más compactos y garantizan una mayor y más reproducible calidad de los efluentes tratados. Su implantación permite asegurar una mayor protección al ambiente, especialmente en el contexto de aguas residuales urbanas. Sin embargo, tanto la inversión como operación son más costosas, y en general son tecnologías de mayor complejidad que requieren operadores y sistemas de operación bien entrenados.

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*Autor para toda la correspondencia

IMMOBILIZATION OF LACCASE ONTO CHITOSAN-COMPOSITEBEADS AND DECOLORIZATION OF TEXTILE DYE REACTIVE BLACK5

Tülin Aydemir* (1); Günay İrem Altındağ (1)

(1) CBU,Manisa,TURKEY

Residual dyes released from textile dyeing industry are visible even in low concentration; they are highly recalcitrant to physical or chemical wastewater treatment systems. In recent years, biological decolorization techniques have been considered as alternative, environmentally-friendly methods for dye detoxification and color removal. Enzyme methods applied in dye degradation have low energy costs, are easy to control and have low impacts on ecosystems application. Many enzymes, such as laccase and polyphenol oxidase are used in the development of biological processes for the treatment of textile effluents. Laccases (benzenediol:oxygen oxido-reductase, EC 1.10.3.2) are excellent biocatalysts for biotechnological and environmental applications because of their high activity, selectivity and specificity, which permit them to perform complex chemical processes under experimental and natural conditions. However, the use of these enzymes for practical applications is still limited due to their low stability and high production costs. So, immobilization of laccases can overcome some of the aforementioned limitations by improving some of the properties of the enzyme. Laccase, immobilized on several supports, has been evaluated for the elimination of pollutants such as phenols and dye decolorization. Enzymes are often immobilized onto or into solid supports to increase their thermostability, operational stability and recover.

Recently, there has been a growing interest in chemical or physical modification of chitosan to improve its solubility and widen its environmental and biomedical applications.

In the present study, laccase from *Trametes versicolor* was immobilized on multi-walled carbon nanotube-chitosan (MWCNT- CS) composite beads by covalent attachment. The prepared beads were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), thermal gravimetric analyze (TGA). The yield of the immobilization and enzyme activity were found to be % 83 and 3.51 U/gr carrier at pH 5.0 and 30°C. improved pH, thermal and storage stabilities of the immobilized laccase were obtained compared to the free counter part. The operational stability was also improved, as 80% of the initial activity was retained after 7 cycles.

Application of the immobilized system in textile dye Reactive Black 5 removal was investigated in a batch system and decolorization values of about 70% were obtained at the end of 6 h.

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*Author for all correspondence

MASTER INTERNACIONAL EN OPERACIÓN Y MANTENIMIENTO DE PLANTAS DE TRATAMIENTO DE AGUAS

Julio Antonio Pérez-Alvarez*(1,7);* Miguel Ferrero-Fuertes (2,7); Rosa Menéndez-López (3,7); Javier Santos-Navia (7); María J. Rodríguez-González (7); Cristina Díaz-Muñiz (4,7); Juan J. Enterria (7); José C. Rubio-Fernández (5,7); Amador Menéndez-Velazquez (6,7)

(1) Acciona Agua
(2) Universidad de Oviedo, Oviedo (Asturias), España
(3) Consejo Superior de Investigaciones Científicas-INCAR, Oviedo (Asturias), España
(4) Confederación Hidrográfica del Cantábrico. (Ministerio de Agricultura, Alimentación y Medioambiente) Oviedo (Asturias), España
(5) Sadyt
(6) Instituto Tecnológico de Materiales de Asturias (ITMA)
(7) Grupo de Tecnología del Agua del Colegio Oficial de Químicos de Asturias y León, Oviedo (Asturias), España

El agua, recurso natural, que interviene directamente en todos los aspectos de nuestras vidas (alimentación, higiene, industria, energía, agricultura, salud…) es un bien que debe ser sometido a criterios de gestión que optimicen su disponibilidad y buen uso.

Políticas internacionales hacen del agua un elemento estratégico, que debe venir acompañado de acciones que permitan, a todos los agentes implicados, mejorar el conocimiento del ciclo hídrico e incrementar su capacidad de administrar y explotar mejor sus recursos hídricos. Para ello debe mejorarse la base científica y tecnológica con el fin de desarrollar métodos para la gestión racional de los recursos hídricos, incluyendo la protección del medio ambiente.

En la coyuntura actual se hace indispensable hablar de la internacionalización entendida como puente de unión entre distintas culturas, por este motivo desde el Grupo de Tecnología del Agua del Colegio Oficial de Químicos de Asturias y León y la Universidad de Oviedo se ha querido dar un carácter internacional al Master abriendo un puente de conocimientos y experiencias entre España y México.

El Título Propio que se presenta aquí pretende potenciar la empleabilidad y el conocimiento de personas que quieran acometer su carrera profesional dentro del área de la Operación y Mantenimiento de Plantas de Tratamiento de Aguas. Por este motivo se ha buscado un enfoque teórico-práctico pero siempre aplicado a las realidades que el profesional se va a encontrar en el día a día al gestionar una planta de tratamiento de aguas.

El curso pretende aportar una visión general de distintos aspectos que afectan a la Gestión del Agua, desde su conocimiento científico-técnico, hasta las aplicaciones y tecnologías actualmente disponibles para su óptima gestión.

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*Author for all correspondence

STUDIES ON BIOREMEDIATION OF PHENOLIC GROUPS IN INDUSTRIAL OIL FRACTIONS DEGRADED BY LIGNINOLYTIC BASIDIOMYCETES

Borja Rodríguez de Francisco (1); Aldo E. González-Becerra (1)*

(1) CBMSO, Microbiology and Virology Department, Madrid, Spain.

Oil is the raw material currently most demanded by developed countries. It is used for transport, manufacture of plastics, detergents, etc., but mainly as a source of energy. It is an insoluble and homogeneous mixture of organic compounds, primarily insoluble hydrocarbons which are recalcitrant to degradation; therefore, oil is a highly polluting material.

In this project the ability of 34 strains of ligninolytic fungi to degrade a fraction of oil was tested. Their enzymatic activities (enzymes Lacase, Manganese-peroxidase, Versatile-peroxidase and Lignin-peroxidase) were quantified and those strains showing greater bioremediation ability, both in solid and liquid medium were selected.

The selected strains were inoculated in Kirk medium (nitrogen and carbon limited medium) with three inductors (Poly-R 478, ABTS and Ramazol blue) which discoloration indicates the presence of the indicated enzymes. In addition to producing these enzymes, the selected strains were able to grow on this minimal medium and to resist the toxicity of inductors. The cytotoxicity of different concentrations of oil (1%, 2% and 4% v / v, equivalent to concentrations of 10.000, 20.000 and 40.000 ppm, respectively) was tested on Kirk solid medium. These high concentrations are unprecedented in these types of studies. Six strains were able to grow at every oil concentration tested (*Ganoderma resinaceum*, *Pycnoporus sanguineus*, *Trametes I-62*, *Laccaria cf*., *Fomes fomentarius* and *Daedalea quercina*). These strains also showed their ability to grow on Kirk medium supplemented with oil but without glucose, suggesting that oil is being used as a carbon source.

The production of degrading enzymes was tested in Kirk liquid medium with 2% v/v of oil. Culture media with or without glucose, and with or without oil, were used as controls. *Ganoderma resinaceum* showed Lacase activity in the media with oil; Laccaria cf. showed Lacase and MnP activity in all media assayed but at low concentrations. The strain *Trametes* I-62 showed activity for all the assayed enzymes, except Lignin-peroxidase, at high concentrations in all tested media arising as the best candidate with the most bioremediation potential.

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*Author for all correspondence

BIOREMEDIATION OF SYNTHETIC DYES AND PAPER INDUSTRY EFFLUENTS BY BIOLOGICAL TREATMENT USING WHITE-ROT BASIDIOMYCETES: PROTEOME STUDY

Marta Otero (1); José M. Carbajo (2); Juan C. Villar (2); Ana I. Marina (1);
Aldo E. González-Becerra*(1)

(1) Department of Virology and Microbiology, Centro de Biología Molecular Severo Ochoa, Madrid (CBM-SO), Spain.
(2) Centro de Investigación Forestal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain.

The importance of white-rot Basidiomycete fungi in bioremediation lies in the main ligninolytic enzyme, laccase, and its high substrate-specificity, that can be used for degradation of recalcitrant aromatic compounds, which are present in wastewaters from textile and paper industry and might cause a serious environmental impact in case of spill.

The aim of this work was to select the best bioremediator of thirty five fungi. For that purpose we studied their growth rate, their ability to degrade different kinds of azo compounds in solid Kirk medium and fungal response in medium with lignosulfonates and alkali lignin (black liquors).

Experiments with dyes resulted in a gradual disappearance of color as the fungi grew. Thus, *Trametes versicolor*, *Trametes I-62*, *Pleurotus sajor-caju* and *Phanerochaete chrysosporium* were selected. The enzymatic activity of the ligninolytic complex was measured under four conditions and showed that laccase (9,61 dA/min), manganese peroxidase (1,65 dA/min) and lignin peroxidase (0,04 dA/min) were mainly detected in the presence of Induline, an alkali lignin that even modified, is still a good ligninolytic system inducer.

Black liquors showed different results depending on their characteristics: in solid medium without glucose and nitrogen appeared a brown halo round the colony, which might be caused by a phenol polymerization. Nonetheless, with glucose and nitrogen the colouring decreased at concentrations of 1% and 2% v/v. In liquid medium there was no clarification and spectrophotometry analysis showed an increase in absorbance below 300 nm, which suggests that phenols were polymerizing as well. Only lignosulfonates contained free carbohydrates before the biological treatment (pentoses and hexoses).

After all these tests we chose the specie that showed the highest bioremediation capacity, *T. versicolor*, whose proteome revealed three different groups of proteins: the ligninolytic enzyme laccase (*Ik2*), a sequence with homologue to an aspartyl protease from *Fibroporia radiculosa* TFFH 294, and the translation elongation factor 1-alpha.

In conclusion, *T. versicolor* is capable of degrading different phenolics compounds, but only at low concentrations. The almost exclusive presence of laccase in proteome analysis suggests that *T. versicolor* is trying to detoxify the medium by polymerizing phenols.

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*Author for all correspondence

ADSORCIÓN DE As(III) Y As(V) EN NANOFERRITAS DE MANGANESO

Elías E. Hernández-Beteta (1); Oscar F. Mijangos-Ricardez (1); Jaime López-Luna*(1)
Sergio Martínez-Vargas (2)

(1) Instituto de Estudios Ambientales, Universidad de la Sierra Juárez, Oaxaca., México.
(2) Facultad de Ingeniería, Universidad Autónoma del Carmen, Ciudad del Carmen, Campeche, México.

El uso de nanopartículas de óxidos de hierro, como una alternativa de bajo costo y alta eficiencia para la eliminación de metales pesados en agua, ha tomado gran importancia en los últimos años. Las propiedades fisicoquímicas de estos nanomateriales facilitan la eliminación de metaloides como el arsénico, incluido en el Listado de Contaminantes Prioritarios de la US-EPA. Las nanoferritas que proceden de la sustitución parcial o total de Fe(II) por Mn(II) en la estructura de la magnetita, ofrecen mayor resistencia a la oxidación sin afectar las propiedades adsorbentes del material. De esta manera, el objetivo de este trabajo fue evaluar la capacidad adsortiva de nanoferritas de manganeso para la eliminación de As(III) y As(V) a escala de laboratorio, mediante el modelo cinético de Pseudo-Segundo-Orden (PSO).

Las nanopartículas se sintetizaron por el método de coprecipitación, empleando una solución mixta de Fe (II), Mn (II) y Fe (III), 0.5, 1.5 y 2 mmol respectivamente. Se obtuvieron partículas con tamaño promedio de 2.5 nm, densidad 5.22 g mL^{-1}, área superficial 81.09 g m^{-2} y punto de carga cero (pzc) 7.5. Las cinéticas de adsorción se realizaron con concentraciones iniciales de 2, 5, 10, 20 y 40 mg L^{-1} de As(III) y As(V), a pH 2 y 8, con una proporción de partícula de 0.4 g L^{-1}. Los tiempos de contacto fueron de 5, 10, 15, 30, 60, 120, 180 y 240 min con agitación oscilatoria de 250 rpm. La concentración inicial y final del metaloide se determinó por ICP-OES.

El porcentaje de remoción disminuyó con el aumento de las concentraciones iniciales, 95%-28% para As(III) y 92%-27 para As(V). La adsorción de ambas especies del metaloide mostró diferencias significativas ($p<0.05$, prueba LSD) entre pH 2 y 8. El modelo de PSO mostró que a pH 2 la velocidad inicial de adsorción h de As(III) se incrementó (1.833-6.986 mg g^{-1} min^{-1}) mientras que la constante de velocidad k_2 disminuyó (0.1514-0.0162 g mg^{-1} min^{-1}) con el incremento de las concentraciones iniciales. De la misma forma, a partir de la concentración inicial de 5 mg L^{-1}, h (2.658-4.938 mg g^{-1} min^{-1}) se incrementó y k_2 (0.018-0.0062 g mg^{-1} min^{-1}) disminuyó para As(V). A pH 8 se observaron tendencias similares pero con velocidades menores. Así, para As(III) se obtuvo h (0.417-3.938 mg g^{-1} min^{-1}) y k_2 (0.0255-0.0034 g mg^{-1} min^{-1}), mientras que para As(V) h (0.197-4.553 mg g^{-1} min^{-1}) y k_2 (0.0134-0.0069 g mg^{-1} min^{-1}).

La adsorción en equilibrio (q_e) para la máxima concentración inicial (40 mg L^{-1}) y pH 2 fue de 20.74 y 27.18 mg g^{-1}, para As(III) y As(V), respectivamente. En tanto que a pH 8 se obtuvieron valores de q_e de 33.96 y 18.46 mg g^{-1}.

Puede concluirse que la sustitución de Fe(II) por Mn(II) mejora la capacidad adsortiva de la partícula, que podría deberse al incremento de grupos hidroxilo en los sitios de sorción.

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*Author for all correspondence

KINETIC RESPONSE AND CHANGES OF CATABOLIC GENES IN A BIOFILM REACTOR USED TO REMOVE MIXTURES OF ORGANOCHLORINE HERBICIDES

Ixchel Nava-Arenas (1); Nora Ruiz-Ordaz* (1); Juvencio Galíndez-Mayer (1); Everardo Curiel-Quesada (1); Marco L. Luna-Guido (2)

(1) ENCB-IPN, México DF, México;
(2) CINVESTAV- IPN, México DF, México.

Organochlorine herbicides that have been commonly reported in the freshwater environment are the chlorophenoxy acid, 2,4-D; atrazine, simazine; and the auxin analogue, diuron. The application of these xenobiotics in agricultural fields can be made simultaneously or sequentially; thus, pollution of water bodies may be caused by a mixture of herbicides.

In this work, the operational stability of a biofilm reactor was studied during the degradation of various mixtures of this organochlorine herbicides sequentially supplied, continuously and with different volumetric loading rates of herbicides. The reactor used is an aerated column packed with fragments of volcanic rock. To the support material, a complex biofilm able of degrade organochlorine herbicides atrazine, simazine, 2,4-D and diuron was attached. The initial community was integrated by *Burkholderia* sp., and two recently isolated microbial communities. One of them could degrade atrazine and simazine, and the other was able to degrade the herbicide diuron; whose members possess genes encoding the main catabolic enzymes *atzABCD, tfdACD* and *puhB*. To acclimatize the integrated community a mineral minimum medium was used. It contained: diuron, atrazine, simazine and 2,4-D, as carbon and nitrogen sources.

Throughout the bioprocess, high removal efficiencies of the herbicides, COD and TOC; as well as herbicide dehalogenation, were observed. Moreover, in the community the genes encoding enzymes of the catabolism of the different herbicides: *tfdACD, atzABC* and *puhB*, were identified. Of the most abundant culturable microorganisms in the community at the end of the acclimatization, two genera agree with those of the original community. Once acclimated the community, the triazine herbicides were removed from the original mixture of herbicides, and the volumetric loading rates of 2,4-D and diuron were increasingly supplied to the reactor. In these new conditions high removal efficiencies for the herbicides, COD, TOC, and herbicide dehalogenation, were obtained like the previous process. It was also observed that only were consistently detected the genes *tfdACD*, *puhB*, and occasionally the genes a*tzA* and *atzC*. Once finished the process, of the most abundant culturable microorganisms present in the community, four matches to those of the original communities and five to the new acclimated community. Subsequently, the herbicides atrazine and simazine were restored to the medium supply and the complete mixture of herbicides was continuously fed. During the treatment of this mixture, high removal efficiencies for the herbicides, COD, TOC, and herbicide dehalogenation, were observed. In this case, the genes encoding the enzymes in the catabolism of different herbicides are consistently detected the *tfdACD* the *puhB* and *atzABC*. Of the most abundant organisms in the community four genera match with those of the original communities, four with the community at the end of the removal process of the 2,4-D and diuron mixture, and four with the community initially acclimated in the complete mixture of herbicides. Considering the set of results obtained, we conclude that the system maintained its operational stability.

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*Author for all correspondence

TEQUILA VINASSES BIOREMEDIATION WITH ULTRAZYME POWDER

Francisco Galnares-de la Garza*(1)

(1) ITESO, Tlaquepaque, México

It is known that for each liter of tequila produced, 1.4 kg of bagasse and 10-12 liters of vinasses are generated. In the majority of the tequila factories, bagasse is converted into compost, however; approximately 80% of the vinasses are discharged directly into water bodies or onto the soil without receiving adequate treatment. This common practice causes deterioration of water bodies due to the low pH, high temperature and elevated organic loads of these effluents.

The aim of this research was to evaluate in a laboratory scale the possible use of UltraZyme Powder® (UZP) in the bioremediation of tequila vinasses.

A simple factorial experimentwas carried out to evaluate: the behaviour of UZP bacteria in both aerobic and anaerobic conditions; the benefits of augmenting the concentration of nitrogen to reach the optimal 20:1 C:N ratio; and the effect of the concentration of UZP bacteria in three levels of addition (none, 5 ml and 10 ml).

Although COD diminishment was none in anaerobic conditions, aerobic conditions presented a very high COD removal from the very first week of reaction. The greatest COD removal was presented in the reactors where urea was added, reaching a 94% of organic matter degradation by the sixth week of reaction in the reactor with the high concentration of UZP bacteria (4,000 ppm of an initial COD value of 77600 ppm).

And even though this value is far bigger than the value stipulated by law to discharge the effluent directly to water bodies, it'll be much easier to implement other kind of treatments with that organic matter concentration.

It is concluded then, that UZP bacteria functions well in aerobic conditions to treat tequila vinasses, but do not have the strength enough to compete with anaerobic indigenous microorganisms present in vinasses. However due to the high hydraulic retention time to reach the standard value to discharge to the environment, it would be very expensive to treat them like this. Further research has to be made to diminish COD before the aerobic digestion.

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*Author for all correspondence

ELIMINACION DE AMONIO Y CRESOLES POR UN LODO NITRIFICANTE EN UN REACTOR SBR

Juan Antonio Salas-Cortés (1); Flor Cuervo-López (1); Anne-Claire Texier*(1)

(1) UAM-Iztapalapa, México DF, México

Los procesos biológicos acoplados de nitrificación y desnitrificación permiten la eliminación de amonio de las aguas residuales en forma de N_2. Recientemente, el uso de consorcios nitrificantes en reactores de lotes secuenciados (SBR) ha sido propuesto como una nueva alternativa para la oxidación simultánea de amonio y compuestos fenólicos de efluentes industriales. Sin embargo, todavía no se tiene información de tipo fisiológica y cinética de la oxidación simultánea del amonio y de los isómeros del cresol por consorcios nitrificantes en reactores biológicos.

El objetivo del trabajo fue evaluar la capacidad metabólica de un consorcio nitrificante para oxidar simultáneamente amonio, *o*-cresol (*o*-cr), *m*-cresol (*m*-cr) y *p*-cresol (*p*-cr) en un reactor SBR.

Se operó un reactor SBR de 1L inoculado con lodo nitrificante (0.2 g proteína microbiana/L) y alimentado con un medio litoautotrófico nitrificante. Los ciclos de 12 h se dividieron en: llenado (0.07h), reacción aerobia (11.26h), sedimentación (0.25h) y drenado (0.42h). Primero, se operó el SBR sin adición de cresoles (fase I: ciclos del 1 al 172) para obtener un proceso nitrificante estable y posteriormente se alimentó con cresoles: fase II: *m*-cr (10, 20 y 30 mg C/L) (ciclos 173-240); fase III: *m*-cr (30 mg C/L) y *o*-cr (10, 20 y 30 mg C/L) (ciclos 241-354); fase IV: *m*-cr (30 mg C/L) con *o*-cr (30 mg C/L) y *p*-cr (30 mg C/L) (ciclos 355-413). En el reactor no alimentado con cresoles (fase I), se estabilizó un proceso respiratorio nitrificante con altos valores de eliminación de amonio (E_{NH4} = 100%). El producto mayoritario fue el nitrato (rendimiento $Y_{NO3} = 0.94 \pm 0.10$ g N-NO_3^-/g N-NH_4^+ consumido) y no hubo acumulación de nitrito. Cuando se adicionaron los cresoles al SBR (fase II a IV), se observó que la actividad nitrificante del consorcio no fue alterada ya que todo el amonio fue oxidado a nitrato (E_{NH4} = 100% y $Y_{NO3} = 0.91 \pm 0.06$). Se evidenció que los tres isómeros del cresol en mezcla no afectaron la nitrificación en el SBR. En todos los casos, los diferentes cresoles fueron totalmente consumidos por el lodo con eficiencias de 100%. Se observó un proceso de adaptación metabólica del lodo para oxidar los cresoles ya que las velocidades específicas (q) aumentaron a través de los ciclos de operación ($q_{m\text{-}cr}$ aumentó de 0.0003 a 0.450 mg C/mg proteína·h en 54 ciclos; $q_{o\text{-}cr}$ pasó de 0.024 a 0.061 en 98 ciclos). El *o*-cresol fue el más recalcitrante de los tres isómeros.

El consorcio nitrificante presentó la capacidad metabólica para oxidar simultánea y totalmente, el amonio y los tres isómeros del cresol. El uso del SBR permitió una adaptación metabólica del lodo en consumir los cresoles. Este tipo de reactor puede constituir una buena alternativa para tratar efluentes industriales en un solo reactor.

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*Autor de correspondencia

ELIMINACIÓN DE HORMONAS PERSISTENTES EN AGUAS RESIDUALES

Ana Fátima Ruiz Esparza-Ortiz* (1); Adrián Rodríguez-García (1)

(1) CIDETEQ, Parque Tecnológico Querétaro s/n, Sanfandila Pedro Escobedo, Querétaro, C.P. 76703 México.

En la actualidad una de las problemáticas más graves que se afronta en el mundo es la falta de suministros de agua, por lo que las operaciones de alimentación concentrada de animales (CAFO) es una estrategia de reciclado de agua factible y económico en los campos agrícolas, brindándoles nutrientes y materia orgánica para el crecimiento vegetal, aun así estas suelen contener altas concentraciones de hormonas consideradas como disruptores endocrinos, con riesgos potenciales en ecosistemas.

El objetivo de este proyecto es desarrollar diferentes técnicas de oxidación avanzada con digestión anaerobia como un proceso acoplado, para tratar altas cargas orgánicas y para la eliminación de estos compuestos recalcitrantes, siendo un proceso de tratamiento viable, eficaz y económico.

Para llevar a cabo el proceso de Digestión anaerobia se trabajó con un reactor UASB de 7 L, con un tiempo de retención de 18 h a un flujo de 6.5 ml/min. Se evaluaron varios parámetros según las normas mexicanas y Standard Methods, los parámetros tomados en cuenta fueron: DQOs, DQOt, Alcalinidad parcial y total, relación α, nitrógeno amoniacal, AGVs, sólidos y grasas y aceites. En los procesos de oxidación avanzada se trabajó con la reacción Fenton para la producción de radicales hidroxilo, mediante la reacción de H_2O_2 y $FeSO_4.7\ H_2O$ para llegar a la mineralización del contaminante; tomando como condiciones de operación, concentración de H_2O_2, concentración de $FeSO_4.7\ H_2O$ y tiempo. Para la determinación de las hormonas se hizo usó del estándar de 17 β estradiol para verificar su eliminación después del tratamiento acoplado; se determinó mediante cromatografía de líquidos de alta resolución. El tiempo de retención del estándar se reportó a los 10 min.

En el proceso biológico la mayor remoción de carga orgánica en DQO fue de un 85% y en carbono orgánico total un 92 %, hubo oscilaciones a lo largo de todas las pruebas debido a los diferentes lotes de agua residual, por lo que se hizo un promedio de eficiencia en eliminación en DQO y TOC obteniendo resultados de 68% y 62% respectivamente, en la reacción Fenton se hizo el análisis de Carbono Orgánico total obteniendo una eliminación de carbono orgánico de 62% obteniendo resultados positivos en la degradación de materia orgánica. Según la literatura la eliminación de hormonas con un proceso de oxidación avanzada puede tardar entre 10 y 30 min por lo que se espera que después del proceso acoplando, la eliminación de hormonas en aguas residuales de granjas lecheras sea exitosa, y junto con esto el efluente de proceso esté dentro de los límites que la norma permite.

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*Autor de correspondencia

STRUCTURE OF TECHNOLOGICAL INSTRUMENTS IN WASTEWATER TREATMENT IN MEXICO

Lilian E. Domínguez-Montero (1); Blanca Jiménez-Cisneros (2);
Rosa O. Cañizares-Villanueva (1); Sergio Caffarel-Méndez (3);
Héctor M. Poggi-Varaldo*****(1)

(1) CINVESTAV del IPN, México DF, México;
(2) II-UNAM, México DF, México;
(3) TESE, Div. Ing. Qca. y Bqca., Edo. de Méx, México.

The development and adaptation of technology is vital for the wastewater treatment (WWT) be efficient, easy to implement, low costs construction and operation. Patents can be a useful indicator for measuring and analyzing these technological issues.

The purpose of this study was to evaluate the existing technological development in Mexico related to WWT. Particularly, we analyzed Mexican patented technology of international and autochthonous origin.

We examined the databases of the Mexican Institute of Industrial Property (IMPI), United States Patent and Trademark Office (USPTO), European Patent Office (EPO) and World Intellectual Property Organization (WIPO). The analyzed period was defined taking into account all standards wastewater since 1996.

The results show that the patented technology in Mexico for WWT is scarce compared to the volume and variety of patents in developed countries of the Organization for Economic Cooperation and Development; North American Free Trade Agreement. Mexico has contributed only with 34 patents our of 4,679 (0.73%) in the period 1996 to 2012 according to the database of WIPO.

Also, it is lower than those of developing countries of similar standing as Mexico, such as Brazil. Furthermore, it was determined that the Mexican technology in Mexican patents is a minor proportion of all Mexican patents in WWT. Since 1996 to 2013, in the database of IMPI were registered 132 patents, of which 42 belong to Mexican companies, institutions and inventors (31.82%).

This reflects that the technological development in the WWT sector in Mexico is weak and is mostly dependent on foreign technology and technological packages originated abroad. This, in turn, is a strong indicator for governmental policymakers. Indeed, they might want to implement stimuli programs at business and educational levels in Mexico in order to foster the generation and application of proprietary technology in WWT.

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*Author for all correspondence

ARBUSCULAR MYCORRHIZA ON WATER CONDUCTANCE AND OXIDATIVE STRESS IN *Medicago sativa* UNDER DIESEL CONTAMINATION

Herminia A. Hernández-Ortega*(1); Paz A. Quintanar-Isaías (2);
Ana T. Jaramillo-Pérez (2), Alejandro Alarcón (1); Ronald Ferrera-Cerrato (1)

(1) Colegio de Postgraduados, Montecillos. Estado de México, México.
(2) Universidad Autónoma Metropolitana, Iztapalapa, Distrito Federal, México.

Some plant species tolerate and grow at soils contaminated with petroleum hydrocarbons (PH). In addition, microbial associations in the rhizosphere contribute on decontamination process of soil. The presence of organic contaminants in soil imposes conditions of oxidative stress and physiological drought interfering with water and transpiration in plants.

This study evaluated the effects of *Rhizophagus intraradices* (AMF) on the growth, water conductance and accumulation of reactive oxygen species in plants of *Medicago sativa* due to diesel-induced stress. Plants were also inoculated with AMF under either contaminated or uncontaminated sand with diesel (9 000 and 18 000 mg kg^{-1}). After 30 days, AMF-plants under diesel contamination (18 000 mg kg^{-1}) had significantly greater dry weight of shoots (>59%), height (>88%) and leaf area (>64%) than non-AMF plants.

Moreover, increasing concentrations of diesel resulted in reduced water conductance of plants; however, the inoculation of AMF induced 35% and 53% more water flow in plants at diesel concentrations of 9 000 and 18 000 mg kg^{-1}, respectively.

Diesel also caused high accumulation of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) in roots, but AMF significantly reduced this accumulation; although AMF colonization was decreased by this contaminant.

In summary, *Rhizophagus intraradices* alleviated the diesel induced-toxicity on *Medicago sativa* by enhancing plant biomass and water conductance, and by reducing the formation of O_2^- and H_2O_2. Work financially supported by the SEP-CONACYT grant 79456.

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*Author for all correspondence

REMOCIÓN DE AZUL ÍNDIGO POR LA BIOMASA SECA DE CUATRO CEPAS DE *Trichoderma sp.*

Erika Sánchez-Torres (1); Oscar García-Barradas (1);
Ma. Remedios Mendoza-López (1); Rosalba Argumedo-Delira*(1)

(1) SARA-UV, Xalapa, Ver., México

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El colorante azul índigo es ampliamente utilizado en la industria textil, ya que es usado para teñir mezclilla, la cual es empleada para la confección de pantalones, siendo México uno de los principales productores de mezclilla. No obstante, el uso de estas prendas por influencia de la moda deja una huella de contaminación que afecta a los ecosistemas acuáticos, debido a la liberación de efluentes coloreados con azul índigo a los ríos mexicanos, primordialmente por textileras informales que no cuentan con plantas tratadoras de aguas residuales.

Con el fin de generar información básica de la interacción *Trichoderma*-azul índigo que pueda ayudar a generar biotecnologías de bajos costos, útiles para el tratamiento de efluentes contaminados con azul índigo, la presente investigación tuvo como objetivo determinar la capacidad de biosorción de la biomasa seca de cuatro cepas de *Trichoderma* sp. (*Trichoderma* sp. H-1, *Trichoderma* sp. H-2, *Trichoderma* sp. H-3 y *Trichoderma* sp. H-4.) ante cinco concentraciones del colorante azul índigo (9, 19, 28, 33 y 39 mg L^{-1}) durante 24 h, 25 °C y pH=1.5.

Los resultados muestran que la biomasa seca de *Trichoderma* sp. H-11 tuvo una remoción del 100% en todas las concentraciones probadas, mientras que la biomasa seca de *Trichoderma* sp. H-1 (95%), *Trichoderma* sp. H-2 (83%) y *Trichoderma* sp. H-12 (79%) presentaron una remoción promedio menor. Los datos obtenidos tuvieron una buena correlación con las isotermas de adsorción de Langmuir y Freundlich, encontrando que la capacidad máxima de adsorción (q_m) para la biomasa seca de *Trichoderma* sp. H-2, *Trichoderma* sp. H-12 y *Trichoderma* sp. H-1 fue de 57.3 mg g^{-1}, 27.1 mg g^{-1} y 3.930 mg g^{-1} respectivamente. En el caso de la cinética de adsorción los datos se describieron mejor con el modelo cinético de pseudo segundo orden, la constante de velocidad de pseudo segundo orden (K_2) indicó que la biomasa seca de *Trichoderma* sp. H-1 adsorbe en menor tiempo al colorante azul índigo bajo las condiciones probadas.

Finalmente la biomasa seca de la cepa *Trichoderma* sp. H-11 demostró ser un eficiente biosorbente fúngico ante el colorante azul índigo, lo que indica el amplio potencial de las especies fúngicas del género *Trichoderma* para el tratamiento de efluentes contaminados con colorantes.

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*Author for all correspondence

DIRECT DYE BLUE FADE 2 BY PEROXIDASES

Gabriela Eleonora Moeller-Chávez* (1); *María Yesenia Vargas- Espinoza* (2)

(1) UNAM, México DF, México;
(2) UNAM, México DF, México.

Peroxidases are emerging as an important class of enzymes that can be used for the efficient degradation of organic contaminants. In this study, two enzymatic methods for the direct treatment of blue coloring 2 (AD2) were used. The catalytic ability of two peroxidase enzymes 1) produced by industrial waste soy 2) the fruit of the chayote was evaluated.

The objective of this study is to bleach the direct blue dye 2 (AD2) in synthetic sea water using the peroxidase enzyme in which various operational parameters that affect the enzymatic degradation such as concentration of the starting dye, dose of hydrogen peroxide time were evaluated contact of the solution pH, amount of peroxidase enzyme. The volumes of water samples were artificially contaminated 30ml treated with a dye concentration of 10, 20, 30, 40, 50, and 60 mg L^{-1}. The strategy of the enzyme dosage was a unique addition to starting treatment.

Finally it should be mentioned that by using chemometric techniques such as design of experiments and response surface methodology was possible to select the best treatment for both peroxidase enzymes.

Only a significant factor the pH, however it was possible to optimize the method for obtaining the partially purified extract of industrial waste soybean obtained to find enzyme activity of 20 to 30 UL, the results of the experimental design showed in the Pareto chart in response graphs shows that if there exists a relationship in the dose of peroxidase and hydrogen peroxide dose of achieving a percentage of fading to 73%, according to the following experimental factors 1000µL enzyme dosages, H_2O_2 dose 2000µL, pH 3.5, 25 °C T and contact time of 2 hours. For the enzyme of the squash fruit enzymatic activity of 30 to 40 was achieved UL, design results showed Pareto chart in two significant effects on pH, and the contact time in achieving a response graphs percentage 96% discoloration with the following experimental factors 2000 µL enzyme dosage, 1000 µL dose of H_2O_2, pH 3.5, T 50 °C and the contact time 6 hours. Effluent toxicity was evaluated by bacteria *Vibrio fischeri*. However an increase in the toxicity evaluated by indicating the formation of more toxic than the original dye substance was obtained.

This study demonstrated that it is feasible degradation dye Direct Blue 2 (AD2) by enzymatic treatments. Thereafter, peroxidase and industrial waste soy chayote fruit could be used in the initial steps of effluent treatment for bleaching followed by conventional treatment plus for degradation and detoxification.

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*Author for all correspondence

INCREASED BIODEGRADABILITY OF THE FUNGICIDE IMAZALIL AFTER PHOTO-PHENTON TREATMENT

Elsa López-Loveira* (1); Federico Ariganello (1); Roberto Candal (1, 2); Gustavo Curutchet (1, 2)

(1) 3iA-UNSAM, Buenos Aires, Argentina;
(2) CONICET, Argentina.

Imazalil ($C_{14}H_{14}Cl_2N_2O$) is a widely used fungicide for the post-harvest treatment of citric fruits. This fungicide is usually commercialized in the form of emulsions containing 50% of the active compound and coadjutants that help to stabilize the emulsion and disperse the product. The presence of Imazalil and the coadjutants in waste waters, produced by washing the fruits before packing, is a potential risk for the living organisms present in the receptor water courses. The treatment of the waste waters containing pesticide is mandatory to preserve environment quality. Imazalil is not easily biodegradable; however, a resistant microbial consortium was isolated from sludge containing the fungicide that was not able to degrade the pollutant in culture with planktonic biomass.

Photo-Fenton is an advanced oxidation process that was successfully used to degrade pesticides in water using light, Fe(III) and H_2O_2, as energy source, catalyst and oxidant respectively. Frequently, it is not possible to completely mineralize the pollutant and only a partial reduction of total organic carbon (TOC) can be accomplished.

However, the partial oxidation of the recalcitrant compound may lead to byproducts with a higher biodegradability than the former pollutant.

In this work, water solutions containing 500 mg/L Imazalil and 500 mg/L TOC were partially oxidized by photo-Fenton process ($FeCl_3.6H_2O$ (0,15 mM), pH = 3.0, 25 ºC and H_2O_2 doses (9 mM)). The reaction was carried out in a batch reactor (250 mL) with top lighting (UVA, 20 W). After 6 h illumination, Imazalil was completely degraded. There was no change in the TOC with 1 and 2 doses of H_2O_2, which means that mineralization was negligible under these conditions, but with 3 doses, TOC mildly decreased. The three remaining solutions were adjusted for biological treatment. The pH was raised to 7.0 and the solution was supplemented with inorganic nutrients ($SO_4(NH_4)_2$, K_2HPO_4, $MgSO_4$ and $CaCl_2$). The solutions (100 mL) were inoculated with a consortium previously adapted to Imazalil, and incubated at 25 ºC. After 5 days of incubation, the TOC decreased by 38%, 53% and 59% in solutions 1, 2 and 3 respectively, indicating that the oxidation treatment produces byproducts with higher biodegradability than the former fungicide.

This coupled process shows to have great potential for treatment of Imazalil for waste water from fruit industry.

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*Author for all correspondence

BIOTECHNOLOGY PROCESS FOR THE VALORIZATION OF THE LIQUID RESIDUAL EFFLUENTS OF THE CITRIC INDUSTRY

Elizabeth León-Becerril (1); César Augusto Zárate-Cruz (1);
José de Jesús Díaz-Torres (1), Alberto López-López* (1)

(1) CIATEJ A.C: - Guadalajara, Jalisco, México

Orange juice production generates important quantities of solid and liquid residues such as citrus peel, including peel, seeds and membrane; these residues can be utilized as raw material in the manufacture of cattle feed or simply burnt, and many times they are considered as wastes, although their composition is high in sugars, cellulose, hemicellulose, pectin, organic acids and essential oils. In order to prevent problems related to the disposal of this product and environmental concerns, this waste must be properly processed. In particular, pyrolysis of citrus peel as a source of energy requires the reduction of humidity from 82% to 35% H_2O (w/w), generating a liquid residual effluent.

This work proposes a biotechnology process for the treatment and utilization of the liquid effluents of the citric industry. Firstly, a physicochemical characterization of the effluent was made; the effluent presents pH= 4.0, high concentrations in organic matter (100.0 g/l COD); total suspended solids (90.0 g/l) total sugars (48.0 g/l) mainly reducing sugars; in addition of value added byproducts such as essential oils (d-limonene), organic acids as ascorbic (1.5 g/l), citric (2.1 g/l) and succinic (3.6 g/l). Then the process consisted on the recovery of d-limonene by extraction; d-limonene is employed in the manufacture of domestic household products; food and medicines as flavoring agents, and cosmetics and bactericide products. In the next stage, an anaerobic process was employed for the treatment of the liquid residual effluent in order to reduce the concentration of organic matter and to produce in parallel methane; the absence of d-limonene avoids the inhibition of microbial activity.

The system consisted on a batch reactor of 2 liters, operating at constant temperature of 37ºC, and controlled pH at 6.8 ± 0.2. The reactor was inoculated with biomass stabilized from an anaerobic wastewater plant. Adaptation and growth of biomass last 20 days, step increments of organic matter in feeding were made, with periods of 2-3 days, until the real concentration in organic matter was reached.

The anaerobic process reached removal efficiencies of organic matter from 70 to 80%, for organic loading rates from 20 to 30 kg/m^{3}d. The production rate of methane was 400 l CH_4/kg COD removed, in operating conditions. Finally, it was established a biotechnology process for recovery of d-limonene, and the subsequent liquid effluent by an anaerobic process, producing biogas with a high concentration in methane, which can be used as an energy source.

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*Author for all correspondence

CHEMICAL PRETREATMENT AND BIODEGRADABILITY OF EFFLUENTS FROM ETHANOL DISTILLERIES

Alfredo Fuentes-Moyado (1); Ignacio González-Martínez (2);
Jorge Vázquez-Arenas (2); Carmen Fajardo-Ortíz (1); Mónica Meraz-Rodríguez*(1)

(1) Dept. of Biotechnology, DCBS, UAM-Iztapalapa, México DF, México;
(2) Dept. of Chemistry, DCBI, UAM-Iztapalapa, México DF, México

Vinasses have a strong environmental impact because the extremely high organic matter content that attains frequently more than 100 gCOD/L.

Several anaerobic treatments have been proposed to diminish its organic matter content and transform it to methane to supply energy to the sugar and distilling industries. Due to the refractory dark color that is not degraded in anaerobic systems, physic-chemical processes has been proposed, such as chemical coagulation and electrofloculation with iron and aluminum, obtaining high organic matter removal efficiencies from the aqueous phase with the formation of sediments that have to be further treated to recover the metallic ions added and to transform organic matter to a biofuel.

In this work a physic-chemical pretreatment was performed adding $FeCl_3$ to raw vinasses to produce sediments with a high organic matter content that were further assayed for biodegradability in acidogenic and methanogenic anaerobic conditions and the biofuel production was evaluated, considering that iron is a cofactor during acetoclastic methanogenesis. Iron as Fe^{3+} in concentrations ranging from 500 ppm to 3000 ppm were added to not diluted vinasses that contained 103 g COD/L, 34 g TOC/L, 68.3g VS/L and 8.2 g VSS/L, for the suspended and dissolved organic matter coagulation-flocculation-sedimentation without pH control.

It was found that at 1000 ppm of Fe^{3+} produced a sediment containing 66.4 g SV/L with a remnant iron content of around 150 ppm. Afterwards, to test sediments biodegradability, acidogenic followed by methanogenic assays in serological bottles were performed filled with phosphate buffer, adding the sediments obtained as substrate and acidogenic/methanogenic sludges as inoculum with and without controlling the S/X ratio for the methanogenic assays.

The acidogenic assay produced 0.2 mL H_2/h with 29.5% SV removal; while the uncontrolled S/X ratio methanogenic assay performed with the predigested sediment produced 0.4 mL CH_4/h, with a 42.5% SV removal. During the acidogenic stage, acetic, propionic and butyric acids were formed (13.3 g VFA/L) and in the methanogenic stage acetic acid was consumed completely at 0.04 g/L·h producing 96.3% methane in the biogas. Also it was noticed that the acetogenesis from propionate and butyrate was inhibited, probably by the iron present in the sediments during the methanogenic assays.

During another acidogenic assay hydrogen was produced at a rate of 0.6 mL/h with a removal of 43.01% SV while 9.8 g VFA/L were formed, in which no acetic acid

was produced and butyric acid predominated on propionic acid. In the methanogenic assay with an S/X ratio of 2.3, it was found that a minimum amount of acetic acid was consumed and butyric acid was consumed almost completely rendering consequently 57.0% methane in the biogas, less than expected. The contrast between methanogenic assays can be explained through the less amount of substrate added to the methanogenic assays to fix the S/X in 2.3 which contained less iron that did not improve the acetoclastic methanogenesis but allowed the butyric acid acetogenesis and further limited methane production.

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*Author for all correspondence

INFLUENCE OF ORGANIC LOADING RATE ON THE HYDROGEN PRODUCTION FROM AGRO-INDUSTRIAL EFFLUENTS AS SUBSTRATE USING AFBR

Samantha Christine Santos (1); Paula Rúbia Ferreira Rosa (2); Edson Luiz Silva*(2)

(1) Department of Hydraulics and Sanitation, São Carlos Engineering School, University of São Paulo, São Carlos, SP, Brazil;
(2) Department of Chemical Engineering, Federal University of São Carlos, São Carlos, São Paulo, Brazil

The aim of this study was to evaluate the influence of hydraulic retention time (HRT) and two different carbon sources (cheese whey and cassava processing wastewater) on the stability and efficiency of anaerobic fluidized bed reactor (AFBR) in hydrogen production.

Four AFBRs were operated for 160 days, with different operating conditions, and temperature control fixed at 30 °C. Two reactors used cheese whey as substrate ($AFBR_1$ and $AFBR_2$), and two other reactors were feed with cassava processing wastewater ($AFBR_3$ and $AFBR_4$). The reactors were inoculated with sludge from a UASB reactor used for swine wastewater treatment. To inhibit methanogenesis in AFBR reactors, it was conducted heat pre-treatment of the inoculum.

At $AFBR_1$ the organic loading rate (OLR) ranged from 8.5 to 15 kg COD m^{-3} d^{-1} (HRT with a range of 14-8 h), thus achieving the highest hydrogen yield (HY) of 2.6 mmol g^{-1} COD at the lowest OLR. For $AFBR_2$, the OLR ranged from 12 to 40 kg COD m^{-3} d^{-1} and the highest HY of 3.2 mmol.g^{-1} COD was obtained also in the lowest OLR.

When cassava processing wastewater was used as substrate for the $AFBR_3$ the OLR ranged from 4 to 12 kg COD m^{-3} d^{-1} with a fixed HRT of 12 h, and showed a maximum HY of 2.0 mmol g^{-1} COD (OLR 10 kg m^{-3} d^{-1}). In $AFBR_4$ the OLR varied from 14 to 30 kg COD m^{-3} d^{-1} using a HRT of 12 hours, and it was obtained a maximum HY of 1.5 mmol g^{-1} COD (OLR 14 kg m^{-3} d^{-1}). The main soluble metabolites observed at all four bioreactors were ethanol, methanol, acetic, butyric, propionic and lactic acids.

Based on these results it was concluded that the ability to produce H_2 continuously, the organic acids distribution and the bioreactor performance were influenced by factors such as modifying the substrate source and also by decreasing the hydraulic retention time and consequent increased organic loading rates applied.

The results show that fermentation of cheese-processed wastewater has a higher potential to produce hydrogen under the studied conditions.

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*Author for all correspondence

INFLUENCE OF SEED SLUDGE AND HIDRAULIC RETENTION TIME IN CONTINUOUS HYDROGEN PRODUCTION FROM A CASSAVA-PROCESSING WASTEWATER USING GLUCOSE MIXTURES

Paula Rúbia Ferreira Rosa (1); Samantha Christine Santos (2);
Isabel Kimiko Sakamoto (2); Maria Bernadete Amâncio Varesche (2);
Edson Luiz Silva*(1)

(1) Department of Chemical Engineering, Federal University of São Carlos, São Carlos, São Paulo, Brazil;
(2) Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo, São Carlos, SP, Brazil;

In this study it was evaluated the potential of fermentation cassava processing wastewater and glucose mixture as substrates for hydrogen production in anaerobic fluidized bed reactors (AFBR).

The effect of reduction the hydraulic retention time (HRT) (10-2 h) and different sources of inoculum on the stability and efficiency of AFBRs in the production of hydrogen were evaluated. The reactors were inoculated with the sludge from a UASB reactor used for the treatment of swine wastewater (SW) and the sludge from a UASB reactor that had been used to treat poultry slaughterhouse wastewater (SP), both heat treated before innoculation. A concentration of 5000 mg DQO.L^{-1} was applied, 50% glucose and 50% of cassava, with a temperature control to 30 °C.

The application of the high HRTs (10, 8 and 6 h) favored the metabolic pathway of ethanol-acetate. Thus, for the sludge from the UASB treatment of poultry slaughterhouse wastewater (SP) showed the highest hydrogen (HY) and ethanol (EtOHY) yields, of 1.0 mmol H_2.g^{-1}COD and 3.0 mmol EtOH.g^{-1}COD by applying the HRT of 10 and 6 h, respectively. For the sludge from UASB used for the treatment of swine wastewater (SW) the results showed maximum HY and EtOHY of 0.65 mmol. g^{-1}COD (HRT of 6 h) and 2.1 mmol. g^{-1}COD (HRT of 10 and 8 h), respectively. Through the application of short HRTs (4 and 2 h), the metabolic pathway of lactate was favored, and the highest yields of lactic acid (LaY) were 2.8 mmol HLa.g^{-1} COD (SW) and 1.1 mmol HLa.g^{-1} COD (SP). Even with reduced HRT application (2 hours), methane was produced with maximum production of 9.68 L CH_4.d^{-1}.L^{-1}, greater than the hydrogen production of 2.08 L H_2.d^{-1}.L^{-1}.

Contributing to the conclusion, the denaturing gradient gel electrophoresis (DGGE) showed that differences were observed in the microbial community in accordance with the HRT reduction. In the bacterial population profile bands there was a similarity of 55% (SW) and 68% (SP). In contrast, for the profile of the DGGE bands of the methanogenic archaea, there were high similarities (over 90%) among the applied HRTs for the reactors. Based on phylogenetic analysis of 16S rRNA, the sequences of the bacteria and methanogenic archaea were similar to *Lactobacillus* and *Methanobacterium*, respectively.

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*Author for all correspondence

STUDY OF THE EFFECT OF STERILIZATION AND INOCULATION OF LIGNOCELLULOLYTIC FUNGI FOR TREATMENT OF THE AZO DYE BLACK TERASYL SR IN PEAT PACKED BIOFILTERS FOR ORGANIC MATTER AND COLOR REMOVAL IN THE TEXTILE INDUSTRY

M. P. Mejía -López (1); G. E. Moeller-Chávez* (2)

(1) F. I. -UNAM, Cuernavaca Mor., México;
(2) UPEMOR, Cuernavaca Mor., México.

Azo dyes are widely used in the textile industry; they are carcinogenic and recalcitrant molecules. This research is focused on the development of a procedure at bench scale to treat an azo dye (Black terasyl SR) widely used in the Mexican textile industry (thread production and dyeing), by a biofiltration process using peat as the packing material.

The main objective was to study the effect of inoculation with *Pleurotus ostreatus* a lignocellulolytic fungi and the effect of sterilization in the performance of the biofilter to remove organic matter, color and toxicity.

The research consisted in constructing, packing, operating and evaluating four reactors. One reactor was packed just with peat, the second one used sterilized peat inoculated with P. ostreatus, the third reactor was packed with sterilized peat and the last one was packed and inoculated with *P. ostreatus* without sterilization. The reactors were operated for five months. Wastewater treated in this work was originated from an effluent having a high concentration of COD (8000 mg/L) and a color concentration of 566 mg/L. When very high concentrations were presented dilutions were made. The highest volumetric organic workload was 624.7 mg/(L.d) this load was used for a short time due to the rapid decline of the inoculum strain. Thus the load was reduced to 161.7 mg/L d.

Results indicated that the biofilter packed with peat with no sterilization had the best performance: 50% color removal and 56% organic matter removal with the complete elimination of toxicity.

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*Author for all correspondence

EFFECT OF THE COSOLVENT ON THE DEGRADATION OF NAPHTHALENE BY CATALYTIC OZONATION

Claudia M. Aguilar-Melo*(1); Jorge I. Chairez-Oria (1); Julia L. Rodríguez-Santillán (2); Tatiana Poznyak (2)

(1) UPIBI- IPN, México DF, México; (2) ESIQIE-IPN, México DF, México.

Naphthalene (NA) is a polycyclic aromatic hydrocarbon that has been listed as a priority pollutant by Environmental Protection Agency of United States (EPA), due to its possibility to act as carcinogenic and teratogenic. This chemical has also been associated to medical disorders in liver, kidney, red blood cells and neurological damage. The NA toxic properties and stability in aquatic systems increase the removal difficulty by conventional methods (for example, the microbiological effect). The ozonation and catalytic ozonation (O_3-cat) process are sound alternatives of degradation for these compounds. The catalytic ozonation consists of combining the ozone and heterogeneous catalyst. This interaction between these compounds produces species of radical type such as OH• that has higher oxidation potential. Therefore, O_3-cat process increases the mineralization degree in organic recalcitrant compounds compared to conventional ozonation (O_3). However, the existence of certain compounds in water, such as: phosphates, carbonates, sulphates, surfactants and co-solvents (in real systems, the chemicals increase the pollutants concentration in water bodies) can be detrimental to the efficiency of O_3 and O_3-cat systems.

In this work, the degradation of the NA (20 mg/L) in the presence of a co-solvent (ethanol) was studied, in order to simulate some aspects of real wastewaters where not only water acts as solvent. Two proportion of the mixtures water:ethanol were selected 70:30 and 50:50 with the purpose of studying the effect of co-solvent in the degradation system of NA by O_3 process. In addition, to increase the concentration of NA, the O_3-cat process was used with nickel oxide as catalyst (100 mg/L). The monitoring of NA was carried out by the technique of high performance liquid chromatography (HPLC). The degradation analysis of NA by O_3-cat in two different proportions of co-solvent showed the following results: in the case of 70:30 (water:ethanol) a 95% of NA degradation in 60 min was obtained, while in the case 50:50 (water:ethanol), only 60% of NA degradation in the same time of treatment was achieved. The O_3 process showed similar results of degradation to the initial compound in comparison with catalytic system. According to these degradation results, exist an inhibition effect in pollutant removal by ethanol. This effect could be related to the proportion in which ethanol appeared when it is combined to NA. The higher ethanol concentration, the lower elimination rate of NA was obtained (by 35% during the 60 min). In the experiments, it was observed that catalyst in the ozonation process has a removal profile comparable with the one of the conventional treatment. This work agrees with previous investigations regarding to the difference in dynamics of byproducts concentrations that indicates a better decomposition of these organics by the O_3-cat process.

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*Author for all correspondence

MULTI-COMPONENT ADSORPTION OF COPPER, NICKEL AND ZINC FROM AQUEOUS SOLUTIONS ONTO ACTIVATED CARBON PREPARED FROM DATE STONES

Farah Bouhamed*

Université des Sciences et Technologies de Lille UMR CNRS- Lille1 8157 Geosystemes Equipe Chimie Analytique et Marine, Bât C8 59655 Villeneuve d'Ascq Cedex France

The adsorption is a separation process widely used for the removal of heavy metals from wastewater due to its versatility and easy operation. Among the most commonly used adsorbents are activated carbon, biosorbents, natural zeolites, and clay minerals.

The individual adsorption of heavy metals from water solution on these adsorbents has been extensively studied; however, industrial wastewater normally contains two or more metals. The presence of another metal in solution affects the adsorption capacity of the single metal. Therefore, it is very important to study the multicomponent adsorption of heavy metals and to find out about the selectivity or affinity of each metal for a given adsorbent and to know if there is competition between the metal cations for the same adsorption sites.

Our recent work demonstrated a successful conversion of date stones residues to activated carbon. The objective of this work was to determine sorption properties of the prepared activated carbon when contacted with a mixture of metal ions (Cu, Ni, Zn) and thus to extend our previous study made on single metal sorption on this material. A synthetic mixture solution of copper, zinc and nickel was arbitrarily selected as a modeled tertiary sorption system. Possible interactions between the metal ions and the surface of the adsorbent in the ternary component sorption were described.

The competitive adsorption of Cu(II), Ni(II) and Zn(II) onto activated carbon prepared from date stones was investigated in ternary system. Mutual interference effects were probed using equilibrium adsorption capacity ratios, qe'/qe, where the prime indicates the presence of two other metal ions. The combined action of the metals was found to be antagonistic, and the metal sorption followed the order $Cu^{2+}>Ni^{2+}>Zn^{2+}$. The behavior of the competitive adsorption of Cu(II), Ni(II) and Zn(II) was successfully described by the original Langmuir model.

The test results using real wastewater indicated that the prepared activated carbon could be used as a cheap adsorbent for the removal of heavy metals in aqueous solutions failure of biological removal mechanisms.

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*Author for all correspondence

PHOTOCATALYTIC AND SONOCATALYTIC OXIDATION OF A DYE OVER TIO_2: KINETIC MODELLING

José A. Ayala-Romero (1); Abhishek Dutta (2); Richard S. Ruíz-Martínez (1); Carlos O. Castillo-Araiza*(1)

(1) Grupo de Procesos de Transporte y Reacción en Sistemas Multifásicos - UAMI, México DF, México; (2) Faculteit Industriële Ingenieurswetenschappen, KU Leuven, Campus Leuven (@Groep T), Andreas Vesaliusstraat 13, B-3000 Leuven, Belgium.

Water pollution is one of the main challenges to be takled on worldwide. Textile dyes, herbicides and pesticides, aromatic compounds, etc., are among most common organic contaminants found in ground water wells and surface waters, causing adverse effects to the environment and to human health. For instance, water scarcity is observed in many developing countries because of growing world population and water pollution. To account for this latter problem, water is normally treated by various mechanical, biological, physical, and chemical processes. Nevertheless, there are certain products, called bio-recalcitrant (non-biodegradable), for which much more effective treatment systems are needed.

Advanced Oxidation Processes (AOPs), and particularly heterogeneous photocatalysis based on the production of hydroxyl radicals (OH•) is recognized today because of its efficiency in degrading a wide range of recalcitrant organic contaminants into CO_2, water and some biodegradable mineral acids. The most common catalysts are based on the industrial TiO_2 Degussa P25. Nevertheless, photocatalysis based on TiO_2 is still experiencing a series of challenges, particularly those based on: (a) developing new catalysts with a strong capacity to mineralize high concentrations of recalcitrant molecules and (b) addressing kinetic modeling and reactor design.

In this work the catalytic photodegradation of a dye on an industrial TiO_2 is carried out simultaneously with catalytic sonodegradation in order to address the challenges of the former technology. These technologies are evaluated in a laboratory scale reactor operated in kinetic control regime.

The results suggest that the catalytic synergy using both technologies simultaneously lead to the strongest capacity to mineralize the recalcitrant molecule. A lumped kinetic model based on Langmuir Formalism for the mineralization of the dye on TiO_2 is developed accounting for both homogeneous and heterogeneous reactions.

The kinetic model predicts intrinsic observations adequately. Kinetic parameters indicate that the use of ultrasonic frequency in photocatalyst improves catalyst activity as a consequence of a major formation of active sites (OH•) on catalyst surface.

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*Author for all correspondence

MICROBIAL DEGRADATION OF THE GEMFIBROZIL DRUG IN A RIVER ECOSYSTEM

Anna Barra Caracciolo* (1); Nicoletta Ademollo (1); Martina Di Lenola (1); Luisa Patrolecco(1); Paola Grenni(1)

(1) National Research Council of Italy, Water Research Institute, Rome (Italy)

Pharmaceuticals are commonly found as water ecosystem microcontaminants (µg-ng/l). Since they are molecules designed to be biologically active at very low concentrations, their presence in the environment is a source of concern for both human and ecosystem health. After therapeutic administration, human pharmaceuticals are generally excreted unchanged and their incomplete removal during biological wastewater treatments is the main source of surface water contamination. Degradation of a chemical in the aquatic ecosystem depends on a variety of factors, including the compound's properties, environmental factors and above all the presence of a natural microbial community able to degrade it via metabolic and/or co-metabolic pathways.

Gemfibrozil, a fibrate drug used as a lipid regulator, has been found in several EU and Italian surface waters, including the River Tiber (Rome). In this context, the present work aims to evaluate the natural attenuation potential of a river ecosystem versus Gemfibrozil, alone or in the co-presence of the non-steroidal anti-inflammatory drug Naproxen. For this purpose microcosms treated with Gemfibrozil or simultaneously treated with Gemfibrozil and Naproxen were set up in order to assess if their presence could affect the natural microbial community. Microcosm degradation experiments were set up using river water samples collected downstream from a wastewater treatment plant. The experiment was performed in autumn and was compared with the same experimental set up performed in spring of the same year to highlight if seasonal differences in the river water influenced the Gemfibrozil degradation. The microcosms were treated with Gemfibrozil or Gemfibrozil plus Naproxen (100 µg/L each) in order to evaluate the disappearance time of 50% of the initial drug concentrations (DT_{50}) by using a HPLC-Fluorescence method. Moreover, the drug effect on the microbial community number was also evaluated using an epifluorescence microscopy method.

The overall results show that the river microbial community had a key role in Gemfibrozil degradation. However, in spring Gemfibrozil halving was about 70 days in the microbiologically active water, while in autumn, it was only partially degraded. 105 day after the microcosm treatment 64% of its initial concentration was still present. This fact could be ascribable to the higher DOC occurrence, which could have a role in the drug adsorption, reducing its bioavailability. Moreover, Gemfibrozil degradation was further lengthened in the co-presence of Naproxen.

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*Author for all correspondence

COMPARISON OF BDD AND SNO_2 ELECTRODES FOR CARBENDAZIM ELECTROOXIDATION

Houceine Bouya*(1); Mohamed Errami (1); Rachid Salghi (1); A. Hormatallah (2)

(1) Equipe de Génie de l'Environnement et de Biotechnologie, ENSA, Université Ibn Zohr, BP 1136, Agadir, Morocco;
(2) Laboratoire des pesticides. Institut Agronomique et Vétérinaire Hassan II, Complexe Horticole d'Agadir, BP 18/S. Agadir. Morocco.

In the recent past two decades, anodic oxidation has been widely investigated for wastewater treatment. Its high oxidation efficiency, fast reaction rate, and easy operation make it a very attractive alternative to the conventional processes for degrading toxic organic pollutants. Specifically, the electro-oxidation process has been shown to be attractive for the mineralization of organic pollutants from wastewater. Concentrated and refractory organics could be mineralized on the electrode during anodic oxidation process. It is well known that proper selection of electrodes is one of great importance for effective and economical operation. Good electrodes should be not only effective for pollutant degradation, but also stable electrochemically. Up to date, numerous types of electrodes including Pt, graphite, PbO_2, SnO_2, IrO_2, Pt–Ir, RuO_2, glassy carbon, fiber carbon, MnO_2, TiO_2, and boron-doped diamond (BDD) have been investigated. SnO2 electrodes are generally believed to be superior to Pt, graphite, PbO_2, and many other common electrodes. Pure SnO_2 is an n-type semiconductor with a band gap of about 3.5 eV. BDD has received growing attention for pollutant oxidation. The anodic oxidation is a promising process for degrading toxic and organic pollutants present in wastewater treatment. Suitable selection of electrodes is the key to reach effective and economic operation.

In this study, two types of electrodes, BDD and SnO_2, which is generally believed that the BDD electrode be superior to the usual electrodes, were compared under the same conditions. It was found that BDD electrode could mineralize our pesticide effectively. But the SnO_2 electrode could only mineralize less than BDD. When oxidizing more refractory pesticide, it demonstrated very poor activity.

The straight experimental comparison in the present study indicates that the BDD electrode is much better than the SnO_2 electrode for pesticide oxidation.

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*Author for all correspondence

REMEDIACIÓN DE AGUAS CONTAMINADAS CON NÍQUEL USANDO *Botryococcus braunii*

Loreta Rojas (1); María Mar Areco * (1); ***Gustavo Curutchet*** (1)

(1) UNSAM. 3IA. Laboratorio de Análisis Ambiental. Buenos Aires. Argentina.

La contaminación de aguas con metales pesados provenientes de descargas industriales es un problema aún no resuelto. Industrias como las curtiembres, galvanoplastias, petroquímicas y mineras, aportan al medio ambiente metales pesados (Ni, Cr, Cu, Zn, etc.) sumados a otro tipo de contaminantes. Las tecnologías tradicionales de tratamiento de efluentes (precipitación, floculación, etc.) suelen no ser efectivas cuando se tratan bajas concentraciones de metales (que aún generan un enorme impacto ambiental) en grandes volúmenes de agua. Tecnologías que involucran el uso de diferentes microorganismos han mostrado potencialidad para resolver algunos casos. *Botryococcus braunii* es una microalga de agua dulce con capacidad de producción de hidrocarburos y otros metabolitos de interés. En trabajos previos hemos mostrado su potencialidad para depurar aguas ácidas conteniendo cobre y zinc, pudiendo acoplarse la depuración con producción de hidrocarburos. El objetivo del presente trabajo es estudiar la remoción de níquel, metal ampliamente utilizado en la industria de baterías, aceros y galvanoplastias, entre otras, a partir de *B. braunii*.

Para ello se determinaron las tasas de crecimiento de *B. braunii* en dos medios de cultivo: Bold Basal Medium (BBM) y Chu 13 modificado; variando las condiciones de pH, a 25 °C y bajo ciclos luz-oscuridad de 16:8 horas. Se evaluó la viabilidad de la cepa en presencia de distintas concentraciones de Ni(II) (0 o control, 1, 2, 3, 5 y 10 ppm) y se realizaron experiencias de remoción del metal durante 35 ± 5 días, a partir de soluciones de Ni(II) de las concentraciones mencionadas en contacto con un inoculo (de concentración celular conocida) de *B. braunii*. Las concentraciones remanentes de Ni(II) se determinaron por absorción atómica y el crecimiento algal a lo largo del experimento por turbidimetría y peso seco. Se evaluaron las tasas fotosintéticas y de respiración del alga en condiciones de luz y oscuridad y en los cultivos con diferentes concentraciones de Ni(II) mediante experimentos con respirómetro.

Los resultados obtenidos permitieron determinar una mayor tasa de crecimiento de *B. braunii* en medio BBM; demostraron la capacidad del alga de remover níquel presente en solución, ya que esta microalga no sólo adsorbe el metal sino que su actividad fisiológica favorece el aumento del pH del medio desde valores de pH de 5 hasta valores de 7, lo que favorece la precipitación de las oxo sales del metal, este proceso colabora con la depuración de metales pesados del medio acuoso, ya que en presencia de *B. braunii* el níquel puede ser removido por adsorción y por precipitación; y, por último, los resultados permitieron analizar relaciones entre el metabolismo oxidativo, la fotosíntesis y el stress con el proceso de remoción de Ni(II).

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*Author for all correspondence

APLICATION OF AN ELECTROOXIDATION PROCESS USING PbO_2 ANODES FOR THE ATRAZINE REMOVAL

Juan Napoles-Armenta (1); Celestino García-Gómez (1);
Pablo Gortáres-Moroyoqui* (1)

(1) ITSON, Biotecnología y Ciencias Alimentarias, Ciudad Obregón, México.

In recent years, concern has arisen from the contamination of water bodies by some contaminants found in low concentrations (ng/L or μg/L). Today we speak about emerging contaminants such as pharmaceuticals, personal care products, surfactants, industrial additives, plasticizers, pesticides and others chemical compounds. Atrazine is an herbicide of interest because of in aquatic species can cause feminization and even toxicity.

The objective of this work was to assess the effect of current intensity (I), electrolysis time (t), and hydraulic recirculation time (HRT) on the removal of atrazine from water. The initial concentration in each of the treatments was 5 mg/L. A cylindrical reactor with concentric electrodes was used. The electrooxidation processes has been investigated using Ti/PbO_2 (anode) and Ti (cathode) circular electrodes in the presence of $NaSO_4$ as electrolyte. Electrodes were distributed, from center to periphery, cathode-anode-cathode. A factorial 2^3 *e*xperimental design with a composite central experimental design, were carried out to evaluate the effect of I, t, and HRT on the atrazine removal.

Five levels were studied for each variable (I, t, and HRT) with a total of 20 different experimental conditions or treatments. Samples were analyzed using UV spectrophotometry at 223nm. Data analysis was performed using the Design Expert program 9.

Micropollutant removal was affected by for all variables studied, showing significant difference. The longer time is the greater the atrazine removed ($F \leq 0.0001$). Therefore, t has a positive effect on atrazine removal. Also atrazine removal is directly affected by current intensity ($F \leq 0.0001$), which implied a positive effect. In contrast HRT showed a negative effect on the atrazine removal ($F \leq 0.0075$). The best removal was 68.04%, which was achieved with 2A of I, 90 minutes of t, and 3.87minutes of HRT.

This process seems to be a promising technology that can be used as tertiary treatment to remove contaminants emerging the water.

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*Author for all correspondence

REMOVAL OF CONGO RED DYE BY ANAEROBIC UASB REACTOR AND ELECTROCHEMICAL SYSTEMS

Itzel Romero-Soto (1); Celestino García-Gómez (1); Luis Álvarez-Valencia (1); Edna Meza-Escalante (2); Pablo Gortáres-Moroyoqui*(1)

(1) ITSON, Departamento de Biotecnología y Ciencias Alimentarias, Ciudad Obregón, México.
(2) ITSON, Departamento de Ciencias del Agua y Medio Ambiente, Ciudad Obregón, México

In this work, the removal efficiency of Congo Red (CR) was evaluated coupling an UASB reactor (0.9 L) to an electrooxidation (1.12 L) or an electrocoagulation (0.95 L) cells as post-treatment. The UASB reactor was inoculated with anaerobic sludge (SSV 23.31g/L) and fed with synthetic water containing RC (100 mg/L) and sodium acetate (1 g/L equivalent to 1066 mg of COD/L), for 3 months at different hydraulic residence times (HRT, 12, 10, 8 h). Removal efficiency of color and chemical oxygen demand (COD) was monitored. The UASB reactor effluent was collected and delivered independently to cells of electrocoagulation or electrooxidation to compare these post-treatments. The system of electrocoagulation (EC) was operated using two pairs of electrodes, anode of Fe and cathodes of stainless steel, with 110 cm^2 of surface area.

In electrooxidación system (EO) two pairs of electrodes were used, with Titanium (Ti) as cathode and Ti coated with PbO_2 as anode. In both systems the effect of current intensity (1 A and 2 A), recycle time (10 min and 30 min) and operation time (for EC, 10 min and 25 min and EO 60 min and 140 min) was evaluated on the removal efficiency of color and COD. A factorial design 2^3 with central composite design was used, with a total of five levels for each variable and a total of 20 different experimental conditions or treatments.

In the UASB reactor an average of 96.6 ± 1.98% and 84.2 ± 3.47%, respectively were obtained, resulting 3.4 mg RC/L and 168 mg COD/L. The maximum removal efficiencies with electrocoagulation were 54.32% to 53.61% for COD and color and electrooxidation exceeded 91.25% and 99% for color and COD respectively.

These results indicate that the electrooxidation is a suitable system as post treatment to ensure complete removal of color and by-products derived from the anaerobic biotransformation of azo dyes in industrial wastewater.

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*Author for all correspondence

OPTIMIZATION OF PHENOL REMOVAL USING TI/PBO_2 ANODE WITH RESPONSE SURFACE METHODOLOGY

Celestino García-Gómez (1); Juan Nápoles-Armenta (1);
Pablo Gortáres-Moroyoqui* (1)

(1) Departamento de Biotecnología y Ciencias Alimentarias. Instituto Tecnológico de Sonora. 5 de Febrero 818 sur. C. P. 85000 Obregón, Sonora, México

Phenolic compounds are commonly present in wastewater produced by several industries, such as petroleum refineries, formaldehyde resins, pharmaceuticals, textile, dyes, coke, plastics, coal conversion and chemical plants. They are among 129 pollutants listed by the Environmental Protection Agency (EPA) due to their toxicity and difficulty to be degraded. Because of their refractory chemical nature, the use of biological treatments becomes inadequate. The electrochemical oxidation technology has become a promising method for the toxic, bio-refractory, and highly concentrated organic wastewater treatment because of its simplicity, easy control, strong oxidation performance, and environmental compatibility.

The main goal of this study was to assess the removal of phenol in aqueous medium (initial concentration of 20 mg L^{-1}) by electrooxidation process using Ti/PbO_2 concentric anode electrode. Response surface methodology (RSM) was applied to evaluate simple and combined effects of the three main independent parameters; current density (1-2 A), electrolysis time (20-40 min) and recirculation flow rate (187-284 ml min^{-1}) on the phenol removal efficiency and optimizing the operating conditions of the treatment process. A factorial experimental design 2^k was firstly used to determine the effect of independent parameters on phenol removal. Afterward, the optimal experimental parameters were investigated by using a Central Composite Design methodology (CCD). A total of 20 different experimental conditions were carried out with five level of each independent variable. Current intensity and treatment time were the main parameters affecting phenol removal rate. Analysis of variance (ANOVA) showed a high coefficient of determination value (R^2 =0.932) and satisfactory prediction first-order regression model was derived. Maximum phenol removal efficiency was predicted and experimentally validated. The optimum values for each variable were current density 1.72 A, electrolysis time 40 minutes, and recirculation flow rate 187 ml min^{-1}. Under optimal conditions, high phenol removal (>90%) was obtained. This study clearly showed that response surface methodology is one of the suitable methods to optimize independent variables to maximize phenol removal.

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*Author for all correspondence, pablo.gortares@itson.edu.mx.

REMOVAL OF COLOR AND COD FROM A TEXTILE EFFLUENT BY ELECTRO-OXIDATION

Vianey A. Burboa-Charis (1); Petia Mijaylova-Nacheva (2); José de Jesús Treviño-Resendez (2); Celestino García-Gómez (1); Itzel C. Romero-Soto (1); Pablo Gortáres-Moroyoqui*(1)

(1) ITSON, Cd. Obregón, Sonora, México;
(2) IMTA, Juitepec, Morelos, México;

The textile industry generates large amounts of wastewater, which represent one of the effluents most difficult to treat. Regulatory requirements and water optimization (including reuse in industrial activities) demands research and innovation around processes to improve the removal of persistent or poorly biodegradable components. The aim of this study is to determine the effect of current intensity and time on the removal of color and chemical oxygen demand (COD) using electro-oxidation (EO) system. The effluent comes from a textile industry in Jiutepec, Morelos, which was previously passed on a membrane biological reactor. Color and COD concentration before to be inside to the EO system were 2493.33 Co-Pt and 2605.45 mg L^{-1}, respectively. In the case of color, were analyzed according to the standard method APHA platinum-cobalt (Manual HACH, 2010) read in a UV-VIS spectrophotometer HACH. The determination of COD was established by the standard method.

A cylindrical acrylic reactor height of 19.5 cm was used, with an operating capacity of 1.25 L, working with three electrodes; two cathodes (Ti) and one anode (TI/PbO2) with cathode-anode-cathode arrangement. Preliminary tests were conducted using different intensity values (3, 6 and 9 Amperes) and two different recirculation flows (120 and 480 ml/min), with a time of 120 minutes. According to the results obtained in preliminary testing an experimental matrix was performed in the statistical package Statgraphics Centurion with a 2^k (2^2) factorial and central composite design method (three central points) and four extreme levels (low and high). Variables studied were current intensity and time, trying five levels each one (low extreme, low, central, high and high extreme) with a total of eleven different experimental conditions or different treatment. Recirculation flow rate was constant (480 ml /min). The optimal conditions obtained were time 151.7 minutes and current intensity 10 A, with a removal of 95.2% for color and 73.3% for COD. The cost, considering only electricity consume, for optimal treatment is 127.19 $/m^3.

Comparing the results with previous, published research, the color was quickly removed, reaching 98% color removal; Furthermore COD reduction reached 90% was high compared to 75% obtained by other authors. This can be ascribed to the difference of the electrode material, the different arrangement of the electrodes or to the variation in operation time and intensity.

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*Author for all correspondence

REMOCIÓN DE METALES PESADOS EN SOLUCIÓN ACUOSA EMPLEANDO LA BIOMASA DE LA PERSEA AMERICANA COMO SORBENTE

Lizbeth L. Díaz-Muñoz (1); Adrián Bonilla-Petriciolet*(1)

(1) Instituto Tecnológico de Aguascalientes, Aguascalientes, México;

La contaminación del agua por metales pesados es un problema ambiental relevante ya que puede causar diversos trastornos y enfermedades en los seres vivos. Actualmente, se dispone de diversos métodos para la remoción de metales pesados entre los que se encuentran el intercambio iónico, ósmosis inversa, precipitación química y procesos de sorción. Específicamente, la sorción presenta diversas ventajas ya que es de bajo costo, sencilla operación y de alta eficacia de remoción. Bajo este contexto, en el presente estudio se ha empleado la biomasa de la Persea Americana (BPA), en su forma natural y modificada con ácido tartárico (AT), como sorbente para la remoción de Cd^{2+} y Zn^{2+} en solución acuosa. Para la modificación química de esta biomasa se realizó un diseño de experimentos basado en un arreglo ortogonal L_{16} donde se estudió el efecto de las variables de impregnación de la BPA sobre la capacidad de remoción de metales pesados en el agua. Estas variables fueron: relación masa – volumen de la BPA y la solución de AT, concentración del ácido tartárico, temperatura de la modificación química y tiempo de contacto. Los experimentos de remoción se realizaron con soluciones mono-componente de estos metales a 30 °C, 150 rpm y 24 h. Los resultados indican que la BPA en su forma natural presenta una capacidad de remoción máxima de 5.60 mg/g para el Cd^{2+}, mientras que para el Zn^{2+}, la capacidad de remoción máxima es 2.59 mg/g. Por otra parte, la BPA modificada con una concentración de 1.5 M de ácido tartárico y empleando una relación masa – volumen de 1/5 g/mL, 120 °C y 8 h mostró las capacidades de remoción mayores para el Cd^{2+} y Zn^{2+}. Los experimentos de remoción de metales pesados utilizando la BPA modificada con AT mostraron una capacidad de remoción de 10.93 y de 7.41 mg/g para el Cd^{2+} y Zn^{2+}, respectivamente. En general, la capacidad de remoción de metales pesados utilizando BPA modificado con AT puede aumentar hasta un 195 y un 286% para el Cd^{2+} y el Zn^{2+}, respectivamente, con respecto al material en su forma natural. En conclusión, los resultados obtenidos indican que la BPA empleada en este trabajo es un sorbente alternativo, de bajo costo y prometedor para remover iones de Cd^{2+} y Zn^{2+} en soluciones acuosas.

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*Autor de correspondencia

CADMIUM REMOVAL CAPACITY FROM WATER AND ITS EFFECT ON *Schoenoplectuspungens*

Carmen Mendez-Hurtado (1), Edith Flores-Tavizón* (1),
Juan Pedro Flores-Marguez (2) Sergio Saúl-Solís (1), Laura Yáñez-Espinosa (3)

(1) IIT-UACJ Ciudad Juárez, Chihuahua. México
(2) ICB-UACJ Ciudad Juárez, Chihuahua. México
(3) IIDZ-UASLP San Luis Potosí, San Luis Potosí. México

The constant anthropogenic activities significantly increased the concentration of heavy metals, such as cadmium (Cd), in human ecosystems. As a result of activities such as mining, industry, use of fossil fuels or household waste, application of phosphate fertilizer and waste incineration, Cd has been pushed in the atmosphere, giving it the way to travel great distances to be subsequently deposited on the ground or water. The presence of high concentrations of Cd in the environment has a negative effect on the metabolism of plants, with the generation of reactive oxygen species and blocking essential functional. *Schoenoplectuspungens* is a species of flowering plant in the sedge family known by the common name Common Three-Square, is used for the stabilization or recovery of metals from contaminated areas; their roots grow easily in the presence of abundant water so it is an excellent choice for soil stabilization in wetlands and clean sites. Also meets the requirements to be used in hydroponics techniques because it grows easily without soil and is tolerant to pH acid characteristics. In this work we determined the capacity for Cd removal from hydroponic solution at concentrations of 0.2, 0.5, 1.0, 3.0 and 5.0 ppm by the *Schoenoplectuspungens* species, as well as damage caused by metal on vegetative structure, chlorophyll content and functional groups present. The observed removal of Cd from the solutions by the plant was more than 90%, being it accumulated in the plant. Finally, Cd affects chlorophyll content which is inversely proportional to the concentration of the metal, because Cd in their ionic form replaces to magnesium on the chlorophyll molecule; while the decreasing the functional groups is because the Cd bind to these groups or is substituted to other elements.

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*Author for all correspondence

SWINE WASTEWATER TREATED BY AN ANAEROBIC SECUENTIAL SYSTEM WITH PULSED RECIRCULATION

Germán Giácoman-Vallejos*(1); Ileana de los Ángeles González-Canché (1); María del C. Ponce Caballero (1)

(1) Universidad Autónoma de Yucatán (UADY), Facultad de Ingenieria, Mérida, Yucatán, México;

The wastewater from intensive pig production represents a serious problem of environmental pollution, particularly in regions with carstic soils with high permeability such as in Yucatan State, México. The state swine production currently generates 9428.37 m^3/d of wastewater, which is mainly directly disposed to the soil, making it vulnerable as well as the surface and groundwater.

Swine wastewater contains high concentrations of organic matter (over 10000 mg TCOD/L), nutrients (over 1000 mg/L both nitrogen and Phosphor), pathogens (over $1*10^{+9}$ NMP), heavy metals (Cu over 3 mg/L and Zn over 54 mg/L) and suspended solids (over 15000 mg/L). As a result of the high organic matter content in swine wastewater, oxygen consumption is increased and its depletion leads to a decrease in ecosystem diversity. The high nitrogen and phosphorus concentrations in this wastewater, can lead to the eutrophication of aquatic ecosystems. Also, the presence of pathogens, could lead to the dissemination of diseases.

To treat swine wastewater, it has been used anaerobic digestion by different type of reactors; one of them is the recently employed anaerobic sequential Batch Reactor (ASBR), normally operated without recirculation.

This study evaluated as an alternative for the treatment of swine wastewater an ASBR with recirculation and under four different sequences of pulsed and rest. It was considered that the inclusion, of a pulsed sequential intermittent recirculation in the ASBR allow increasing the contact between the substrate and the microorganisms through the promotion of short mixing cycles. These short mixing cycles also minimizes the suspension of solids due to the release of trapped gas bubbles contained therein. This in fact prevents the formation of flocculent solids and instead promotes sedimentation of dense material generating a granular sludge.

From the results, it was observed efficiencies greater than 90% removal of total COD for all cases tested and 75% removal efficiency of soluble COD in the case of two hours of rest with pulsated recirculation with a highly significant statistical difference of 0.006 with respect to other sequences. Regarding the total phosphorus, was obtained a removal above 75% for the four cases evaluated. The results make clear that ASBR with pulsated recirculation is an alternative for the treatment of swine wastewater.

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*Author for all correspondence

CONTRIBUTION TO THE ASSESSMENT OF THE HEALTH STATUS OF THE COAST OF SIDI IFNI: BIOMONITORING OF THE IMPACT OF WASTEWATER

Mohamad Abbassi; Abderrazak Kaaya*; Ali Banaoui

Laboratoire des "Systèmes Aquatiques: Milieu marin et continental ", Faculté des Sciences - Université Ibn Zohr, Agadir - Maroc

The present study constitutes a contribution to the assessment of the marine ecosystem health in the town of Sidi Ifni (south of Morocco). So far, there are similar studies reported in the open literature, in spite of the history of wastewater discharges in that zone. The objectives of this work were to perform the environmental characterization of the site by physicochemical analyses and to evaluate the extent of chemic pollution by biological methods.

For the chemical approach, analysis of physicochemical parameters of marine water (Temperature (T°C), pH, conductivity and salinity) were carried out. Our results, at both sites studied, show a real ecosystem disturbance of Sidi Ifni. This led us, in the present conditions, to strengthen coastal surveillance of the city and neighboring sites and to develop effective prevention against pollution. In addition, think long term, to treat all wastewater from the city.

Preliminary measurements of biochemical indicators such as *Acetylcholinesterase, Glutation S-transeferase*, *Catalase* and *Malondialehyd* (AChE, GST, CAT and MDA) in the Mediterranean mussel (*Mytilus galloprovincialis)* collected from the two sites on the coast of Sidi Ifni, showed that these activities are measurable and inducible by pollution existing in the study sites. It would now be useful to characterize the best expressed activities and study their induction in situ, their qualitative and quantitative correlation with contaminants in this environment.

Seasonal variations of the measured parameters show that it is imperative to take into account such variations in the development and validation of biomarkers.

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*Author for all correspondence

EMPLEO DE ULTRASONIDO DE POTENCIA DE ALTA FRECUENCIA PARA LA DESACTIVACIÓN DE AGENTES CONTAMINANTES BIOLÓGICOS EN AGUAS RESIDUALES

Orlando Rodríguez-Rúa*

ICIMAF, La Habana, Cuba.

Al presente valiosas reservas de aguas se encuentran en riesgo de contaminación o se hallan francamente en esta condición. La desactivación de no pocos agentes contaminantes del ambiente resulta un verdadero reto para la tecnología en uso. Importantes contaminantes son de carácter biológico, muchos de los cuales son resistentes a su eliminación por los métodos tradicionales, básicamente empleando la cloración. Se reporta además, que la desinfección de agua por cloración genera variados DBPs (*disinfection by products*) potencialmente cancerígenos para los seres humanos.

Es en este escenario donde han sido propuestas diferentes alternativas para el tratamiento del agua de consumo humano y de otros residuales acuosos por medio de distintos procesos de oxidación avanzada o AOP (*advanced oxidation processes*), siendo uno de estos implementado a través del uso ultrasónicos (US). Entre las ventajas de la aproximación se encuentra la no introducción de productos químicos contaminantes ni generados por la acción de las ondas acústicas, la no afectación del método cuando en el líquido se encuentran sólidos en suspensión y la posibilidad del uso combinado de la técnica ultrasónica con otros AOPs, lográndose una notable acción sinérgica.

Este trabajo presenta un análisis de la utilidad del empleo de US de potencia de alta frecuencia (>100 kHz) para la desactivación de contaminantes biológicos como de virus, bacterias y oocitos de *Cryptosporidium parvum* en residuos acuosos, discutiendo resultados experimentales obtenidos en la eliminación de estos últimos. Esta tecnología ultrasónica puede ser de interés en otras aplicaciones de desarrollo sostenible, como es para la degradación de pesticidas y compuestos aromáticos en diferentes residuales.

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*Author for all correspondence

BIOSORCION DE METALES PESADOS UTILIZANDO SISTEMAS INMOVILIZADOS DE *Spirulina maxima*

A.R. Domínguez-Bocanegra

CINVESTAV- IPN, México DF, México

En el presente trabajo se estudió la capacidad que presenta la biomasa de *Spirulina maxima (Arthrospira)* inmovilizada en alginato de sodio y agar-agar para crecer, adsorber y remover cadmio y plomo presentes en aguas residuales.

Los experimentos se llevaron a cabo en matraces Erlenmeyer de 1000 mL de capacidad total, con 800 mL de medio de cultivo Zarrouk modificado y 100 g de pellets con y sin células de *S. maxima* en fase exponencial de crecimiento, adicionando simultáneamente y de forma paralela los metales cadmio y plomo a 5 ppm, 10 ppm, 20 ppm y 50 ppm a partir de soluciones patrón de acetato de cadmio $(CH_3COO)_2\,Cd{\cdot}2H_2O$ y carbonato de plomo $PbCO_3$ respectivamente. Los matraces se incubaron a temperatura ambiente (28 ± 2ºC), aireación continua 0.5 vvm, agitación 100 rpm y fotoperiodo natural (luz solar). Para evaluar la capacidad de adsorción de la biomasa de *S. maxima* y de los soportes (alginato de sodio y agar-agar) se tomaron muestras durante la primera hora cada 10 min, en la segunda hora cada 20 minutos y durante la tercera hora cada 30 minutos; a partir de la cuarta hora se tomaron muestras cada 60 minutos hasta completar 8 h, posteriormente cada 24 h. Se cuantificó peso seco, concentración de clorofila “a” y concentración de metal utilizando un espectrofotómetro de absorción atómica marca Varian.

Los resultados obtenidos indican que la máxima adsorción de los metales en la biomasa inmovilizada de *Spirulina maxima* se llevó a cabo durante la primera obteniendo una remoción del 84% en presencia de 20 ppm Pb^{+2} en alginato de sodio y un 79% con agar-agar, mientras que en presencia de 20 ppm Cd^2 los porcentajes de remoción fueron menores obtenido un 24% con alginato de sodio y 19.7% en agar-agar. La capacidad máxima de biosorción ($q_{máx}$) de *S. maxima*, bajo estas condiciones, para cadmio fue de q_{max} 0.85 g de Cd/g biomasa con alginato de sodio y de q_{max} 0.56 mg de Cd/g biomasa en agar-agar, mientras que para plomo fue de q_{max} 11.61mg Pb^{2+}/g biomasa en alginato de sodio y de q_{max} de 1.5 mg Pb^{2+}/g biomasa en agar-agar.

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*Author for all correspondence

PHENOLIC MARKERS OF STRESS IN PLANTS *IN VITRO* OF *Vallisneria americana* WITH ENRICHMENT AND ENVIRONMENTAL UNBALANCE N:C

P. Cruz-Cerino (1); V. Ruiz-Carrera* (1); C.A. Ledesma-Escobar (2), M.A. Salcedo-Meza (1); A.J. Sánchez (1); V.J. Robles-Olvera (3)

(1) UJAT-CICART, Villahermosa, Tabasco. México.
(2) Campus Rabanales, Universidad de Córdoba; Campus de Excelencia Agroalimentaria ceiA3. Córdoba, Córdoba. España.
(3) ITV-UNIDA, Veracruz, Veracruz. México.

In coastal lagoons of the American continent the angiosperm submerged *Vallisneria americana* and associated environmental services are threatened by the global change in the N and C biogeochemical cycles. In experimental approach were analyzed on leaves of *V. americana* the contents of flavanols, flavonoids and phenolic compounds to define if are metabolic markers of stress by effect bivariated of increase of N and CO_2 in the water.

With this goal, intention *in vitro* rooted plants in the water column were exposed at environmental unbalance N/C ($N\text{-}NO_3$ y $C\text{-}HCO_3$) in ratios from 80 to 483. The N/C relations were designed with increases in the nutrient concentrations of 50, 25 and 12.5% the culture medium MM, the relations 48 to 72 of N/P and from 0.15 to 0.6 Mm $C\text{-}HCO_3$.The cultures stayed ambient controlled up 75 days.

The plants died in concentration of nutrients from 50%. Extracts were obtained with 80% ethanol of the fresh and frozen biomass with leaves viable. The flavanol (vanillin-acidified method) and flavonoid (aluminum chloride method) contents were estimated with standard (±) Catechin (Ct), and the total phenolics (Folin-Ciocalteu method) with Gallic Acid (GA).

In the three determinations were used techniques of addition of standard or evaporation. The linearity of the three analytes presented very high regression and correlation coefficients (r y $r^2 > 0.99$; $p<0.0001$). In the average content of total phenolics (0.36 ± 0.074 mg Eq AG/g) predominated the flavonoids (0.33 ± 0.12 mg Eq Ct/g) and was low flavanol content. The relation N/C affected the content of flavonoids ($p<0.015$). The flavonoids were markers of stress in *Vallisneria americana* with the variation environmental N/C caused by the enrichment of N.

*Author for all correspondence

DIRECT ANAEROBIC SEWAGE TREATMENT: A SUSTAINABLE SANITATION OPTION FOR DEVELOPING COUNTRIES

Adalberto Noyola*

Instituto de Ingeniería, UNAM

According to the World Health Organization (WHO), the expected investments in Latin America and the Caribbean region for accomplishing the Millennium Developing Goals (target 10) just considering sanitation, not full wastewater treatment, are 8.9 billion USD for the 2011-2015 period, and an additional amount of 39 billion USD for attaining 100% sanitation coverage. The lack of wastewater infrastructure, a major problem for public health and environmental protection, may be regarded as an opportunity to select more sustainable treatment technologies and make optimal use of the huge investments needed for the new facilities.

In the case of developing countries with warm climate conditions, the anaerobic option is being increasingly applied to direct sewage treatment using anaerobic reactors, such as the upflow anaerobic sludge blanket (UASB) reactor. A recent survey in the Latin American region identified three major treatment technologies for municipal wastewater, activated sludge leading on a flow basis, and stabilization ponds (SP) on the number of facilities. Nearly 60% of the accumulated design flow in the sample (2734 WWTP in six countries) is treated in activated sludge systems.

The trend of increasingly adopting direct anaerobic process for sewage treatment in developing countries is based on a more sustainable option facing conventional activated sludge or low cost stabilization ponds. The UASB reactor have low energy needs and includes an arrangement for biogas capture and burning, still being a compact technology, requiring a very small foot print, an important advantage for urban areas. However, its lower effluent quality needs a post-treatment step in most cases.

A drawback of direct anaerobic sewage treatment is that 20 to 30% of the CH_4 produced in the process leaves the system as dissolved gas in the effluent. Depending whether a post-treatment is considered, this may be a direct emission of GHG. Also, biogas produced in small anaerobic WWTP cannot be used for energy purposes, due to the low production as a result of the limited amount of organic matter in conventional sewage (400 to 600 mg/L of chemical oxygen demand, COD). In these cases, biogas should be burned in order to reduce the GWP of these emissions; however, this may not be assured in very small facilities, even if the equipment is installed, due to neglected operation that usually accompanies this kind of municipal infrastructure in developing countries.

The adoption in developing regions of anaerobic treatment technologies for the future sewage treatment facilities would reduce GHG emissions, accomplishing at the same time lower capital investments and reduced operational costs, if compared to conventional full aerobic options. Research efforts are needed in order to develop efficient technologies for dissolved methane recovery and for small cogeneration units. These would be valuable elements to strengthen the anaerobic option for sewage treatment, resulting in a lower carbon footprint of the water sector.

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*Author for all correspondence

PLANTA DE TRATAMIENTO DE AGUAS RESIDUALES ATOTONILCO: LA APUESTA DE SUSTENTABILIDAD DE MÉXICO

Julio Antonio Pérez-Alvarez*(1,3)*;* José Flores-Bado (2);
Cristina Hernández-Romero (2);

(1) Acciona Agua
(2) ATVM-Acciona Agua
(3) Grupo de Tecnología del Agua del Colegio Oficial de Químicos de Asturias y León, Oviedo (Asturias), España

La PTAR de Atotonilco es la instalación más grande del mundo construida en una sola fase y la tercera en capacidad de tratamiento. En la actualidad, las aguas residuales procedentes del Valle de México se utilizan directamente en el Valle del Mezquital del Estado de Hidalgo, sin tratamiento alguno desde hace más de 100 años. Utilizan la técnica de riego por inundación, principalmente para cultivos de maíz y forraje.

Es el proyecto de mayor envergadura en saneamiento y depuración de México con notables beneficios sociales, ambientales y de sustentabilidad. El proyecto se asignó en Octubre de 2009, la construcción se inició en el año 2010 y, actualmente está en su fase final de construcción. La puesta en marcha está prevista para el segundo trimestre de 2015 y para inicios de 2016 entrará en operación.

Esta planta beneficiará a 700,000 personas en el Valle del Mezquital, al sanear el 60% de las aguas residuales del Valle de México. Las bases de diseño implican la producción anual de 1,041 Hm3 de agua tratada con calidad de agua para riego sin restricciones. Los caudales promedio de diseño son de 35 m3/s y un máximo de 42 m3/s en la época de lluvias. El caudal máximo es de 50 m3/s. La producción anual de arenas y basuras previstas es de 100,178 Tn/año con destino a un relleno sanitario. Los biosólidos generados se estiman en 837,408 Tn/año (28% de materia seca) con destino a un monorrelleno sanitario propio de 100 Ha donde recibirán un tratamiento y disposición final. El biogás generado será aprovechado internamente en energía térmica y eléctrica. La generación de electricidad prevista es de 197,291,002 kW-h.

Entre los beneficios sociales cabe mencionar que el agua producida tiene por objeto fundamental su reutilización mejorando las prácticas y ampliando las zonas agrícolas actuales. Supone una notable mejora sanitaria y medio ambiental de su ámbito de influencia de riego y de la presa de Endho.

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*Author for all correspondence

REMOVAL OF PESTICIDES PRESENT IN AGRICULTURAL WASTEWATER

Juvencio Galíndez-Mayer (1), Nora Ruiz-Ordaz (1)

(1) ENCB-IPN, México DF, México;

To prevent that urban and industrial wastewater discharges that pollute water bodies, systems for wastewater collection and transportation to treatment plants, as well as a variety of processes and equipment to remove the main pollutants have been designed. Although the agricultural sector consumes a proportion of water greater than that destined to municipal and industrial use, for that sector an equivalent degree of development has not been reached. This fact can be attributed to problems such as drainage, collecting and transport of agricultural wastewater to specific areas where toxic and recalcitrant pollutants can be removed.

Usually, surface water bodies are only perceived as reservoirs for landscaping, recreation and as a source of potable water. In addition, they should be viewed as aquatic ecosystems that must be protected from contamination. The fundamental purpose of agricultural wastewater treatment should be the protection of these aquatic ecosystems, preventing or at least reducing their pollution by compounds harmful to aquatic biota. Consequently, the sustainable use of water as a natural resource would favor its preservation and improving its quality.

The difficulty of channeling and treatment of agricultural wastewater is that contamination usually occurs diffusely in large land areas, which may have very different orographic and hydrographic profiles. However, there are several alternatives that can reduce the deterioration of water bodies. These alternatives range from those that simply favor the natural attenuation, to others with a different degree of technological advance. In this presentation, the practical possibilities and limitations of some of them are presented.

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*Author for all correspondence

A BIOREFINERY FOR BIOFUELS PRODUCTION FROM MACROPHYTES TREATING A POLLUTED URBAN RIVER AND MICROALGAE GROWN IN ANAEROBIC EFFLUENTS

Eugenia J. Olguín* (1)**,** Gloria Sánchez-Galván (1); Héctor Hernández-García (1)

Institute of Ecology, Environmental Biotechnology Group, Biotechnological Management of Resources Network, Xalapa, Veracruz, México. Carretera antigua a Coatepec # 351, El Haya, Xalapa, Veracruz, 91070, México

Biorefineries for the production of biofuels are a modern and pertinent option to contribute to the mitigation of the global change negative effects. However, there are still a number of technological, environmental and economic issues to solve before they become commercially viable. Our research group has been working at the lab scalel to establish the conditions for scaling up of a biorefinery for the production of biogas from macrophytes treating a polluted urban river and biodiesel from microalgae utilizing anaerobic effluents from pig waste. Thus, it is expected that the economic viability and the water footprint of the whole dual-purpose integrated system will improve compared to a biorefinery utilizing fresh water and fertilizer for cultivation of the microalgae. Lagoons with floating macrophytes have shown to be efficient for the removal of nutrients, heavy metals and pathogens.

In the above mentioned biorefinery, lagoons with *Pistia stratiotes* have been established for the treatment of an urban river receiving domestic effluents obtaining very high biomass productivities. Being this macrophyte rich in cellulose and hemicellulose, its use for biofuel generation (biogas) is a pertinent goal. In this respect, the hydrolysis-acidogenesis of the plant biomass for volatile fatty acids production results to be the limiting step of the anaerobic digestion, therefore the use of specific microbial inocula and reactors has been tested. In relation to the cultivation of microalgae for biodiesel production using anaerobic effluents from pig waste, good biomass and lipid productivities have been found. It was concluded that the use of macrophytes for biogas production and treatment of wastewater as well as the use of mixotrophic microalgae cultures for the production of biodiesel in biorefineries are a feasible and convenient combination.

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*Author for all correspondence, E-mail: eugenia.olguin@inecol.mx ,Tel: +52 2288421848, Fax:+52 2288187809

SEWAGE EPIDEMIOLOGY APPLIED TO ILLICIT DRUGS IN WASTEWATER OF FORT-DE-FRANCE URBAN AREA: POPULATION CONSUMPTION ESTIMATION AND WASTEWATER TREATMENT PLANT EFFICIENCY IN MARTINIQUE (FWI)

Damien A. Devault* (1); Thomas Nefau (2); Sara Karolak (2); Hélène Pascaline (1); Yves Levi (2)

(1) University of the French West Indies, EA 929 AIHP-GEODE "BIOSPHERE", Martinique;
(2) Univ. Paris Sud, Faculté de Pharmacie, UMR 8079, CNRS, AgroParisTech.

Drugs of abuse are increasingly consumed worldwide. Such consumption could be back-calculated based on wastewater content. The West Indies, with its coca production and its thriving illicit drug market, is both a hub of world cocaine trafficking and a place where its consumption is prevalent particularly in the form of crack. The present study will investigate Caribbean consumption by a daily 5 to 7 day sampling campaign of composite wastewater samples from the four wastewater treatment plants of the Martinique capital, including working and non-working periods, during 2013. The experiment was repeated during 2014, focusing on the mean wastewater treatment plants, one based on physic-chemical and one based on biological treatment, in order to determine their respective efficiency. Results obtained during the 2013 campaign were confirmed even strengthened: concentration in sewage are the highest values reported before excepted for prison sewage.

The local consumption of cocaine is ten to thirty times higher than OECD standards because of the prevalence of crack. The excretion coefficient for crack consumption and the impact of temperature on drug stability need further investigation. However, the low diversity of illicit drugs consumed and the crack prevalence suggest practices driven by the transiting of drugs for international trafficking: 75% of cocaine dealt in Europe are transiting by west indies.

Biological wastewater treatment is more efficient than physic-chemical, close to 100% for cocaine and metabolites compared to 85%, respectively. Morphine, mainly due to codeine-rich pharmaceuticals, and 11-nor-delta-9-hydroxytetrahydrocannabinol due to cannabis consumption are less efficiently treated by physico-chemical processes.

Notwithstanding, temperature and sun impact is to determine because the more efficient plant was outdoor (exposed to sun and outdoor temperature) when physic-chemical one was indoor. If the wastewater temperature, close to 26-28°C in the sewers, could be evoked for justifying a putative degradation increase in wastewater treatment plants, intense sludge contamination is possible too, leading to threat the compost quality make with.

Considering the treatment efficiency due to temperature, the corresponding impact on illicit drug content should affect too the sewage before the treatment plant. Then, consumption evaluation could be minimized by such unexpected degradation.

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*Author for all correspondence

IMPROVEMENT OF TERTIARY WASTEWATER TREATMENT TECHNOLOGIES: THE CHALLENGES OF RENUTRES NETWORK IN BRAZIL

Lourdinha Florencio* (1); Sávia Gavazza (2); Mario T. Kato (1)

(1) Laboratory of Environmental Sanitation, UFPE, Brazil.
(2) Laboratory of Environmental Engineering, UFPE, Brazil.

Nutrient removal of sanitary wastewater is required not only to meet the water quality standards, but also to reduce the eutrophication of water bodies, that are of increasing concern worldwide for many wastewater treatment companies. On the other hand, the rapid depletion of natural reserves of nutrients, such as sulfur and phosphorus, has been reflected in the increasing price of products and fertilizers. In Brazil, considering the urgency of these issues, we established a research network (Renutres) with the objective to develop and improve technologies for tertiary wastewater treatment. This network aims to work in the removal and recovery of nutrients in an attempt to save scarce resources and eventually explore aggregate economic aspects. Nutrients can be removed from wastewater through reactions mediated by bacteria that have the ability to transform and incorporate these compounds. In this context, the network is studying various configurations of systems available that promote removal of nutrients simultaneously.

Several parameters and system configurations are being studied, such as Bardenpho, UCT, traditional simultaneous nitrification-denitrification, sequential batch systems for nitrification-denitrification with phosphorus accumulation, alternative sources of carbon for removal in activated sludge, and others. Regardless of the system, the phosphorus transfer to the biological sludge may facilitate their exploitation and subsequent recovery as struvite. The precipitated magnesium ammonium phosphate, when produced in a controlled manner/way, can become a solution to the problem of reduction of rock phosphate and thus, it can be a sustainable alternative.

Another valuable nutrient is the sulfur that can be removed or recovered by the partial oxidation of organic sulfide present in the wastewater. Thus, the biological sulfur recovery methods are advantageous because they operate at room temperature and atmospheric pressure and, therefore, it can become an alternative of low cost and high efficiency for removal of H_2S. Another way of nutrient recovery being studied is by fixing N and P in algae production. It is well known that many species of microalgae have high lipid content that can be used in the production of oil (biodiesel). In summary, the network is working to improve tertiary wastewater treatment for nutrient removal technologies, as well as the recovery in the form of struvite, elemental sulfur, and biomass, for energy production.

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*Author for all correspondence

BIOLOGICAL TREATMENT OF TEXTILE EFFLUENTS: CHALLENGES AND CONSOLIDATED ISSUES

Sávia Gavazza* (1)**;** Mario T. Kato (2), Lourdinha Florencio (2)

(1) Laboratory of Environmental Engineering, UFPE, Brazil.
(2) Laboratory of Environmental Sanitation, UFPE, Brazil.

Pernambuco State in Northeast Brazil has shown an increasing economical growth in the last ten years with the investments distribution in local production arrangements (LPA). One of these LPA is the Agreste Clothing Pole (ACP). This sector has been generating more than one billion reais (US$ 500 million) in the region economy, the production of 700 million pieces of clothes and the employment of more than 90 thousand people.

However, a concern has been the danger of environmental pollution due to the production of high volume of effluents and sludge. Additionally, the effluent characteristics show important quality variation, especially concerning the chemical and biochemical oxygen demand, pH, color, and salinity. The composition of the wastewaters depends on the used organic compounds, chemicals and dyes during the clothes processing steps.

It is very relevant the development of research looking for technological alternatives to improve, or replace, the actual technology used by the industries of the LPA Agreste Clothing Pole of Pernambuco (coagulation, flocculation, and sedimentation). Biological treatment applied in pilot scale to treat this kind of effluent showed to be a promising technology (Ferraz Jr. et al., 2011). After the treatment, a liquid effluent that complied with Brazilian legislation was produced, reducing the impacts related with sludge production and management, commonly found for physical-chemical process, like the used by most of the textile industry in Pernambuco. However, the treatment system previously used (anaerobic + aerobic) was later transferred to another textile industry, aiming to investigate the system's behavior when fed with a wastewater with different composition.

At the new industry, a sulfate concentration of 700 mg/L was detected at the wastewater. The sulfate availability changed the anaerobic metabolic pathway of organic matter degradation, thus making sulfide formation, followed by sulfide-metals precipitation. In this case, the sulfate reduction was preferred in relation to dyes reduction, leading to very low color removal efficiencies (16%) (Amaral et al., 2014). Thus, it is still necessary to widen the knowledge to contribute with the technological development to the industrial textile sector. The investigation of the individual factors interfering on the treatment system is required. Although the huge amount of information available at literature, this kind of effluent require almost individual attention due to the differences in the chemical compounds used by each industry.

Additionally, the mission of reducing toxicity is added to the traditional requirement of removing azo dyes, organic matter, and sulfate, with the challenge of using one single unit for the treatment. Bioeletrochemical systems also started to be applied for the treatment of textile effluents in lab scale. There are plenty of fundamental research concepts to be investigated that still covers some borders of the knowledge.

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*Author for all correspondence

Chapter 8.
Solid Waste Management and Treatment

APPLICATION OF A BIOLOGICAL TREATMENT, AFTER FENTON TREATMENT, TO A MATURE LEACHATE FROM SOLID WASTE LANDFILL

Marbella Amador-Cruz (1); Patricia E. Cruz-Ortega (2); Rosa Salgado-Brito (3); Gabriel Pineda-Flores* (2)

(1) Professional Interdisciplinary Biotechnology Unit-IPN, México DF, México
(2) Mexican Cleaner Production Centre- IPN, México DF, México
(3) Simon Bolivar University, México DF, México

The Solid Waste Mature Leachate (SWML) is a liquid waste with a high level of organic matter.It is formed from the solid waste deposited in confinement sites in longer periods to 10 years. In the ecosystems, the SWML affects the balance of nutrients, produce genotoxic effects on local biota and contributes to soil erosion. Chemical methods have been applied to SWML, as Fenton oxidation, which are effective in removing recalcitrant organic waste. However, it is possible to increase the removal of these compounds using a method of biological oxidation after the Fenton oxidation. The objective of this work was to apply a treatment by activated sludge after Fenton oxidation to a sample of SWML to increase the removal of organic matter.

The SWML was characterized by the determination of the BOD, COD, total nitrogen, total phosphorus and an UV visible scan of a SWML sample. The Fenton method was applied with a ratio of 0.027 Fe^{+2}/H_2O_2. After Fenton treatment, the activated sludge treatment was applied using a glass column bioreactor with bubbling and an inoculum of 10% of activated sludge at ambient temperature. The removal of organic matter was determined by assessing the COD every 24 hours for 5 days.

The Fenton treatment reduced BOD, COD and total nitrogen by 49.06, 91.53 and 73.52%, respectively, and total phosphorus in an undetectable level. After applying the biological treatment the COD was reduced by 99.37%; the BOD and total nitrogen in an undetectable level. The result of the UV visible scan showed a change in the region sweeping trend from 100 to 200 nm. This was interpreted as a modification of the compounds with aromatic structures in the SWML after applying the treatment.

The Fenton treatment reduced total phosphorus to a level below the Mexican official maximum limits. After applying the biological treatment, BOD and total nitrogen were also reduced to permissible levels. It can be concluded that the sequential application of series Fenton-activated sludge treatment methods reduced effectively the organic pollutant content in the SWML.

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*Author for all correspondence

BIODEGRADATION OF ECOVIO® COMPOSTABLE PLASTIC BY COMPOSTING AND BIOAUGMENTATION

Xochitl Quecholac-Piña* (1); Mariel A. García-Rivera (1); Rosa M. Espinosa-Valdemar (1); Alethia Vázquez-Morillas (1); Margarita Beltrán-Villavicencio (1)

(1) Universidad Autónoma Metropolitana, Unidad Azcapotzalco, México DF, México.

Biodegradable plastics have been developed as an option to diminish the environmental impact of these materials at the end of their useful life. The aim of this research was to assess the biodegradation of the compostable plastic film Ecovio® in a bioaugmented composting system, mixed with organic waste.

Two 200 l reactors were used to contain 50% of organic waste, 25% of mulch and 25% of dry leaves, in weight basis. Bioaugmentation was performed by adding 4 kg of brown sugar loaf and 1.4 kg of yeast in each reactor. One hundred Ecovio® strips (1.5cm x 12 cm) were added to one of the reactors. The initial waste mixture was characterized by measurement of pH, humidity, organic matter, ashes and nitrogen. Temperature, volume and weight of the waste were monitored along the process. Manual aeration of the mixtures was performed daily in the first 15 days and every two weeks afterwards. The degradation of the plastics was evaluated measuring its elongation at break. Plastic strips were sampled on days 10, 15, 20, 25, 45 and 57. In those samplings pH, humidity, organic matter, ashes and nitrogen were evaluated in the compost. At the end of the process Ecovio® has been degraded in 61.57% (mass basis), and it was not possible to recover complete strips for the elongation test. The composts obtained in both reactors complied with the parameters of organic matter and nitrogen set in the local regulation for composting processes, NADF-020-AMBT-2011. No differences were found between the reactors with and without plastic, so this material did not affect the composting process.

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*Author for all correspondence

BIOSURFACTANT PRODUCTION USING WASTE COOKING OIL AS CARBON SOURCE

Juan L. Santiago-Rosales (1); Gladys Castorena-Cortés (1); Patricia Olguín-Lora (1); Teresa Roldán-Carrillo* (1)

(1) Instituto Mexicano del Petróleo

Vegetable oils (VO) are basic products in the human life; their principal use is as food. Annually, Mexico produces about 1360 tons of VO. After VO is used, they are mainly discarded into the drainage, which severely damages the environment. One liter of waste cooking oil (WCO) contaminates 1,000 liters of water. An alternative use for this kind of waste is the production of biodiesel and biosurfactants[2]. Biosurfactants are amphiphilic molecules produce by microorganisms, that reduce the surface tension (ST) and interfacial (IT) between fluids. These compounds may have application in the petroleum, pharmaceutical, food industry, and in the bioremediation. Currently, biosurfactants have acquired great interest due to their advantages as biodegradability, low toxicity, and capability to work in drastic conditions (temperature, salinity and pH). Biosurfactants can be produced from renewable resources. The aim of this work is to evaluate the production of biosurfactants using WCO as carbon source.

The IMP-X strain was isolated from a soil contaminated with hydrocarbons. It was used to produce biosurfactante using WCO as substrate at different concentrations. The best treatment was compared with biosurfactant production using soybean oil (not used) as substrate. Biosurfactant production was evaluated by ST measurement with a DuNoüy tensiometer. Microbial growth was determined by protein quantification using Bradford method[3].

The IMP-X strain produced biosurfactant, since the culture broth was able to reduce ST from 54.6 to 28.3 mN/m. The biosurfactant produced by the IMP-X strain had a critical micelar concentration (CMC) of 3,000 mg/L, and its yield was 15 g/L as a crude product. The production cost of biosurfactant decreased 51% using WCO as substrate, compared to the cost of biosurfactant obtained with a similar commercial grade substrate (soybean oil). WCO can be used as an alternative source for biosurfactant production, with an economic and environmental benefit

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*Author for all correspondence

EFFECT OF pH AND TEMPERATURE ON MINERALIZATION OF ORGANIC FRACTION OF MUNICIPAL SOLID WASTE (OFMSW)

F. J. Martínez-Valdez (1); C. Martínez-Ramírez (1); L. Martínez-Montiel (1); E. Favela-Torres (1); N. O. Soto-Cruz (2); F. Ramírez-Vives (1); G. Saucedo-Castañeda* (1)

(1) Metropolitan Autonomous University, Iztapalapa Campus, Mexico City
(2) Durango Institut of Technology, Durango, Dgo., Mexico

Inadequate disposal of the organic fraction of municipal solid wastes (OFMSW) has enlarged with the increasing of population; causing problems related to human heath and environment The urban waste generation in Mexico in 2011 was 40 000 million tons with a contribution of 12 % coming from the Valley of Mexico City. In this area OFMSW is about 44 % of total waste. Composting is the aerobic mineralization of organic matter carried out by microorganisms forming CO_2, H_2O and compost as the final product. It could be a good alternative for the treatment of OFMSW. The aim of this work was to study the effect of temperature and pH upon the mineralization of the OFMSW under controlled conditions.

The mineralization at lab scale of OFMSW from Mexico City was studied at different pH (5, 6, 7and 8) and temperatures (27, 31, 35, 42, 47 and 50 °C). It was found that at slightly acidic pH (5 and 6) increased the mineralization in terms of CO_2 production. Total mineralization was 229.5 and 337.2 mg of CO_2/g IDM at an initial pH of 5 and 6, respectively, reached at 50 h of process. Temperature has no important effect on the total mineralization of organic matter (2.5 – 3.2 mmol/g IDM). However, it shown an effect on the constant rate of mineralization, the highest value (2.16 d^{-1}) was observed at 27 °C. At 50°C an increase of reducing sugars in media were observed, possibly due to an increase in hydrolytic enzyme activities (pectinases and xylanases).

The respiratory quotient (RQ) was determined at 5 temperatures tested. It is defined as the ratio of the amount of CO_2 produced (mmol) related to the amount of O_2 consumed (mmol). In all cases the RQ increased to a maximum value during the first 10-15 h of the assay. The maximum value of RQ observed was 1.9 corresponding to 47° C. This increase may be due to the respiratory activity of vegetable waste as well as the oxidation of short-chain organic acids (malic acid and citric acid) present in some fruits and vegetables. Subsequently RQ values drop close to unity. These results suggest that the process of mineralization of OFMSW is similar to the stoichiometry of oxidation of hexoses. The maximum constant rate of mineralization obtained (5.28 d^{-1}), at 35 °C and pH 6, is at least 3.5 times higher to that reported in literature (1.5 d^{-1}). As conclusion the first step of mineralization of OFMSW, under controlled conditions, can be achieved on a short period of time. This fact can reduce the size of bioreactor or increase the capacity of composting plants, after that stabilization of organic solid can be realized under conventional conditions.

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*Author for all correspondence

POLLUTION OF SOILS IN AGGREGATES TREATMENT PLANT ON FLUVIAL TERRACES. CASE OF STUDY: TORMES RIVER (SALAMANCA, SPAIN)

Antonio Martínez-Graña* (1); Jorge Federico-Landa-Arreguín (1)

(1) Department of Geology. Faculty of Sciences. University of Salamanca, Salamanca, Spain.

This work analyzes the environmental problematic of an aggregates treatment plant of 166,453 m^2 on terraces of Tormes river (Salamanca, Spain). First are identified, in a preliminary research, the indications of pollution by means of a historical study of uses of the soil, the analysis of the physical way (geology, geomorphology, pedology, hydrology-hidrogeology and vegetation); the potential pollutants and environmental problematics (persistence, degradation, toxicity…). Secondly, one proceeds to an exploratory research of the identified points, analyzing the type and distribution form of every pollutant; and physical associate parameters (permeability, viscosity….), establishing the speed of the pollutants in the terraces determining the groundwater and piezometric level. Thirdly one proceeds to the research detailed to determine the values of reference and the extension of the pollution, valuing the environmental risks.

The analyzed pollutants, both in waters and in soils, are substances derived from oil: mineral oils, traces of petrols, and aromatic polycyclical hydrocarbons (PAH's): naphthalene, anthrocene, phenanthrene, fluoranthene, benzo, crisenthene, benzo(a)pyrene, benzoperithene and benzoflurantene. In fourth place, the analysis of results they show an absence of pollution of the superficial and underground waters of the river Tormes, indicating that the pollution is immobile in sandy superficial levels, not coming at the level of gravels that communicates directly with the riverbed of the river.

In fourth place, the analysis of results, shows an absence of pollution of the superficial and underground waters of the river Tormes, indicating that the pollution is immobile in sandy superficial levels, not coming at the level of gravels that communicates directly with the riverbed of the river. In soils, the chromatographic profiles indicate elements you plan for spillage of hydrocarbons (gas oil, fueloil…) and mineral oils. The compounds PAH's; the naphthalene, phenanthrene and anthrocene, they present lower values than 0.1 mg/kg; indicating absence of pollution, as the benzo (A) anthrocene presents values 0.3 and 0.7, the benzo (K) fluoranthene with values of 0.1 and 0.3 and the benzo (G, H, I) pyrene with values of 0.3 and 0.7. The concentration in mineral oils of 1485 mg/kg, and the presence of fluoranthene with values of 0.5 and 1.1 and of chrysene with values of 0.2 and 0.6; indicate a significant pollution. The polluted sectors present visible effects in 2.060 m^3. The depth of the contaminated level is 1 meter underground (on the basis of the analytical ones and soil pits); generating a volume of polluted soil with of 2.060 m^3.

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*Author for all correspondence

BIODEGRADABLE WASTES COMPOST LEACHATES TREATMENT IN AN ACIDOGENIC-METANOGENIC-NITRIFICATION-DENITRIFICATION SYSTEM FOR CARBON TRANSFORMATION TO METHANE AND NITROGEN REMOVAL

Vianka Hernández-Fydrych (1); Karen Cárdenas-Medina (1);
Carmen Fajardo-Ortiz (1); Mónica Meraz-Rodríguez* (1); Patricia Castilla-Hernández (2)

(1) Universidad Autónoma Metropolitana-Iztapalapa, México DF, México.
(2) Universidad Autónoma Metropolitana-Xochimilco, México DF, México.

Compost leachates are the liquid effluents drained from the composting piles used to stabilize the garbage biodegradable fraction consisting mainly of at the source-separated food waste, branches, leaves, grass and trees clippings. Garbage is stabilized during 30 to 40 days in windrow composting piles of 300 m^3 that may generate around 200 m^3 of leachates each day during the dry season. Samples of these effluents were collected at the Composting Plant during 2012 and 2013 and were characterized physic-chemically. A mean organic matter content as COD of 90 g/L was found for samples taken during the rainy season and of 119 g/L during the dry season.

A two-phase acidogenic-methanogenic anaerobic treatment was performed diluting the effluent with municipal waste water to attain feeding concentrations that were increased from 4.9 to 26.5 gCOD/L. A mean organic matter conversion to VFA of 52.7 % was attained in the acidogenic reactor. This pre-treated effluent was fed to the methanogenic reactor, which attained a methane productivity of 5.9 $LCH_4/L_R \cdot d$ equivalent to 0.78 kW-h. The acidogenic-methanogenic system maintained a mean removal efficiency of 95.3%. Nevertheless the methanogenic reactor effluent contained from 215 to 889 mg NH_4^+/L and 281 to 1386 mg DQO/L that were not removed during the anaerobic treatment. The effluent was polished by means of a nitrification-denitrification coupled process in a SBR of 1L volume. The operation sequence consisted of 210 min for continuous feeding-aeration-stirring for the nitrification stage and of 130 min stirring only for the denitrification stage, with a subsequent sedimentation time of 10 min and 10 min for withdrawal and 4 cycles per day.The assays were performed in two stages, one with the methanogenic reactor effluent only at an organic loading rate of 578 mg COD/ $L_R \cdot d$ and a nitrogen loading rate of 239 mg NH_4^+-N/ $L_R \cdot d$ for a ratio of 2.4 mg COD/mg NH_4^+-N. Under these conditions the COD and nitrogen removal efficiencies were of 18% and 29%, respectively. In the second stage the reactor was fed with the methanogenic reactor effluent amended with the acidogenic reactor effluent to supply the necessary available organic matter for both processes. The loading rates were of 572 mg COD/$L_R \cdot d$ and 174 mg NH_4^+-N/ $L_R \cdot d$. Under these conditions both COD and nitrogen removal efficiencies improved to 54.5% and 65%, respectively.

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*Author for all correspondence

LIXIVIACIÓN FUNGICA DE ORO A PARTIR DE PLACAS DE CIRCUITO IMPRESO DE TELÉFONOS CELULARES UTILIZANDO UN CULTIVO ESTACIONARIO

Brenda J. Soto (1); Ma. Esther Díaz-Martínez (1); Alejandro Alarcón (2); Ronald Ferrera-Cerrato (2); Rosalba Argumedo-Delira* (1)

(1) SARA-UV, Xalapa, Ver., México;
(2) Colegio de Postgraduados, Montecillos. Estado de México, México;

Los teléfonos celulares obsoletos se encuentran dentro de los Desechos de Equipos Eléctricos y Electrónicos (DEEE o WEEE por sus siglas en inglés). Como resultado de la innovación tecnológica en las características de los equipos, la vida útil de los teléfonos celulares es aproximadamente de un año, lo que crea un flujo importante de estos dispositivos móviles obsoletos. Millones de estos son sustituidos y desechados cada año, contribuyendo con la generación de la denominada basura electrónica. Los teléfonos celulares están constituidos por materiales valiosos como plástico, Fe, Cu, Al, Au, Ag, Pd principalmente. Para la recuperación de los metales valiosos presentes en las Placas de Circuito Impreso (PCI) de los teléfonos celulares se han empleado procesamientos hidrometalúrgicos y pirometalúrgicos, sin embargo, estos presentan desventajas ambientales y energéticas, por lo que la generación de nuevas técnicas de recuperación de metales a partir de las PCI de bajo impacto ambiental y energético es cada vez más necesario. Considerando lo anterior, la presente investigación tuvo como objetivo evaluar la biolixiviación de Au a partir de PCI de teléfonos celulares por *Aspergillus niger* MXPE6 y un consorcio fúngico (*A. niger* MXPE6 + *A. niger* MX7) en un cultivo estacionario a 25 °C y pH = 4.45.

Los resultados indican que los tratamientos con PCI e inoculados con *A. niger* MXPE6 presentan valores mayores de pH (3.5) en comparación con sus respectivos controles (2.8), este cambio no se observó en los tratamientos con PCI y los controles inoculados con el consorcio fúngico, ya que el pH (2.8) se mantuvo constante en ambos casos. Además se encontró que la biomasa seca obtenida fue mayor en los tratamientos con PCI que en los controles tanto para *A. niger* MXPE6 como para el consorcio fúngico. Con lo que respecta a la lixiviación la utilización del consorcio fúngico incrementó la lixiviación de Au considerablemente, ya que lixivió un 56% en comparación con el uso de un solo hongo que presentó una lixiviación del 17% bajo las condiciones probadas.

Es evidente que la composición de las TCI, las condiciones de cultivo utilizadas afectan la eficiencia de biolixiviación fúngica de Au. Por lo tanto la modificación de algunas de estas condiciones podría incrementar dicha eficiencia.

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*Author for all correspondence

SOIL AND WASTE AS MANDATORY TOPIC IN CURRICULUM OF ENVIRONMENTAL EDUCATION

Dante Camarillo-Ravelo* (1); Diana G. Castro-Frontana (1);
Katia J. López-Mendieta (1)

(1) ENCB-IPN, México D.F., México.

The curriculum for Environmental Systems Engineering (ISA), of the National School of Biological Sciences - National Polytechnic Institute (ENCB-IPN, in Spanish), dates back to 1998. This curriculum requires to be redesigned according to current needs and future lines of action of the Environmental Engineering.

After analysis of internal and external references, and the definition of graduate profile and a new mission and vision for future environmental systems engineers, three curricular paths that respond to current trends in the formation of environmental engineers were created. These paths are: "water and liquid waste", "air, energy and climate change" and "soil and waste". The objective of this study is to explain the elements that were taken into account for the creation of the path "soil and waste". The creation of a career that includes the issue of soils and solid wastes, is justified by the existence in Mexico of the National Contaminated Soils Remediation Program (published at the end of 2010), as well as by the existence of the National Program for Waste Prevention and Integral Management.

The path “soil and waste” aims to (*i*) contribute to the prevention, minimization and control of waste (municipal and hazardous), through actions such as minimizing, the separation at source, reuse and recycling, material and energy recovery and safe disposal as the last option and, finally, (*ii*) giving students essential tools about soil characterization, remediation and restoration, so they could be part of work groups in this key area, to achieve the public health’s and environmental protection. All of the above is framed within management systems, which are technically feasible, economically viable and socially acceptable. To achieve the objectives and competencies associated with this path, the following subjects are proposed: Integrated Solid Waste Management; Hazardous Waste Management; Sampling and Characterization of Soils and Solid wastes; Physical and Chemical Treatments (of soil and wastes); Biological Treatments (of soil and waste); Computer Modeling; Soils Remediation and Restoration; and Selected Topics of Soil and waste Remediation/Restoration.

The basic information for analyzing was provided by the Redesign Committee of the Environmental Systems Engineering (ENCB-IPN, ISA).

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*Author for all correspondence

THE ELECTRONIC WASTE IN MEXICO: A REVIEW

Christian E. Hernández-Mendoza

Universidad del Mar Campus Puerto Ángel, Oaxaca, México.

The electronic waste (e-waste) can contain heavy metals (e.g., lead, mercury, etc.) and other hazardous materials. Due to this, they have to be processed carefully but this does not always happen. The e-waste production worldwide was 49 million tons in 2012 and it is expected to rise 33% by 2017. The quantity of e-waste produced worldwide is growing almost 3 times faster than the municipal wastes. In Mexico, the e-waste production increased 167% from 1998 to 2006. In 2012 the e-waste production in the country was 1.138 million tons with an average production per inhabitant of 9 kg/year. This accelerated growth results in high e-waste quantities that cannot be treated as municipal solid wastes. These facts have attracted the attention due to its negative impacts on the environment and human health. In Mexico, there are some studies about the e-waste but the information is dispersed. Thus, the objective of this work was to review the e-waste production and management in Mexico.

In Mexico, the official studies are mainly focused on the e-waste generation at the north and central part of the country. While in the south part of the country there is not an official study about e-waste. The e-waste handling in Mexico is as follows: 40-50% is stored (e.g., into houses, offices, storehouses, etc), 3-11% is recycled, and 20% is refurbished. The national law does not consider the e-waste as hazardous. Instead, it is considered as special waste that is subject to the execution of plans for its management. Nonetheless, 5-30% of this waste is disposed of with municipal waste. It is known that the components of the electric and electronic equipment are not hazardous while they are into its cases. However, once they get mixed with the municipal wastes they can be broken and the hazardous materials are released or lixiviated causing negative impacts. It is estimated that this wastes are responsible for 70% of the toxins emission in landfills. Although the Mexican Official Norm NOM-161-SEMARNAT-2011 sets the criteria to classify the special wastes, it is not specialized in e-waste management. Another factor affecting the e-waste production and management is the import of e-waste. TVs and monitors are the main e-wastes imported from USA to Mexico because most of the processing facilities are located in Mexico and India. Mexico has some facilities authorized to handle the e-waste but their capacity is limited.

In conclusion, there is not enough information about e-waste in Mexico and the existing one is dispersed. The e-waste management is not properly done although there are authorized processing facilities in the country. This may be due because of the inexistence of a specialized law on e-waste management. Furthermore, if the e-waste production trend continues as at the present and no further actions are taken, it is probably that Mexico could become the next e-waste dump of the world.

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*Author for all correspondence

BIODEGRADATION OF PLASTIC FILMS BY ANAEROBIC DIGESTION

Gabriela A. Santiago-Juárez * (1); A. Vázquez-Morillas (1); Maribel Velasco-Pérez (1); Margarita Beltrán-Villavicencio (1)

(1) Universidad Autónoma Metropolitana- Unidad Azcapotzalco, D.F., México.

Plastic films are widely used for their versatility and low cost. However, high rate of consumption, discard and stability in the environment, transform them in a waste stream of complicated disposal and expensive treatment. In México City, the local government has promoted the use of compostable and oxodegradable plastics as a way to lessen the environmental impact of plastics. At present, it is evaluating alternatives to allow the treatment of plastic films along with organic waste. For that reason, this study assessed the degradability of oxodegradable HDPE (oxidized and non- oxidized), conventional HDPE and compostable film Ecovio® in anaerobic digestion conditions, following the guidelines established by the ASTM D5511-11.

The process was carried out in hermetic sealed reactors with a volume of 1000 cm^3. Each device contained cow manure as anaerobic inoculum (187.5 cm^3), dry grass as substrate, (150 cm^3), water (562.5 cm^3) and 30 plastic strips (1x15 cm). The experiment was performed with a control device (without plastic) and in triplicate for each plastic. Oxygen in devices was removed with nitrogen at the beginning of the experiment. The anaerobic digestion process lasted 40 days and was maintained at 52 ± 2 °C. Decrease of elongation at break was measured at the beginning and the end of anaerobic digestion; it was used as indicator of plastic films degradation.

At the end of the experiment, elongation at break decreased 91% (σ=19) for Ecovio®, 41% (σ=51) for oxodegradable oxidized HDPE, 40% (σ=53.5) for oxodegradable HDPE and 39% (σ=61) for conventional HDPE. The biogas production was also used as indicator of degradation of plastic films. The greater amount of biogas was produced by oxodegradable non-oxidized HDPE (14360cm^3), followed by Ecovio® (11520cm^3), oxodegradable oxidized HDPE (10780 cm^3) conventional HDPE (10150cm^3) and the control (7850 cm^3). Thus, it was concluded that the anaerobic digestion process produces some level of degradation in the studied plastics, even if they have not been made to degrade in absence of oxygen.

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*Author for all correspondence

EXPERIENCIA EN LA PRÁCTICA DEL COMPOSTAJE EN UN CENTRO DE TRABAJO

Maricela Esquivel-Martínez* (1)

(1) GEIC- CFE, Boca del Río, Ver, México;

El compost, es un fertilizante natural, considerado como un mejorador de suelo, obtenido a partir de la degradación aerobia de la materia orgánica en un tiempo relativamente largo. La práctica del compostaje a pequeña escala puede ser una solución al problema de la disposición de los residuos sólidos orgánicos, los cuales contribuyen hasta en un 60% al volumen de los residuos totales de una población.

Los objetivos de este trabajo fueron; aprender, adquirir y transmitir experiencia en el tema del compostaje con las condiciones climáticas del puerto de Veracruz y difundir la práctica del compostaje a pequeña escala hacia los empleados. Este proyecto se llevó a cabo en un centro de trabajo (CT) de la CFE, ubicado en Boca del Río, Veracruz, donde laboran entre 60 y 90 personas. El composteo se dividió en tres etapas; separación desde origen, recolección y tratamiento. Se diseñaron y colocaron cinco recipientes de 6 L, para acopiar los residuos composteables (residuos de frutas y verduras, principalmente), posteriormente recolectarlos y transferirlos a un compostero de 450 L de capacidad.

Antes de iniciar el proceso de composteo se difundió información relacionada con el tema al 71% de la población del CT, la cual fue en forma personalizada. La generación de residuos composteables (RC) dentro del CT fue de 1-2 kg/día (material fresco). La producción de compost fue de 25 kg/mes (base seca) aproximadamente, los cual se han utilizado para fertilizar algunas plantas de ornato del CT y se han donado 105 kg a 21 trabajadores, quienes lo han utilizado con buenos resultados en sus hogares. Seis trabajadores aportan de 1-4 kg/d de RC generados en sus hogares.

Después de un poco más de un año de practicar el compostaje en el centro de trabajo, se concluye que éste ofrece mejores resultados AMBIENTALES, cuando se combina con la separación, acopio y reciclado de residuos inorgánicos, reduciendo hasta en un 70% el volumen de deshechos que van al tiradero o al relleno sanitario. Sin embargo, el porcentaje de empleados interesados y coparticipes del proyecto va en aumento muy lentamente, aun cuando se difunde la información y se dan todas las facilidades para el acopio, tanto de RC como inorgánicos reciclables. Habrá que seguir buscando formas de llegar a una conciencia y responsabilidad colectiva del cuidado del medio ambiente.

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*Author for all correspondence

ELIMINACIÓN DE HIDROCARBUROS EN LODOS RESIDUALES PROVENIENTES DE CENTRO DE LAVADO AUTOMOTRÍZ

Verónica Lavadores-González (1); Sergio E. Vigueras-Carmona (1); Graciano Calva- Calva (2); Josefina Pérez-Vargas*(1)

(1) TESE, División de Ingeniería Bioquímica, Edo. Mex, México;
(2) CINVESTAV- IPN, Biotehcnology and Bioengineenig Department, México DF, México.

La eliminación de hidrocarburos contaminantes de lodos provenientes de centros de lavado automotriz es un problema de contaminación ambiental, y además de un gasto de agua que puede ir desde 50 hasta 1000 litros por auto dependiendo del servicio solicitado. Debido a la irregularidad de estos centros se les ha pedido que deben tratar sus aguas antes de ser vertidas al drenaje por lo que algunos de estos centros han implementado el uso de pequeñas plantas de tratamiento, sin embargo tienen aún el problema de disposición de lodos residuales que se generan. Se ha podido determinar que estos lodos tienen un alto contenido de hidrocarburos por lo que se les ha propuesto tratarlos con BFNA que se sabe degradan hidrocarburos del petróleo. Se ha demostrado que bacterias de vida libre fijadoras de nitrógeno atmosférico (BFNA) aisladas de suelos contaminados pueden remover exitosamente 120 gramos de petróleo por kilogramo de suelo contaminado, por lo que se espera que el uso de estas BFNA se favorable en la eliminación de hidrocarburos presentes en los lodos obtenidos del tratamiento del agua utilizada para el servicio del automóvil.

En los estudios realizados se ha podido encontrar que un periodo de 8.5 días la cantidad de sólidos totales se ve disminuida hasta en un 67% de una cantidad inicial de 1800 ppm de sólidos totales. El lodo residual tiene un color casi negro y en el tratamiento en columna de burbujeo con estas bacterias y en un lapso de 48 horas se obtuvo una eliminación del color hasta un líquido claro amarillento, y la cantidad de hidrocarburos totales disminuida hasta en un 75%. Este tratamiento permite asegurar la disminución de sólidos totales y una posible disposición final sin ningún tratamiento adicional, debido a que estos residuos ya no tienen hidrocarburos. Este puede ser un tratamiento adicional que elimina estos contaminantes en un periodo corto, y permite reusar el agua proveniente del lodo haciendo un uso eficiente del agua. Esto también disminuye el costo hacia el centro de lavado porque paga menos debido a que no usa tanta agua para su mantenimiento.

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*Author for all correspondence

TRATAMIENTO ANAEROBIO DE LOS LIXIVIADOS DE DESECHOS MUNICIPALES DE TEPATEPEC, HIDALGO, PARA LA PRODUCCIÓN DE BIOGÁS

Angélica Jiménez González* (1); Manuel A. Lizardi Jiménez (1);
Cuauhtémoc F. Pineda-Muñoz (1); Carlos A. Lucho-Constantino (1);
Sergio A. Medina Moreno (1)

(1) Universidad Politécnica de Pachuca, Hidalgo, México.

Los residuos sólidos generados en los mercados municipales, por los general se manejan y se disponen junto con los restos de residuos, esto genera, contaminación por los lixiviados que infiltran en las aguas subterráneas y suelos.

El propósito de este estudio fue evaluar el tratamiento anaerobio de los lixiviados de los desechos orgánicos y la producción de biogás empleando un reactor UASB. Los desechos orgánicos se colectaron del Mercado Francisco I Madero en Tepatepec, Hidalgo.

En la primera parte experimental se realizó la caracterización físico-química de los lixiviados, se obtuvo una concentración alta de materia orgánica (30 g/L) y con pH fuertemente ácido (3.87), posteriormente se realizaron pruebas de biodegradabilidad anaerobia donde se encontró, que la mayor eficiencia de eliminación de materia orgánica fue a una concentración inicial de 8.5 gDQO/L de lixiviados. En el tratamiento de los lixiviados de desechos orgánicos se empleó un reactor anaerobio tipo UASB, la carga del influente fue de 7.9 gDQO/L*d, con una eficiencia de remoción del 73%, la producción de biogás fue del 50 % comparada con el valor teórico esperado.

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*Author for all correspondence

HYDROLYSIS OF SUGARCANE BAGASSE WITH CELLULASES FROM *Trichoderma reesei*, AND SIMULTANEOUS FERMENTATION WITH *Zymomonas mobilis* AND *Saccharomyces cerevisiae* IMMOBILIZED IN ALGINATE-CHITOSAN BEADS

Darcy Rebolledo-Rodríguez (1); ***Enrique Alarcón**** (1); Isabelle Gaime-Perraud (2); Anne-Marie Farnet (2); Beatriz Gutiérrez (3); Victoria I. Espinosa (1); Christian Hernández (1); Josué Santiago (1)

(1) INBIOTECA- UV, Xalapa Ver., México;
(2) IMBE-AIX MARSEILLE, Marseille, France;
(3) ITSTB, Tierra Blanca, Ver., México.

Despite the worldwide effort to generate ethanol from lignocellulose, there is still no an optimal process. More research is needed in order to optimize the bioethanol production from organic matter (OM) wastes. The aim of this research was to evaluate the ethanol production process from sugarcane bagasse integrating the chemical pretreatment of OM; the enzyme hydrolysis (saccharification) and fermentation simultaneously (FSS), using *Zymomonas mobilis* and *Saccharomyces cerevisiae* immobilized in chitosan-alginate beads.

Sugarcane bagasse was treated with NaOH (2 % w/v) at 121 °C and 1.1 kg/cm^2 at four different times (15, 30, 60, 120 and 240 min). After this chemical treatment, the bagasse solution was diluted with water (1:3, v/v) and cellulases (2.9 UI mL^{-1}) from *Trichoderma reesei* were added to the batch reactor (5L). Saccharification was performed at 50°C, pH 4.9 and at 110 rpm. At the highest reducing sugars concentration, the bioreactor was adjusted at 50°C and the immobilized microorganisms (50/50 w/w) were added to perform the fermentation-saccharification. The batch bioreactor was operated for 72 hours. Sodium alginate (2%) and chitosan (0.25%) were used to immobilize the microorganisms. The beads were maintained in $CaCl_2$ (1%) at 4°C until fermentation. The concentration of glucose, arabinose, xylose, glycerol, ethanol and sucrose were determined by HPLC. Cellulase activity, total reducing sugars and total proteins were also determined.

The production of reducing sugars was similar between the chemical treatments (6 gl^{-1}), with no statistical significance (P< 0.05). After 72 hours, 50% of the reducing sugars were removed, but the cellulose activity does not stop; 30% of the activity remains at the end of fermentation. Ethanol production was low probably due to the chitosan-alginate beads avoid microorganisms contact with sugars.

It is concluded that saccharification and fermentation could be performed simultaneously using microorganisms immobilized, but ethanol yields are low.

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*Author for all correspondence

APROVECHAMIENTO DE RESIDUOS DE JITOMATE (*Lycopersicon esculentum*) HIDROPÓNICO COMO SUSTRATOS PARA LA PRODUCCIÓN DEL HONGO SETA (*Pleurotus ostreatus*)

Alma D. Sánchez-García * (1); Karla González-Velázquez (1);
Rosa A. Guillen-Garcés (1)

(1) Universidad Politécnica del Estado de Morelos, Ingeniería en Tecnología Ambiental, Morelos, México

La producción de jitomate (Lycopersicon esculentum) ya sea de riego, temporal, en suelo a cielo abierto, en suelo bajo cubierta y semihidropónico, ha sido por muchos años considerada como un producto agrícola sobresaliente a nivel nacional y estatal.Sin embargo, pese a su importancia, no se le ha prestado interés al manejo adecuado y sustentable del rastrojo que genera su producción. Se ha calculado que en el estado de Morelos se generan alrededor de 1 360 ton/ha/año de rastrojo de jitomate hidropónico. Como una alternativa para el manejo de estos residuos y el aprovechamiento de los nutrientes incorporados a los sistemas hidropónicos, se propuso como objetivo general, evaluar el rastrojo de jitomate (RJ) hidropónico como sustrato para la producción del hongo comestible *Pleurotus ostreatus*, en diferentes mezclas (75%RJ-25% PT, 50% RJ-50% PT, 25% RJ-75% PT), empleando como control positivo paja de trigo (PT) al 100%, y como control negativo el rastrojo (100% RJ).

La metodología empleada, incluyo pruebas fisicoquímicas (potencial hidrógeno, conductividad eléctrica, materia orgánica, carbón orgánico, nitrógeno total y relación carbono nitrógeno) de los sustratos empleados, así como la eficiencia biológica (EB), ciclo de cultivo (CC) y tasa de producción (TP) obtenida de cada sustrato.

Los resultados indican que las mezclas con mayor proporción de RJ (100% y 75%) no fueron viables, sin embargo las mezclas con 50% y 25% de RJ mostraron una EB de 39 y 52.75%, así como un CC 84 y 117 días y una TP de 0.47% y 0.31% respectivamente, en comparación con el control positivo que mostro una EB del 16.33%, un CC de 115 días y una TP de 0.28%. Como conclusión, es posible emplear el cultivo del hongo *Pleurotus ostreatus*, como una alternativa para proporcionar un valor agregado al rastrojo de jitomate hidropónico.

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*Author for all correspondence

IMPACT OF THE SPREADING OF URBAN WASTE ON AGRICULTURAL SOIL BACTERIAL COMMUNITIES IN THE PERIPHERY OF OUAGADOUGOU, BURKINA FASO

Edmond Hien* (1); Sabine Favre-Bonté (2); Salomon Bouda (1); Aboubakar Sidiki Ouattara (1); Dominique Masse (3); Sabine Houot (4); Sylvie Nazaret (2)

(1) Université de Ouagadougou, Burkina Faso ;
(2) UMR CNRS 5557, Villeurbanne, France,
(3) IRD,UMR 210 Eco&Sols, Montpellier, France
(4) UMR EGC Equipe Sol, INRA, Thiverval-Grignon, France

Recycling organic wastes is of interest in agriculture and horticulture for improving soil quality, favouring plant development.Recently, usual farmers' practices in Burkina Faso involved the use of untreated domestic and urban wastes. However raw organic wastes may contain undesirable constituents, e.g. heavy metals, toxic organics, pathogens, with adverse impacts on soil and environmental quality, and possible hazards to human health.

The objective of this study was to evaluate the influence of anthropogenic activities such as the application of urban waste on agricultural land which can promote the emergence and / or spread of pathogens. Assessing the influence of the spreading of raw municipal waste on soil bacterial communities and the abundance of opportunistic pathogens of man was realized.

Soils amended or unamended (control) with waste has been sampled at three sites in the suburban area of Ouagadougou (Burkina Faso). The impact on the total indigenous bacterial community and the risk of spread of pathogens has been assessed by counts of culturable bacteria (heterotrophic cultivable microflora, faecal indicator bacteria and pathogenic species of humans), as well as an analysis of the genetic structure of bacterial community in a culture independent approach (ARISA Method for Automated Ribosomal intergenic Spacer Analysis). The results showed a total cultivable bacteria enrichment and modification of the genetic structure of bacterial communities in the amended plots. The lack of detection of pathogens such as *enterococci* and *fecal coliform*, *Staphylococcus aureus* and opportunistic human pathogens (*Pseudomonas aeruginosa*) suggested that the amendments are not sources of these pathogens and do not select the communities. However, monitoring populations of the species *Stenotrophomonas maltophilia*, an opportunistic pathogen of man frequently found in soils, showed the enrichment of these populations in amended plots.

This work showed that human activities spreading of waste on agricultural land may pose health and environmental risks.

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*Author for all correspondence

Chapter 9.
Hazardous Waste Management and Treatment

DESULFURACIÓN POR *Geobacillus thermoglucosidasius* DEL CATALIZADOR MOS_2 DESACTIVADO

Teresa Torres-Pérez* (1); Martha Poisot (2); Teresa Hernández-Quiroz (3)

(1) División de Estudios de Posgrado, Universidad del Papaloapan, Campus Tuxtepec, Circuito central 200, Col. Parque Industrial, Tuxtepec, Oax. C.P. 68301, México.
(2) Instituto de Química Aplicada, Universidad del Papaloapan, Campus Tuxtepec, Circuito central 200, Col. Parque Industrial, Tuxtepec, Oax. C.P. 68301, México.
(3) Centro de investigación en Micro y Nanotecnología, Universidad Veracruzana, Calzada Ruiz Cortínez 455, Col. Costa Verde, Boca del Río, Ver. C.P. 94292, México.

Los catalizadores derivados de los procesos de hidrodesulfuración son pequeños granos de MoS_2 gastados cuya eficiencia catalítica se agotó y es altamente tóxico. Al aumentar la demanda de catalizadores nuevos, aumenta la cantidad de catalizadores desactivados que se descargan como desechos sólidos y en consecuencia la problemática del manejo de los mismos.

Se han llevado a cabo numerosos estudios sobre la desulfuración de los hidrocarburos por medios químicos y biológicos, así también para la remoción de metales de los catalizadores desactivados. Pero en cuanto a la regeneración de los catalizadores existe muy poca información y mucho menos sobre la utilización de bacterias para activar catalizadores agotados.

Por lo mencionado anteriormente, este proyecto de investigación persigue el objetivo de determinar si la bacteria *Geobacillus thermoglucosidasius* es capaz de utilizar el azufre que satura al catalizador desactivado MoS_2, obteniendo así la reactivación del catalizador.

Este proyecto se realizará a nivel laboratorio, se determinarán las condiciones óptimas de crecimiento de la bacteria con dibenzotiofeno como molécula modelo y posteriormente se realizará la desulfuración del MoS_2 en un biorreactor de 0.5 L.

Para determinar la eficiencia del proceso se comparará la actividad catalítica del catalizador reactivado contra la de un catalizador comercial en la reacción de HDS de DBT en un reactor batch trifásico.

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*Author for all correspondence.

LIXIVIACIÓN FUNGICA DE ORO A PARTIR DE PLACAS DE CIRCUITO IMPRESO DE TELÉFONOS CELULARES UTILIZANDO UN CULTIVO ESTACIONARIO

Brenda J. Soto (1); Ma. Esther Díaz-Martínez (1); Alejandro Alarcón (2); Ronald Ferrera-Cerrato (2); Rosalba Argumedo-Delira* (1)

(1) SARA-UV, Xalapa, Ver., México;
(2) Colegio de Postgraduados, Montecillos. Estado de México, México;

Los teléfonos celulares obsoletos se encuentran dentro de los Desechos de Equipos Eléctricos y Electrónicos (DEEE o WEEE por sus siglas en inglés). Como resultado de la innovación tecnológica en las características de los equipos, la vida útil de los teléfonos celulares es aproximadamente de un año, lo que crea un flujo importante de estos dispositivos móviles obsoletos. Millones de estos son sustituidos y desechados cada año, contribuyendo con la generación de la denominada basura electrónica. Los teléfonos celulares están constituidos por materiales valiosos como plástico, Fe, Cu, Al, Au, Ag, Pd principalmente. Para la recuperación de los metales valiosos presentes en las Placas de Circuito Impreso (PCI) de los teléfonos celulares se han empleado procesamientos hidrometalúrgicos y pirometalúrgicos, sin embargo, estos presentan desventajas ambientales y energéticas, por lo que la generación de nuevas técnicas de recuperación de metales a partir de las PCI de bajo impacto ambiental y energético es cada vez más necesario. Considerando lo anterior, la presente investigación tuvo como objetivo evaluar la biolixiviación de Au a partir de PCI de teléfonos celulares por *Aspergillus niger* MXPE6 y un consorcio fúngico (*A. niger* MXPE6 + *A. niger* MX7) en un cultivo estacionario a 25 °C y pH = 4.45.

Los resultados indican que los tratamientos con PCI e inoculados con *A. niger* MXPE6 presentan valores mayores de pH (3.5) en comparación con sus respectivos controles (2.8), este cambio no se observó en los tratamientos con PCI y los controles inoculados con el consorcio fúngico, ya que el pH (2.8) se mantuvo constante en ambos casos. Además se encontró que la biomasa seca obtenida fue mayor en los tratamientos con PCI que en los controles tanto para *A. niger* MXPE6 como para el consorcio fúngico. Con lo que respecta a la lixiviación la utilización del consorcio fúngico incrementó la lixiviación de Au considerablemente, ya que lixivió un 56% en comparación con el uso de un solo hongo que presentó una lixiviación del 17% bajo las condiciones probadas.

Es evidente que la composición de las TCI, las condiciones de cultivo utilizadas afectan la eficiencia de biolixiviación fúngica de Au. Por lo tanto la modificación de algunas de estas condiciones podría incrementar dicha eficiencia.

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*Author for all correspondence.

BIOSORPTION OF COBALT (II) CADMIUM (II) AND ZINC (II) FROM AQUEOUS SOLUTION USING IMMOBILIZED STRAW MUSHROOM

Mongkol Phensaijai*

Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, 1 Chalongkurng Rd., Ladkrabang Bangkok, 10520, Thailand

Straw mushroom *(Volvariella vovacea*) was immobilized with calcium alginate and polyvinyl alcohol in the experiment. The effects of metal sorption time and quantity of immobilized mushroom on heavy metal sorption were studied. Cobalt (II), Cadmium (II) and Zinc (II) ions were absorbed with 2, 4, 6, 8 and 10 % (w/v) of mushroom's concentrations at pH 7.0 and 0.5 ml/min flow rate. At 30 minutes, the 2 % (w/v) immobilized with polyvinyl alcohol gave the best result in cobalt sorption (26.08 mg/l), whereas the 6% (w/v) mushroom immobilized with calcium alginate gave the best result in cadmium sorption (20.28 mg/l) and the 10% (w/v) mushroom immobilized with polyvinyl alcohol gave the best result in zinc sorption (24.67 mg/l).

Keywords: Straw mushroom, *Volvariella vovacea,* Biosorption, Cobalt, Cadmium, Zinc.

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*Author for all correspondence.

DESAROLLO DE UNA BIOTECNOLOGÍA PARA LA DEGRADACIÓN DE COMPUESTOS ORGÁNICOS VOLÁTILES EN SOPORTES SÓLIDOS: RESIDUOS

AGRÍCOLAS Vs. MATERIALES INORGÁNICOS

M. Esther Hernández-Espino (1); Eloy Conde-Barajas (2); Alicia del Real-López (3); J. Luis Hernández-López* (1)

(1) CIDETEQ, Querétaro, México;
(2) ITC, Div. Ing. Amb., Celaya, México;
(3) CFATA-UNAM, Querétaro, México.

La biofiltración durante los últimos años ha incrementado su uso dentro de las tecnologías biológicas de tratamiento de efluentes gaseosos, en particular para compuestos orgánicos volátiles (COV´s). Desde un punto de vista, principalmente ambiental, benceno, tolueno, etil-benceno, o-xileno (BTEX) son disolventes industriales importantes que se encuentran con frecuencia en operaciones industriales y sitios contaminados. El objetivo de este trabajo fue evaluar en un sistema microcosmos la capacidad de degradación de BTEX a través de su asimilación como sustrato por un consorcio microbiano previamente adaptado, empleando diferentes soportes: cáscara de cacahuate y montmorillonita. El consorcio microbiano se conformó por 4 cepas provenientes de nichos ecológicos cercanos a fuentes fijas de contaminación de COV´s.

Se presenta la caracterización microbiana mediante pruebas de microbiología y bioquímicas convencionales. Las bacterias identificadas corresponden a: *Pasteurella pneumotropica, Myroides spp, Brevundimonas vesicularis y Mannheimia haemolytica.* Durante las cinéticas de biodegradación se emplearon concentraciones 200 ppm_v a 1000 ppm_v de BTEX a través del tiempo, se observó que en un tiempo total de 45 días y sin control de temperatura (temperatura ambiente promedio de 24 °C) se obtuvieron en cáscara de cacahuate porcentajes de degradación superiores al 80 % para la mayoría de los componentes de BTEX, en el caso de la montmorillonita se obtuvieron eficiencias de degradación en un rango del 60%. Así mismo, se realizó un seguimiento cuantitativo de las bacterias a lo largo de la cinética de biodegradación en microcosmos a través del tiempo, en cáscara de cacahuate hubo un incremento de la población microbiana del orden de 10^5 a 10^6 UFC/gss. Para el caso de la montmorillonita la población microbiana se mantuvo en el orden de 10^5 UFC/gss.

Se concluye que la cáscara de cacahuate puede ser un material de soporte ideal en biofiltración, ya que permite mantener la viabilidad, compatibilidad y capacidad de degradación del consorcio microbiano en un rango de concentraciones 200-1000 ppm_V de COV´s y sin añadir nutrientes.

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*Author for all correspondence.

PESTICIDE WASTES MANAGEMENT IN SOME HORTICULTURAL FARMS

A. Hormatallah * (1); R. Salghi (2); Lh. Bazzi (3)

(1) Laboratoire des pesticides. Institut Agronomique et Vétérinaire Hassan II, Complexe Horticole d'Agadir, BP 18/S. Agadir. Maroc;
(2) Laboratoire d'Ingénieries des Procédés de l'Energie et de l'Environnement, Ecole Nationale des Sciences Appliquées, B.P 1136. Agadir, Maroc;
(3) Laboratoire Régionale de l'Etablissement Autonome de Contrôle et de Coordination des Exportations d'Agadir, Maroc.

Horticulture is one of the most relevant segments of the Moroccan agriculture sector. Morocco country reaches the self-sufficiency in vegetable production. This sector provides also an employment for the working rural population and important source of hand currency towards exportation to the European Union, Canada, Russia and USA. The intensive conditions of the horticulture crops (citrus, tomato, pepper, green bean…) seem to be favorable to many insects (leaf miners, thrips, Whiteflies ..) and the development of some diseases (powdery mildew, downy mildew, gray mold…). That causes more damage and affects the yield quality. Growers are often applying pesticides to protect their production and the yield quality. Pesticide waste disposal is a concern for Moroccan horticultural producers. In order to meet the objectives of "Green Morocco Plan", to complain to national Legislation (Law 42-95 regarding pesticides regulations, Law 28-00 related to waste management and their disposal) and international regulations (FAO's Code of Conduct for Pesticide use and disposal) and the requirements of Quality Control standards of agricultural products and the environmental management (GlobalGap, Nature'choice, ISO 14000, BRC, it will be very important to know the practices used by farmers to manage pesticide wastes.

60 horticultural producers of the Souss Massa region were interviewed personally with a questionnaire focused on Pesticides waste Management. The Souss Massa, located in the south Atlantic coastal, is a major horticultural producing and exporting area of Morocco. The survey indicated that the majority of pesticide wastes were related to empty pesticide containers and pesticide effluents. All farmers are aware of human health risk and the environment pollution potential of pesticide wastes. The producers are facing problems in terms of eliminating the empty containers due to the absence of facilities for pesticides containers recycling. 80 % of farmers are stored empty containers under lock and key. 7% reuse empty containers for cultural operations after cleaning them. 13% of producers state that they burn empty containers. Burning plastic bottles and pesticide sachets can lead to the formation of toxic gas in the air. Concerning leftover spray solutions, most farmers (73%) was reported that they apply them to the border lines of the field area or to weed grasses until the spraying tank is empty. 16% of farmers release the leftover spray solutions into an evaporation area and 11% were used in a bioremediation system in biobed or phytobac.

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*Author for all correspondence.

DESEMPEÑO DE BIORREACTORES DE LECHO FLUIDIZADO ACOPLADOS A FILTROS ABIÓTICOS EN EL TRATAMIENTO DE ALTAS CONCENTRACIONES DE PERCLOROETILENO

Luz Bretón-Deval (1); Elvira Ríos-Leal (1); Katy Juarez-López (2); Omar Solorza-Feria (1); Héctor M. Poggi-Varaldo* (1)

(1) CINVESTAV- IPN, México DF, México;
(2) IBT-UNAM, México DF, México.

El percloroetileno (PCE) es un compuesto clorado recalcitrante y tiende a depositarse en el fondo de los acuíferos siendo una fuente prolongada de contaminación. El uso de tensoactivos dentro del proceso de biorremediación ha permitido solubilizar mayor concentración de PCE y minimizar los tiempos del tratamiento. Las herramientas abióticas como el hierro metálico (ZVI) permite una remoción de contaminante más eficiente además de disminuir la concentración de metabolitos producto de la deshalogenación reductiva. Los objetivos de este trabajo fueron *(i)* evaluar el desempeño de reactores de lecho fluidizado (RLF) a concentraciones altas de PCE así como las herramientas abióticas: ZVI y el tensoactivo Tween 80, *(ii)* evaluar la degradabilidad de Tween 80 como única fuente de carbono por el consorcio microbiano utilizado, y *(iii)* fabricar nanopartículas (NP) económicas de Fe-Pd y determinar su eficiencia en el tratamiento de agua contaminada con PCE.

Se implementaron cuatro RLF, dos con tenían filtro de ZVI. Todos los bioreactores fueron alimentados con concentraciones crecientes de PCE (80, 165, 278, 393 mg/L). Se utilizó un consorcio metanogénico alimentado a 1 g DQO/L. Durante los incrementos de PCE (80-393 mg/L) los RLF mostraron remociones eficientes del contaminante en los primeros tres periodos. Durante el tercer periodo (393 mg/L) los RLF necesitaron un tiempo de aclimatación largo para comenzar a deshalogenar eficientemente el PCE. Los RLF que tenían acoplados los filtros de ZVI mostraron un tiempo de aclimatación más corto además de una mayor remoción de contaminante y menor producción de metabolitos. El uso del tensoactivo Tween 80 permitió un incremento en la solubilización del PCE de 12 veces más como lo confirmó el parámetro K_m; además este tensoactivo demostró ser más eficiente que tensoactivos como el SDS y el UH. Los resultados de biodegradabilidad mostraron que el tensoactivo puede ser utilizado como fuente de energía bajo diferentes aceptores de electrones sin que resulte tóxico al consorcio.En otro experimento se sintetizaron NP de Fe-Pd, variando la cantidad de Pd para utilizar la nanopartícula más económica y reactiva. Se encontró que a las 4 h de tratamiento en lote las NP Fe-Pd 1:1 habían removido el 80% del PCE (200 mg/L), las NP Fe-Pd 2:3:1 y 9:1 removieron 70 y 58% respectivamente. Después de 16 h las remociones fueron 85% para la 2:3:1, 83% y 76% para la 1:1 y 9:1 respectivamente. Se concluye que: -Los RLF son una herramienta eficiente en el tratamiento de altas concentraciones de PCE. Los RLF acoplados a filtros de ZVI mostraron mayores remociones de PCE y menor concentración de metabolitos. -El Tween 80 mostró ser un tensoactivo factible para usarlo dentro del proceso de biorremediación debido a que solubiliza altas concentraciones de PCE, no es tóxico para el consorcio, y es biodegradado en condiciones metanogénicas y desnitrificantes. -La remoción más eficiente de PCE en tratamiento en lote en 4h se observó con las NP Fe-Pd 1:1. Sin embargo, las NP 9:1 en un tratamiento de 16 h alcanzan una remoción satisfactoria de PCE de 76%, con un ahorro económico en los materiales de las NP de 40%.

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*Author for all correspondence.

CONGO RED REMOVAL BY BIOPOLYMERS FROM SHRIMP EXOSKELETONS

Austria Zúñiga-Zamora (1); Argentina Zúñiga-Zamora (1); Jaime García-Mena (2); Elsa Cervantes-González*(1)

(1) UASLP/COARA, San Luis Potosí, México;
(2) CINVESTAV- IPN, México DF, México.

Azo dyes have been the largest chemical class of dyes frequently used for textile dyeing and paper printing. These industrial effluents often contain significant amount of residual dye due to inefficiency in the dyeing processes. It affects the water quality and may become a threat to public health, since certain azo dyes or their metabolites (aromatic amines) are highly toxic and potentially carcinogenic. Some azo dyes found in industrial effluents are: yellow aniline, methyl red, congo red, chrysoidine, reactive red 272 and others.

Thus, in the present study an analysis of the adsorption of congo red on chitin and chitosan from shrimp exoskeletons was carried out in laboratory level. The evaluated adsorption capacity was Box-Behnken experimental design with three variables at three levels each. Based on the evaluated results of the design, the adsorption of chitosan is better than chitin, the optimum parameters were obtained with pH 7, temperature 25 °C and contact time of 3 hours. The equilibrium adsorption with chitin was best described by the Langmuir adsorption model; meanwhile the chitosan was described by Freundlich adsorption isotherm.

The corresponding thermodynamic parameters (ΔG^o, ΔH^o and ΔS^o) were calculated to evaluate the nature of the adsorption process. Gibbs free energy was calculated according to $\Delta G^o = RT\ln K_a$, where K_a the adsorption equilibrium constant from Langmuir. Entropy and Enthalpy changes were calculated by $\Delta G^o = \Delta H^o - T\Delta S^o$ in a plot of ΔG^o versus T. The results showed, spontaneous nature of adsorption at 313.15 K using chitin, meanwhile at 323 K showed the opposite of nature adsorption due to the Gibbs energy free was 13.88 kJ/mol, the q_{max} was 138.93 mg/g at 313.15 K, the negative value of ΔH^o indicates that the adsorption reaction was exothermic. In addition, the negative value of ΔS^o also suggests that the adsorption was enthalpy driven and spontaneous in nature. For chitosan the negative values of both temperatures for Freundlich isotherms have confirmed the feasibility of process and the spontaneous nature of adsorption, the values of ΔH^o and ΔS^o were also negatives as chitin.

It is concluded that chitin and chitosan are a high capacitate, the 1 g of chitin can adsorb 140 mg of congo red in a favourable condition, meanwhile the 1 g of chitosan can adsorb 320 mg of congo red. Therefore chitin and chitosan are the favourable alternative of congo red removal from water.

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*Author for all correspondence.

DEGRADACIÓN DE DIURÓN Y LINURÓN EN UN REACTOR DE LECHO EMPACADO DE TRIPLE ETAPA

Erick Marrón-Montiel (1); Nora Ruiz-Ordaz (1); Cleotilde Juárez-Ramírez (1); Fortunata Santoyo-Tepole (1); Emmanuel Lima-Martínez (1); Juvencio Galíndez-Mayer* (1)

(1) ENCB-IPN, México DF, México;

El diurón y el linurón son herbicidas derivados de la urea, ambos son usados ampliamente a nivel mundial para el control pre- y post- emergente de “malezas” de hojas anchas anuales y perennes. Estos compuestos están clasificados como ligeramente tóxicos, sin embargo, existen estudios que los señalan como disruptores endócrinos y generadores de efectos tóxicos en el ambiente. Ambos herbicidas y sus derivados han sido detectados en cuerpos de agua superficial, subterránea y agua de mar en diferentes partes del mundo. Por tal motivo el objetivo principal de este trabajo fue evaluar la capacidad para degradar diurón de una comunidad microbiana establecida en el lecho de un reactor de triple etapa con módulos de aeración superficial que ha demostrado una alta eficiencia en la remoción de linurón, lo anterior basado en que la estructura química de ambos es muy similar, la diferencia radica en los sustituyentes de la urea, para el caso del linurón son *N-metoxi-N-metil*, y para el diurón *N, N-dimetil*.

El reactor fue alimentado de manera continua con medio mínimo mineral suplementado con formulaciones comerciales de linurón y diurón como única fuente de carbono, nitrógeno y energía. La concentración teórica de cada principio activo fue de 25 mg L^{-1} (la composición del medio se mantuvo constante). La evaluación del reactor se realizó en 3 diferentes condiciones de operación (3 velocidades de alimentación 100, 200 y 400 mL h^{-1}), bajo estas condiciones de operación los valores de carga volumétrica en el caso del linurón ($B_{V\ Linurón}$) variaron de 1.28 a 5.11 mg Linurón L^{-1} h^{-1}, para el diurón ($B_{V\ Diurón}$) variaron de 0.68 a 2.71 mg Diurón L^{-1} h^{-1} y en el caso de la DQO ($B_{V\ DQO}$) variaron de 4.10 a 16.42 mg DQO L^{-1} h^{-1}. Los resultados muestran que la eficiencia de remoción (**η**) de linurón fue 100% en las tres condiciones de operación, para el caso del diurón los valores de **η** obtenidos fueron 18%, 14% y 12%, el valor de 18% corresponde al valor más bajo de $B_{V\ Diurón}$ y la eficiencia disminuyó conforme se aumentó el valor de la carga volumétrica. La eficiencia de remoción de la DQO no se vió afectada por las condiciones de operación evaluadas, con valor máximo de 68%.

A pesar de que el linurón y el diurón tienen una estructura muy parecida, la comunidad microbiana establecida en el reactor no fue capaz de degradar con la misma eficiencia el diurón que el linurón. Es probable que las enzimas involucradas en la degradación del linurón presentes en los microorganismos de la comunidad sean muy específicas.

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*Author for all correspondence.

DISTRIBUCIÓN Y CUANTIFICACIÓN DE ARSÉNICO ADSORBIDO AL SISTEMA RADICAL DE *Sedum praealtum*, POR MICROSCOPÍA ELECTRÓNICA (SEM-EDXA)

Angélica Rodríguez-Dorantes* (1); Mayra Cristina Paz-Paredes (1); María S. Vázquez-Murrieta (1); Marina O. Franco-Hernández (2); Alfredo Cruz-Orea (3); Leonor A. Guerrero-Zúñiga (4)

(1) Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México, D.F., México
(2) Unidad Profesional Interdisciplinaria de Biotecnología, Instituto Politécnico Nacional, México, D.F., México
(3) CINVESTAV, Instituto Politécnico Nacional, México, D.F., México
(4) Instituto Mexicano del Petróleo, México, D.F., México

La fitoextracción es una técnica útil para fuentes de contaminación no puntuales como una forma efectiva de la remediación del suelo bajo niveles moderados de metales. Para incrementar la cantidad de metales tóxicos en los contenedores vegetales se han empleado varios compuestos sintéticos como el ácido etilen diaminotetra acético (EDTA), como agentes quelantes con resultados muy prometedores. El análisis de la dispersión por rayos X, después de un tratamiento de congelación de muestras biológicas permite la localización precisa de muchos elementos químicos en las paredes celulares, y con ello brindar información sobre los mecanismos implicados en la toxicidad de metales y su tolerancia. El presente estudio evaluó la respuesta del efecto de la adición de agentes quelantes químicos en la remoción de arsénico a través del análisis de la distribución y cuantificación de arsénico adsorbido al sistema radical de *Sedum praealtum* por microscopía electrónica de barrido y análisis de la energía de dispersión por rayos X.

Se emplearon plantas de *S. praealtum* de 30 días crecidas y expuestas en cultivo hidropónico a arsénico, EDTA y ácido oxálico. Se obtuvo la biomasa foliar, radical y del tallo de las plantas y se realizó la cuantificación de arsénico adsorbido por la técnica de microscopía electrónica de barrido (SEM: Scanning electron microscopy) y análisis de la energía de dispersión por rayos X (EDXA: Energy dispersive X-ray Analysis).

La cantidad de arsénico cuantificado en las raíces de las plantas expuestas a éste mas EDTA, mostraron que se detectó una mayor adsorción del metal a las raíces que en la presencia de ácido oxálico, además de que la zona más evidente de esta adsorción fue la zona de la cofia radical, y que esta adsorción decreció a medida que la distancia se hace evidente con respecto a esta. El efecto de la presencia de arsénico en el crecimiento de estas plantas de *S. praelatum* evidenció un daño en su desarrollo, pero la adición de los agentes quelantes sintéticos: el EDTA y el ácido oxálico resultaron eficientes al facilitar la biodisponibilidad de este metal, que pudo ser absorbido pero también adsorbido como lo mostró el análisis de microscopía electrónica de barrido al detectar la presencia de arsénico en el perfil radical obtenido.

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*Autor de correspondencia.

DISEÑO DE UNA TECNOLOGÍA PARA OBTENCIÓN POLVOS METÁLICOS A PARTIR DE PILAS SECUNDARIAS (NIMH) DE DESECHO

Blanca Cuevas-González* (1); Adriana Velasco-Hernández (1); M. Islas-Valdespino (1)

(1) TESE, Div. Ing. Qca. Y Bqca., Edo. deMéx, México.

Nowadays, rechargeable Nickel Metal Hydride (NiMH) are widely used as electrochemical power sources in portable technology such as mobile phones, personal computers, video cameras and hybrid electric vehicles, all these high-demand and consumption be indispensable devices in everyday life, with a tendency to increase their use in the coming years. These batteries contain metallic components (Ni, Co, rare earths etc.) That can be recovered once their useful life and are discarded, to obtain alternative sources of raw materials in the production of Ni-base alloys.

In this paper a characterization of the components of NiMH batteries to a defined brand and model, these are subjected to grinding with each other in an autogenous mill to separate the plastic cover enclosing them, are then fragmented in a blender which are carried by sieving to separate the coarse particles from the fine and a magnetic system for separating the components of the non-metallic metal. Metal part obtained is processed to obtain powders in a mechanofusion reactor.

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*Author for all correspondence.

Chapter 10.
Environmental Toxicology

ASSESSMENT OF GENOTOXIC POTENCY OF MERCURIC CHLORIDE IN GILL CELLS OF MARINE GASTROPOD *Planaxis sulcatus* USING COMET ASSAY

Jacky Bhagat* (1); Baban Ingole (1)

(1) National Institute of Oceanography, Dona Paula, INDIA

Heavy metals are of great ecological concern due to their potentially toxic and persistent nature. Mercury is a global pollutant and is widely spread in biosphere, from both natural and mad-made activities. The amount of mercury has risen steadily in both marine and freshwater ecosystems due to anthropogenic emissions.

In this study, *in vivo* and *in vitro* exposures were used to investigate the genotoxicity of mercuric chloride ($HgCl_2$) to the marine snail, *Planaxis sulcatus*. The alkaline single cell gel electrophoresis (comet) assay on gill cells was used and two parameters - percentage tail DNA and olive tail moment (OTM) were chosen as a measure of DNA damage. The comet assay protocol was validated on gill cells exposed *in vitro* to hydrogen peroxide (H_2O_2, 0–50 μM). For the *in vivo* experiments, the group of 10 snails per concentration were exposed for 96 h to $HgCl_2$ (10, 20, 50 and 100 ppm). Our results show significant concentration-dependent increases in the percentage of DNA in the % tail DNA and OTM (mean values±SD) for all doses compared with controls ($P<0.05$). For the *in vitro* exposure, the gill cells were treated with 10, 20, 50 and 100 ppm of $HgCl_2$ for 1h in the dark at 4°C. Incubation of gill cells with $HgCl_2$ resulted in significantly higher values of the comet parameters at all concentrations. Our results showed that DNA damage increased in the gill cell with increasing exposure time. This study shows the usefulness of comet assay to study the genotoxic damage in snails due to heavy metal pollution.

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*Author for all correspondence.

ECOTOXICITY OF COMPOSTING OBTAINED OF DISPONSABLE DIAPERS ON TWO PLANT SPECIES

Perla X. Sotelo-Navarro* (1); Rosa M. Espinosa-Valdemar (1);
Alethia Vázquez-Morillas (1); Margarita Beltrán-Villavicencio (1);
Cecilia Agapito-Abraham (1); Juan C. Álvarez-Zeferino (1)

(1) Universidad Autónoma Metropolitana – Unidad Azcapotzalco, México D.F., México

This work presents the evaluation of ecotoxicity of two composts. The first compost was obtained from used disposable diapers mixed with garden waste (PU), and the second one from garden waste (RJ).

The assessment was performed considering the effects on seedling emergence and early growth of the plants. Two plant species commonly used in Mexico were used: tomato (*Physalis ixocarpa*) and red ball tomato (*Lycopersicon esculentum*). The viability of the two species was assessed by placing 20 seeds evenly distributed over a layer of cotton in a Petri dish, to which 20 mL of distilled water were added. The Petri dishes were sealed with parafilm and kept at a constant temperature of 25 °C. After 96 h, the number of germinated seeds was counted. The assay was performed with five replicates for each specie. Once the seeds were considered viable, the ecotoxicity tests were conducted in glass rhizotrons with 5 cm diameter and 25 cm in height. The rhizotrons were filled with mixtures of compost and an inert substrate (sand sea) and one control filled with soil was used as a reference. Three seeds of each specie were placed in each rhizotron. The assay was performed with five replicates for each specie and each mixture. The experiment lasted 21 days after the time when 50% of seeds were germinated, as indicated by the 208 method of the OECD (Organization for Economic Cooperation and Development). Afterwards, the plants were extracted and washed. Percentage of germination, height, depth of roots, and weight of wet and dry biomass were determined of each plant.

Statistical analysis of each of the parameters allowed to conclude that the presence of diapers in the composting process did not affect the quality of the resulting compost, which is suitable as a soil amendment for growth of the tested species.

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*Author for all correspondence.

MICROENCAPSULACIÓN DEL EXTRACTO ESPORA CRISTAL DE *Bacillus thuringiensis* HD1 Y SU EVALUACIÓN BIOLÓGICA CONTRA INSECTOS PLAGA

Erika González-Álvarez (1,2); Rocío Castro-Ríos (3) Hugo A. Luna-Olvera (2); Sergio A. Galindo-Rodríguez (1); ***Abelardo Chávez-Montes**** (1)

(1) Depto. Química, Facultad de Ciencias Biológicas, UANL, Cd. Universitaria s/n, San Nicolás de los Garza N.L., México;
(2) Instituto de Biotecnología, Facultad de Ciencias Biológicas, UANL, Cd. Universitaria s /n, San Nicolás de los Garza N.L., México;
(3) Depto. Química Analítica, Facultad de Medicina, UANL, Av. Madero, Monterrey N.L. México.

Bacillus thuringiensis (Bt) es una bacteria gram positiva formadora de esporas y cristales proteicos (extracto) con toxicidad específica para ciertos insectos plaga en su fase larval. Sin embargo, la efectividad de dicho extracto se ve disminuida al ser expuesto a la radiación electromagnética UV (REM UV) principalmente. Sobre los métodos para proteger de REM UV, la microencapsulación es una alternativa eficiente que permite la formación de microcápsulas (MP) a base de polímeros naturales o sintéticos (Eudragit®), con tamaños de 1 a 1000 µm para la encapsulación de materiales biológicos como proteínas y esporas.

Este trabajo describe un proceso de microencapsulación del extracto espora cristal producido por la cepa de *Bacillus thuringiensis* variedad *kurstaki* HD1 y su evaluación biológica contra insectos plaga modelo. La metodología se basó en la técnica modificada de emulsión múltiple ($W_1/O/W_2$) *salting out* y evaporación de solvente, y fue implementada para producir MP de Eudragit® S100 con tamaños alrededor de 30 µm con la capacidad de proteger al extracto de factores ambientales, como REM UV. Inicialmente, se obtuvo una encapsulación eficiente de alrededor del 66 % de proteína total del extracto incorporado en las MP, la cual se confirmó mediante microscopía confocal laser de barrido (MCLB), observándose que el extracto se encontraba embebido en las MP. Posteriormente, se realizó la liberación del complejo de las capsulas en un medio alcalino semejante al del intestino de ciertos insectos plaga, y se observó que al minuto 5 de exposición las MP se habían solubilizado permitiendo la liberación del extracto. Finalmente, se evaluó la actividad insecticida del extracto libre, encapsulado MP y co-encapsulado con diferentes protectores de luz UV (benzofenona, eosina amarilla y rojo sudan) para evaluar su capacidad protectora, al ser expuesto a REM UV *in vitro*, contra larvas de *T.ni* y *S. exigua*. Se determinó que la presencia de benzofenona en las formulaciones de MP confirió la mejor protección del extracto ante REM UV. Estos resultados sugieren que la formulación obtenida provee una metodología prometedora que permite la protección del extracto espora-cristal del ambiente y su liberación específicamente en el intestino diana además siendo una formulación bioinsecticida con alta capacidad residual.

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*Author for all correspondence.

TRANSFORMACIÓN OXIDATIVA DE FENANTRENO UTILIZANDO QUERCETINA COMO MEDIADOR NATURAL

Noemí A. Rivera-Casado (1); Armando Ariza-Castolo (2); Josefina Pérez-Vargas (3); Octavio Gómez-Guzmán (1); Graciano Calva-Calva* (1)

(1) CINVESTAV- IPN, Biotechnology and Bioengineenig Department, México DF, México;
(2) ENCB-IPN, Chemistry Department, México DF, México;
(3) TESE, Div. IBQ, Edo. Méx, México.

El mecanismo enzima-mediador-sustrato (EMS) como alternativa para la remoción de compuestos xenobióticos se ha estudiado principalmente con sistemas enzimáticos de hongos filamentosos. Se ha demostrado que el uso de mediadores metabólicos, tanto sintéticos como naturales, pueden favorecer la remoción de hidrocarburos poliaromáticos hasta en un 90% del compuesto (Majcherczyk, A. *et al.* 1998; Torres-Duarte, C. *et al.* 2009). En estudios previos con procesos de fitorremediación en invernadero de suelos provenientes de sitios intemperizados después de ser impactados por derrames de petróleo crudo, se ha demostrado que los sistemas vegetales, específicamente *Cyperus laxus*, también poseen las herramientas bioquímicas y enzimáticas para poder llevar a cabo la remoción de hidrocarburos poliaromáticos (HPA) mediante mecanismos similares. *C. laxus*, es capaz de fitorremediar suelos conteniendo hasta 325 000 ppm de hidrocarburos totales del petróleo (HTP) y su perfil de compuestos fenólicos y actividad enzimática oxidativa de diversos tejidos demuestra la formación de conjugados HPA-fenoles vegetales, lo que sugería la interacción química y enzimática entre el xenobiótico y los metabolitos vegetales (Rivera-Casado, N. A. *et al.* 2010). Resultados similares se observaron con sistemas asépticos de plantas cultivadas *in vitro* en presencia de fenantreno, antraceno y queroseno. Continuando con esos estudios, el objetivo del presente trabajo fue evaluar la capacidad de la quercetina, uno de los fenoles más abundantes en el sistema de fitorremediación con *C. laxus*, como mediador metabólico en un proceso de oxidación utilizando peroxidasa como sistema enzimático modelo.

Interesantemente, los resultados de las reacciones enzimáticas *in vitro* sugieren que la transformación de fenantreno está regulada por la concentración de H_2O_2 en el medio, el cuál es fundamental para la activación de la peroxidasas; sin embargo el peróxido por sí solo también es capaz de formar el conjugado quercetina-fenantreno. Así, con una concentración de H_2O_2 de 100 µM se estimó una remoción enzimática del 30% de 60 µM de FNN en 30 minutos de reacción, mientras que en ausencia de enzima con 10µM de H_2O_2 se tiene una remoción del 38 % de FNN en un lapso de 5 h. De estos resultados se pueden concluir dos cosas: primero, que la conjugación de metabolitos vegetales como la quercetina con los HPA sí puede ser uno de los mecanismos de remoción de estos compuestos en los sistemas de fitorremediación; y segundo, que la formación del complejo EMS no es indispensable para el proceso de remoción.

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*Author for all correspondence.

BIOENSAYOS DE TOXICIDAD CON CLADÓCEROS Y UNA MICROALGA ANTES Y DESPUÉS DE UN TRATAMIENTO DE BIORREMOCIÓN DEL COLORANTE ROJO CONGO

Miriam Hernández-Zamora (1), Fernando Martínez-Jéronimo (2), Rosa Olivia Cañizares-Villanueva* (1)

(1) CINVESTAV-IPN. Departamento de Biotecnología y Bioingeniería, Laboratorio de Biotecnología de Microalgas. Av. IPN 2508, San Pedro Zacatenco, C.P. 07360, México DF, México. Tel.: 52 (55) 50613800 ext.4342. E-mail: rcanizar@cinvestav.mx
(2) ENCB-IPN. Departamento de Zoología, Laboratorio de Hidrobiología Experimental. México D.F, México.

La descarga de aguas residuales textiles que contienen colorantes azo puede afectar negativamente los ecosistemas acuáticos y la salud humana. Para poder evaluar los efectos tóxicos de los colorantes azo, es necesario realizar bioensayos de toxicidad con microalgas y cladóceros que son representativos de las comunidades en los ambientes acuáticos. En estos últimos se evalúa su efecto a través de estudios de toxicidad aguda por medio de respuestas letales (mortalidad, inmovilidad). Dentro de la variada gama de organismos utilizados en este tipo de prueba se encuentran los cladóceros *Daphnia magna* y *Ceriodaphnia dubia*. Desafortunadamente, son pocas las investigaciones sobre la toxicidad que causan los colorantes azo que ingresan como contaminantes a los sistemas acuáticos; por esta razón, el objetivo del presente estudio fue evaluar el efecto tóxico del colorante rojo Congo en el afluente y efluente en dos niveles de la cadena alimentaria: productores primarios (*Chlorella vulgaris*) y consumidores secundarios (*Daphnia magna* y *Ceriodaphnia dubia*).

La prueba consistió en bioensayos de toxicidad aguda antes (afluente) y después (efluente) de un tratamiento de biorremoción del colorante rojo Congo. Las concentraciones de rojo Congo ensayadas para el afluente fueron: 5, 10, 15, 20 y 25 mg L^{-1} y para el efluente: 0.84, 1.85, 3.72, 5.7 y 10.4 mg L^{-1}. Las pruebas de toxicidad aguda empleando la microalga *C. vulgaris* se realizaron de acuerdo al procedimiento de la OECD-guideline 201 (OECD, 1984), mientras que los bioensayos con los cladóceros fueron de acuerdo al procedimiento de la Norma Mexicana NMX-AA-087-1995-SCFI, 1995 y la U.S.EPA, 2002.

Los resultados de los bioensayos con la microalga y los cladóceros demostraron que el afluente del colorante rojo Congo es tóxico a las concentraciones ensayadas; sin embargo, en los efluentes la toxicidad disminuyó sin provocar un efecto letal en el 50% de los organismos expuestos. En los bioensayos agudos *Daphnia magna* resultó la especie menos sensible al colorante rojo Congo (CE_{50} 17.0 mg L^{-1}), seguida por *Chlorella vulgaris* (CE_{50} 5.14 mg L^{-1}) y *Ceriodaphnia dubia* (CE_{50} 3.32 mg L^{-1}). Así mismo se demostró que *Daphnia magna* y *Ceridaphnia dubia* pueden ser utilizadas como organismos de prueba para la realización de estudios toxicológicos en afluentes y efluentes que contengan colorantes tipo azo.

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*Author for all correspondence.

ANALYSIS OF GENOTOXICITY BIOMARKERS IN HUMAN LEUKOCYTES BY TO PAH EXPOSURE

Erik Uribe-Vega (2); María L. Vega-Barrita (1); Eva Ramón-Gallegos (1); Raúl Uribe-Hernández* (1)

(1) ENCB-IPN, México DF,
(2) FQ-UNAM, México DF,

The DNA molecule is very stable but may undergo spontaneous chemical degradation. However oxidative stress caused by toxic compounds accelerates DNA damage significantly. In this study was evaluated the potential genotoxicity of two polynuclear aromatic hydrocarbons (PAH), exposed cultured human leukocytes in vitro using two types of biomarkers genotoxicity: DNA fragmentation (F-DNA) and adduct formation (DNA-PAH). Determining these biomarkers in human leukocytes culture was performed to 24 hrs exposure. Different concentrations of anthracene and benzo(a)pyrene (BaP) were used. Both the production of DNA fragmentation as well as the formation of adducts DNA-PAH were determined by fluorescence spectroscopy with previous solvent extraction.

In case of the fragmentation DNA, ther was no significant effect with anthracene, whereas the B(a)P was obtained a statistic significant ($p < 0.05$) DNA fragmentation at concentrations of 0.23-2.35 mM, increasing to 15 times the F-DNA. Regarding the formation of DNA-PAH adducts, in case to anthracene said response was not observed, whereas DNA-BaP adduct formation induced a 5.73-fold increase ($p <0.05$) at a concentration of 2.0 mM. The results show that there is a consistent relationship between the productions of both F-DNA as well as adducts DNA-BaP, suggesting the possibility of specific mechanisms associated with the in vitro genotoxicity of BaP. We conclude that the biomarkers may be used as suitable discriminates of genotoxic agents as well as of environmental pollutants with genotoxic potential and for application in studies of environmental risk assessment and in hazardous waste evaluation.

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*Author for all correspondence.

BIOMARKERS IN BENTHIC FISH TO ASSESS THE ENVIRONMENTAL IMPACT IN THE SOUTHERN GULF OF MEXICO: 12 YEARS OF MONITORING PROGRAM.

O. Zapata-Pérez, J.* (1); Jorge Rubio-Piña (1); Victoria Patiño-Suárez (1); Ana. Fuentes-García (1); Marcela del Rio-García (1); Addy Solis-Un (1); Arnulfo Albores-Medina (2).

(1) CINVESTAV- IPN, Mérida, Yucatán, México;
(2) CINVESTAV- IPN, México, D.F., México;

Definitely one of the great human challenges is the early detection of the effects in marine organisms' exposure to contaminants, assess the biological consequences and avoid large changes in the ecosystems biological diversity. For this reason, a way to establish the connection between the levels of pollutants and their harmful effects on organisms are monitoring programs that incorporate biological tools such as biomarkers. A Twelve-year of biomonitoring program was conducted between 2002-2014 in the southern Gulf of Mexico, using benthic fishes like *Ariopsis felis* (hardhead) and *Scyacium gunteri* (flatfish) as a sentinel species.

Two thousand and three hundred fishes (1250 *Ariopsis felis* and 1050 *Scyacium gunteri*) were collected in four rivers and 55 sampling sites along the Gulf of Mexico including areas of oil exploitation. Quantitative differences on liver mRNA of Cytochrome P4501A1 (*CYP1A1*), Glutathione-*S*-Transferase (*GST*), Metallothionein (*MT*) and Vitellogenin (*VTG*) gene expressions were used as markers of exposure to polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and toxic metals.

At the molecular level, results showed that the highest expression of *CYP1A*, *GST*, *MT* and *VTG* genes were detected in fish collected near the coast of Tabasco (Dos Bocas, 18°26'16" N; 93°11'25" W), and the exclusion zone in the oil region, probably among the most polluted zones. The association between gene expression and PAHs in fish liver was low, although its main association was with 2.6-dimethyl-naphthalene and 2,3-dimethyl-naphthalene, indicating that organisms have a high metabolic activity related to the processing and disposal of low molecular weight PAHs of petrogenic origin. On the other hand, four catfish collected in the Términos Lagoon showed endocrine disruptive effects at the histopathological level. In conclusion, the high biomarkers expressions at the exclusion zone suggests that the organisms are responding to the presence of other types of chemical compounds or mixtures like dispersants, detergents or emulsifiers that cause characteristic oxidative and endocrine effects.

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*Author for all correspondence

CARBON NANOMATERIALS: NEW PRODUCTS REQUIRE NEW APPROACHES OR IS OUR HEALTH AT RISKS?

Arnulfo Albores* (1); Yury Rodríguez-Yañez(2)

(1) Departamento de Toxicología. Cinvestav, México, D.F., México.
(2) Hospital General de Ticomán. SS, D.F., México.

Carbon nanomaterials became a generic name given to carbon objects which pose at least one dimension equal or smaller to 100 nm, these materials could be of inorganic origin and may have quite diverse structures, molecular compositions and properties that greatly differ from bulk materials. Carbon nanomaterials attract attention because novel: molecular structures, physical and chemical properties, applications and environment and human health risks that require the development of new technologies and new safety regulations. Carbon nanotubes (CNT) encompass a large superfamily of materials: Pristine carbon nanotubes are single- (SWCNT) or multi-walled; un-substituted (pristine) or substituted with diverse chemical functions, each one with their own set of properties. SWCNT, in particular, consist of a graphene sheet forming empty capped or uncapped cylinders that, due to their synthesis, may contain metal contaminants that modify their biological effects. The exceptional CNT physicochemical properties has increased their applications, but their effects on living beings differ among them and are unknown, thus sanitary measurements on industrial handling and routinary use should be implemented. Taking as background the basal contamination particulate material in the atmosphere, biologically relevant information can be obtained by using experimental models exposed to relevant environmental contamination levels, for example, the pristine SWCNT effects on fibrinolysis by using primary endothelial cell cultures obtained from the umbilical vein (HUVEC) where cell translocation of the material, the expression of tissue plasminogen-activators (*PLAT*, *PLAU*, *KLK1*), and the inhibitor gene *Serpine1* can be assessed by real-time PCR and the proteins tPA, uPA, KLK1 and PA1-1 can be quantitated. In summary, SWCNT damaged to HUVEC either by direct contact and/or internalization can be visualized and gene expression or protein levels alterations in fibrinolysis can be examined. The SWCNT capacity to cause circulation damage, although is an issue that requires careful consideration and accounted when developing sanitary regulations. (Acknowledgements: Supported by: SSA/IMSS/ISSSTE/CONACYT (162391), ICyTDF51/2012 and YRY CONACYT Grant (203482).

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*Author for all correspondence

PHOTOTHERMAL TECHNIQUES APPLIED TO THE DETECTION OF ENVIRONMENTAL POLLUTANTS

Alfredo Cruz Orea*

Departamento de Física CINVESTAV- IPN, México DF, México.

Photothermal phenomena represent currently a very active and widespread field of theoretical and experimental research carried out within both the commercial as well as the academic institutions. The field of photoacoustic and photothermal science and technology has reached a level of maturity and diversity such that it is quite difficult for an individual researcher to be aware of many of the new and important developments. In the case of photoacoustic spectroscopy (PAS) several studies have been done in the environmental and biotechnology fields. Among the studies developed by PAS in the environmental field we exposed two investigations performed in the Physics Department of CINVESTAV-IPN. The first one corresponds to the *Cyperus hermaphroditus* plant, which has the capability to remove phenanthrene of highly contaminated soil. Removal occurs by adsorption of this contaminant on the plant roots and can be assessed by analyzing the evolution of the absorption spectrum of the root system. Photoacoustic spectra of this system indicate that higher amounts of phenanthrene are adsorbed with increasing phenanthrene concentrations, suggesting the use of *Cyperus hermaphroditus* for phenanthrene removal.

The second study was developed in lettuce leave under the action of one herbicide. Depth profiles of double layer biological samples (lettuce leave), obtained by photoacoustic spectroscopy have been studied using the phase-resolved method. The application of the method was demonstrated by insulating out the spectra of the cuticle and the pigment layers of a lettuce leaf. Using this method, we were able to monitor the phase shift between the cuticle and the pigment layers as a function of the time elapsed after herbicide spraying. The temporal changes occurring in lettuce leaves under the action of dehydration were also investigated.

Also another photothermal technique, thermal lens spectrometry (TLS), has been used in the determination of environmental pollutants, for example in detection of pesticides in water.

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*Author for all correspondence

CHEMICAL POLLUTION IN THE ATOYAC RIVER: ENVIRONMENTAL DEGRADATION AND HEALTH RISK FACTORS

Regina Montero Montoya*(1)

(1) Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México

The Atoyac River is the main stream of the Alto Balsas basin in the state of Tlaxcala and has been the source of freshwater for human activities of small agricultural communities and a few industries.

A petrochemical plant was installed in site by the early 90's and other industries followed: metal-mechanics, paints, textile, mosaics, denim laundry and others, constituting industrial corridors that are devoid of technology to protect the environment. Consequently, the wastewater they generate is collected in pipelines where they combine with municipal waste and are ultimately discharged into the river.

This situation is a source of distress for the communities living at the banks of the river and their concerns have been attended only by NGOs and the Academia, generating actions and studies that underline deficiencies in the environmental law as well as gaps in sanitary planning and attention in polluted areas, which impede the recognition of the problem, its management and the application of solutions.

Assessment of environmental quality and health risks using animal models and human monitoring have been used to diagnose the current environmental situation and to identify hazards for human health.

The challenge is to find an interlocutor among the responsible authorities in order to establish permanent monitoring programs for polluted areas like this, in order to recover environmental quality to protect human health.

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*Author for all correspondence

FLUORIDE IN DRINKING WATER AND DENTAL FLUOROSIS: A SERIOUS CONCERN

Luz M. Del Razo* (1); Carmen González-Horta (2); L. Ballinas-Casarrubias (2); Mónica I. Jiménez Córdova (1); Mariana C. Cárdenas Gonzalez (1); Olivier C. Barbier (1).

(1) Departamento de Toxicología, CINVESTAV- IPN, México DF, México;
(2) Facultad de Ciencias Químicas. Universidad Autónoma de Chihuahua, Chihuahua, México.

Most of the population worldwide depend on groundwater sources for potable water. In Mexico, 16% of identified aquifers and 53% of the most important aquifers are overexploited. Some of these aquifers are naturally enriched with inorganic fluoride at concentrations that may be hazardous to human health. Naturally occurring fluoride concentrations in groundwater range from 0.5 to 30 mg/L depending on geological factors have been reported in many “hot spot” geographic areas in Latin America, Asia and Africa. Here, fluoride levels frequently exceed the corresponding World Health Organization maximum contaminant level of 1.5 mg/L. In comparison, the recommended range of fluoride levels for the maximum protection from dental caries is 0.5-1 mg/L. The presence of fluoride in groundwater has been related to leaching from the fluoride-bearing rocks like fluorspar, cryolite, fluorapatite and hydroxyapatite or deposition and weathering of atmospheric volcanic particles, hydrothermal activity and contamination with fluoride-containing pesticides.

High levels of chronic fluoride exposure (>1.5 mg/L in drinking water) has been associated with dental and skeletal fluorosis, decrease in fertility, diminished intellectual capacity, immune dysregulation, renal damage, atherosclerosis, vascular stiffness, and myocardial cell damage. In Mexico, several areas with dental fluorosis, an unattractive discoloration and mottling of the teeth, have not received enough attention, although indicate chronic overexposure to fluoride. Thus, it can be assumed that people with dental fluorosis have more risk to present the several effects associated with chronic fluoride exposure, as shown the recent results obtained in residents from some localities of Chihuahua, Mexico, exposed to 0.07-4.28 mg/L in drinking water, where people with dental fluorosis showed more risk of having kidney injuries.

Given the potential of adverse health effects, immediate measures should be taken to reduce the fluoride exposure, particularly for vulnerable population and specifically for children. Considering the current conditions of overexploitation of the aquifers in Mexico, the groundwater levels will continue to decrease and consequently the fluoride concentration will continue to rise; thus, further studies require addressing the development and implementation of a functional method for defluoridation of water, and the appropriate disposal of the generated waste.

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*Author for all correspondence

EXPRESSION OF BIOMARKERS IN *TILLANDSIA USNEOIDES* AS INDICATORS OF THE PRESENCE OF AIR POLLUTANTS IN HIDALGO, MEXICO

Carlos A. Lucho Constantino*

Universidad Autonóma del Estado de Hidalgo, Centro de Investigaciones Químicas, Mineral de la Reforma, Hidalgo, México. CP. 42184. Tel. 01 771 717 2000, ext. 2216, e-mail: a_lucho@yahoo.com.mx

Natural environments located near urban and industrial areas are usually contaminated by pollutant discharges. The physical and chemical analysis is often performed in order to detect the presence of potentially harmful chemicals to the environment and to human health.

Air pollutants cannot be confined close to the emission source, they are extend to large distances, depending on topographical and meteorological conditions. Collecting samples of particulate matter in the air is done by 1) direct collection by precipitation, and total deposition and 2) accumulated by biomonitors. In the second alternative, the use of plants offered as potential assessment of the effects of pollutants on living beings, by tracking changes in the body's internal monitor (using biomarkers). A biomarker is a measurement that reflects an interaction between a biological system and a chemical, physical or biological substance from the environment. Therefore, the aim of this study was to determine the expression of the biomarkers studied in *Tillandsia usneoides* L. in response to the presence of air pollutants in the industrial region of Tula-Tepeji, Hidalgo. *Tillandsia usneoides* was exposed to similar environmental conditions in two sites: 1) a receiving-pollutants site and 2) a site with few industrial activities over a period of eight months in the industrial region of Tula-Tepeji, Hidalgo. Indeed biomarkers were analyzed as chlorophylls, total protein, peroxidase (POX), ascorbic acid, superoxide dismutase (SOD) and nitrate reductase (NR) and as a biomarker of exposure, the accumulation of plant elements (Ni, Cd, Ca , Mg, Fe, Mn, Cu, S, and Pb) for which parameters are useful as biomarkers in the presence of atmospheric pollutants. Parameters such as ascorbic acid, and nitrate reductase peroxidase were the parameters showed significant difference.

Key words: Biomonitoring, air pollutants, industrial zone

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*Author for all correspondence

Chapter 11.
Microbial Ecology

CHARACTERIZATION OF *Azotobacter* AND *Pseudomonas* WITH POTENTIAL FOR PLANT GROWTH PROMOTION ISOLATED FROM CROPS OF THE MORELOS AND GUERRERO STATES

María A. García-Rivera (1); Laura C. Fuentes-Vázquez (1); Blanca G. Gómez-Sotelo (1); Miguel A. Rodríguez-Barrera (1); Jeiry Toribio-Jiménez (1); Yanet Romero-Ramírez*(1)

(1) Laboratory Research in Biotechnology and Microbial Genetic, Academic Unit of Science Chemistry Biology, Autonomous University of Guerrero, Mexico.

The most common way to incorporate nutrients to the soil has been in recent decades, the use of chemical fertilizers that have generated negative environmental impacts. The alternative to the use of these substances has been proposed the use of Rhizosphere bacteria with effect on plant growth and development. These bacteria are able to stimulate plant growth directly and indirectly and have a number of complex mechanisms to establish beneficial relationships, especially with the roots of plants. *Azotobacter* and *Pseudomonas* have innumerable applications of biotechnological importance such as the production of biosurfactants, alginate and poly-β-hydroxybutyrate (PHBs), additionally, their ability to fix nitrogen, produce siderophores, phytohormones and solubilize phosphates which allows to consider them as plant growth promoting bacteria (PGPR).

In this study we isolated and characterized bacteria of the genus *Azotobacter* and *Pseudomonas* with potential plant growth promoter, from several economically important crops (maize, beans, coffee, sorghum, and jicama) of the Morelos and Guerrero states. Using Burk-sucrose (BS) and Ashby-mannitol nitrogen-free microaerophilic medium, three bacteria strains of the genus *Azotobacter*, and eleven of the genus *Pseudomonas* were recovered from the rhizosphere, rhizoplane, and surface-sterilized plant tissues. All isolates of *Azotobacter* were characterized biochemically and phenotypically by cyst formation, siderophores, PHBs, Indole Acetic Acid and alkylresorcinols production and genotypically by amplification of *ArsA* and *ArpR* genes by multiplex PCR using strains of *Azotobacter vinelandii* SW136, UW136 and UW RsmA as reference. Isolates of *Pseudomonas* were phenotypically characterized by the production of pigments in the middle PPGAS, PHBs and siderophores in CM medium. This study represents the first survey of crops associated bacteria of Morelos and Guerrero states and unveiled a set of strains with potential for use as inoculants.

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*Author for all correspondence

CHARACTERIZATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) ISOLATED FROM CROPS CORN (*Zea mays*) OF TLACOAPA, GUERRERO

Eva Espinosa-Escamilla (1); Zuleyma Flores-Rogel (1);
Miguel A. Rodríguez-Barrera (1); Yanet Romero-Ramírez (1); Jeyri Toribio-Jiménez* (1)

(1) Laboratory Research in Biotechnology and Microbial Genetic, Academic Unit of Science Chemistry Biology, Autonomous University of Guerrero, Mexico.

The group of Plant Growth Promoting Bacteria (PGPR) is defined as the root-colonizing bacteria that significantly stimulate the growth of plants. PGPR have been isolated from soils where the main bacterial species are associated with grasses: *Azospirillum lipoferum, A. brasilense, A. amazonense, Herbaspirillum seropedicae, Acetobacter diazotrophicus, Enterobacter agglomeran, E. cloacae, Bacillus azotofixans, B. polymyxa, Alcaligenes faecalis, Klebsiella sp*. and bacteria of the genera *Azotobacter* and *Pseudomonas*. PGPR have mechanisms that promote plant growth include: production auxins and gibberellins, which activate multiple responses in the plant cell, biochemical, physiological and morphological level.

In this study, 15 bacterial strains capable of promoting growth were isolated from soil and roots of maize. From the overall morphological, biochemical and molecular characterization studies, the isolates were identified and designated as: *Enterobacter aerogenes* y *Enterobacter cloacae, Staphylococcus lentus, Streptococcus thoraltensis y Acinetobacter lwoffii johnsonii*. All isolates were tested for ability to produce auxins and gibberellins, production of siderophores, solubilization phosphate and fix nitrogen. The bacteria that have more than one mechanism were: *Enterobacter cloacae, Enterobacter aerogenes, Acinetobacter johnsonii lwoffii* and *Streptoccoccus thoraltensis*. Of all the isolated bacteria only *Enterobacter cloacae* and *Enterobacter aerogenes* have been reported in previous works, the other bacteria have not been reported.

These isolates will be conducted to evaluate the plant growth promoting efficiency of combined inoculation of rhizobacteria on Corn plants. This study represents the first report of autochthonous bacteria capable of promoting plant growth in maize crops of Tlacoapa, Guerrero.

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*Author for all correspondence

EVALUATION OF COMPOST CONTAINING HEAVY METALS ON GROWTH OF *Azospirillum brasilense* AND *Glomus intraradices* INOCULATED IN *Zea mays*

Jenny M. Vázquez Jacinto (1); Augusto Rojas Aparicio (1);
Nelly R. Romero Gomezcaña (2); Jeiry Toribio-Jiménez (1);
Miguel A. Barrera-Rodríguez (1); Yanet Romero-Ramírez* (1)

(1) Laboratory Research in Biotechnology and Microbial Genetic, Academic Unit of Science Chemistry Biology, Autonomous University of Guerrero, Mexico.
(2) Ministry of Rural Development. Chilpancingo, Gro.

Compost is organic material obtained as a result of the controlled microbial action on organic waste. The green biotechnology is responsible for integrating the use of bio-fertilizers and compost, which represent an important alternative to reduce the use of chemical fertilizers. In this study the compost with presence of heavy metals on the growth of *A. brasilense* and *G. intraradices* inoculated in corn was evaluated. The study was conducted in the experimental field of Guerrero Produce Foundation in open field conditions, with a maximum temperature of 39.5 ± 2 ° C, a low of 13.8 ± 2 ° C and 47% relative humidity. We use an experimental design completely randomized using 3 maize genotypes with 7 treatments, each genotype had five replicates per treatment. The results were subjected to analysis of variance (ANOVA) and when we observed significant differences in each treatment the Tukey´s test with p = 0.05 was performed.

The results showed that the best genotype in adapted was Criollo, in comparison with V-236 (open-pollinated variety) and H-565 (hybrid), as it is native to the region where the project was conducted. The treatments 1 and 2 (with compost) in all genotypes presented the best features of development, this is due to physical and chemical attributes such as mineral elements that prove this organic fertilizer, so promote the nutritional requirements of the plant without the use of biofertilizers.

Microbiological analysis of the compost used in this study was determinate, we observed the presence of *P. aeruginosa* present but don not of *E. coli, S. aureus, Shigellaspp*, and *Salmonella sp*. In conclusion the use of compost not proves adverse effect on the growth of *A. brasilense* and *G. intraradices*, however, when the compost is used in absence of biofertilizer, the plants develop better anatomical features.

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*Author for all correspondence

SCREENING OF *Burkholderia* SP. STRAINS NATIVE FROM CHIAPAS FOR BIOSURFACTANT PRODUCTION, USING LOW-COST SUBSTRATES

Paola *Vázquez-Vázquez* (1); Lizbeth Reyes-López (2); Arnoldo Wong-Villarreal (2); Gustavo Yáñez-Ocampo* (1)

(1) UPChiapas, Tuxtla Gtz., Chiapas, México
(2) UTS, Ocosingo, Chiapas, México

Biosurfactants are a heterogeneous group of surface-active molecules with both hydrophilic and hydrophobic domains, synthesized by native microorganisms isolated from polluted sites such as *Pseudomonas* sp., *Burkholderia* sp. and others. Their principal properties are emulsifying and reduce surface and interfacial tension between the oleaginous and aqueous phases. Therefore, improve the solubility of persistent organic compounds. In Chiapas state México, organic wastes like banana and crude glycerol are low cost substrates that can be used as carbon source to biosurfactant production. Thus, the aim of this research was to screen *Bulkholderia* sp. strains isolated from agricultural soils of Chiapas, to produce biosurfactants using waste banana and crude glycerol as carbon source.

In the present study, seven strains of *Burkholderia* sp. were studied in different solid culture media added with waste banana and crude glycerol (20mL/L) as only carbon source, in this stage growth halo were observed and compared with regard to biosurfactant production. Only two strains named as Bk36a2 and Bk37a4 were selected and tested in kinetic assays in Erlenmeyer flasks with mineral salts medium (80mL) and crude glycerol (20mL/L). Surface tension and emulsion index (EI_{24}) were measured during 72 hours, using as positive control *Pseudomonas aeruginosa* ATCC 27853. All assays were done in triplicates and sterile conditions, using culture medium without inoculum as negative control.

Both strains Bk36a2 and Bk37a4 reduced surface tension of the medium to 29 mN/m at the end of the cultivation, meanwhile positive control (*Pseudomonas aeruginosa* ATCC 27853) achieved 25 mN/m. In contrast, the EI_{24} of Bk36a2 and Bk37a4 was 55 % and 52 %, respectively; *Pseudomonas aeruginosa* ATCC 27853 had 37 % EI_{24}. After this assays we concluded that *Burkholderia* strains Bk36a2 and Bk37a4 isolated from agricultural soils of Chiapas were capable to produce compounds with surface active properties, using crude glycerol as carbon source. Besides *Burkholderia* sp. has been few reported as a biosurfactant producer.

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*Author for all correspondence

DRASTIC CHANGES IN BACTERIAL MAT COMMUNITIES FROM AN ANCIENT AQUATIC ECOSYSTEM IN RESPONSE TO LONG-TERM ENVIRONMENTAL STRESS

Silvia Pajares-Moreno* (1); Valeria Souza (2)

(1) University of Oregon, Eugene, USA;
(2) UNAM, México DF, México.

Understanding the response of bacterial communities to global climate change is remarkably important for predicting the effect of biogeochemical modifications in the ecosystems functioning. Cuatro Cienegas is an oasis in the Chihuahua Desert (Mexico) that hosts a wide diversity of microbial mats and stromatolites in their pools, which have survived in extremely oligotrophic waters. However, the resilience of these unique microbial communities to climate change is so far unexplored.

We propose a method to study such ecosystems of the past by using a mesocosms experiment with different temperature and UV conditions. For this purpose, 15 mesocosms were seeded with both “synthetic” microbial mats and water from Cuatro Cienegas pools and formed by 5 treatments: a) same temperature (30 ºC) and UV as the pools (Control), b) fluctuating temperature (Fluct), c) temperature at 40 ºC (40C), d) with UV radiation (UVplus), and f) with UV filter (UVmin). 16S rRNA gene clone libraries were obtained from microbial mats at the end of the experiment (eight months) and were analyzed using multivariate and phylogenetic tools.

A total of 250 bacterial OTUs (97% cut-off) were recovered out of 600 sequences from both the natural pools and the mesocosms. Sequences were assigned to 13 major lineages, among which Cyanobacteria-Chloroplast (41%), and Alphaproteobacteria (25.3%) were the most abundant. Bacterial mat community from the most stable treatments (Control and UVmin) had a more similar composition and distribution of the phylogenetic groups with the pools than the most extreme treatments (UVplus, 40C and Fluct), which showed drastic changes in the community composition and structure, indicating a community response to each different environment. An increase in bacterial diversity was found in the UVmin treatment, which suggests that stable environments promote the establishment of complex bacterial community. The long-term impact of increasing UV radiation (UVplus) and temperature (40C) produces a sharp decline in richness and a selection pressure on different members of these microbial communities.

This type of experiments combined with multivariate and phylogenetic analyses of molecular data demonstrates the utility of microbial mats as models to answer ecological questions that are difficult to address using other systems.

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*Author for all correspondence

IDENTIFICATION BY MOLECULAR BIOLOGY OF BACTERIA IN THE DIGESTIVE TRACT OF THE EARTHWORM (*Eisenia fetida*) WITH REMOVAL POTENTIAL OF POLYCHLORINATED BIPHENYLS (DECACHLOROBIPHENYL)

Juan José Villalobos-Maldonado* (1); Norma Alejandra Mancilla-Margalli (1); Rocío Meza-Gordillo (2); Martha Alicia Rodríguez-Mendiola (1); Teresa del Rosario Ayora- Talavera (2); Carlos Arias-Castro (1); Reiner Rincón-Rosales (2).

(1) Instituto Tecnologico De Tlajomulco, Tlajomulco de Zuñiga, Jalisco, México;
(2) Instituto Tecnologico De Tuxtla Gutierrez, Tuxtla Gutiérrez, Chiapas, México.

Polychlorinated biphenyls (PCB´s) belong to the persistent organic pollutants (POP´s), which are a group of synthetic substances of high risk to human and environmental health. These substances have been found around the world, including those remote areas where such contaminants were used, for example, polar regions, human populations and breast milk.

The aim of this research was the use of cultivable methods that allowed the isolation and genotypic characterization of the bacteria associated with the gut of the earthworm (*Eisenia fetida*), identifying some reported genus with removal potential of (COP's), creating a mutualistic endosymbiosis. The worm analyzed in addition of peat moss and rabbit excrete at laboratory level, they integrated a vermicomposting system that was able to remove 96%, the pollutant decachlorobiphenyl at a concentration of 200 ppm during 91 days.

From eight pure bacterial strains isolated from the gut of the earthworm, thus total genomic ADN was extracted and ERIC-PCR (genomic fingerprints) technique was applied and later the 16S rDNA gene was amplified using technique of polymerase chain reaction (PCR). Using primers: fd1 (5'-AGAGTTTGATCCTGGCTCAG-3 ') and rd1 (5'-AAGGAGGTGATCCAGCC-3'). The PCR product was purified and subsequently sequenced. Sequences were analyzed using the program BioEdit v.6 and taxonomical position; reconstruction of phylogenetic tree of bacteria was performed by program MEGA v.5, using the Tamura-Nei evolutionary model. Phylogenetic study based on 16 S rDNA gene in the digestive tract of the earthworm *Eisenia fetida* allowed the identification of the bacterial genus *Bacillus, Bacterium, Caryophanon, Staphylococcus, Paenibacillus, Pseudomonas,* and *Stenotrophomonas Solibacillus.*

It is concluded that the genus *Bacillus* was that given the greater abundance and identified to the genus *Pseudomonas*, which has been studied in the removal of some (POP´s), in this relevant case by remotion of decachlorobiphenyl most toxic congener, and researched other sustainable method complementary to those reported in the NOM-133-SEMARNAT-2000, environmental Protection polychlorinated biphenyls (PCB's).

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*Author for all correspondence

POST-FIRE RECOVERY OF ALGERIAN SOIL PHYSICO-CHEMICAL AND MICROBIOLOGICAL PROPERTIES (FOREST OF FÉNOUANE, WILAYA OF SAÏDA)

Amine Habib Borsali* (1, 3) ; Kheloufi Benabdeli (2) ; Raphaël Gros (3)

(1) Université « Dr Moulay Tahar », 20 000 Saïda et Université « Aboubeker Belkaid », 13000 Tlemcen, Algérie
(2) Laboratoire géo-environnement et développement des espaces, Faculté des sciences et de la vie, Université de Mascara, Algérie
(3) Institut méditerranéen de biodiversité et d'écologie, Faculté des sciences et techniques de Saint-Jérôme, Aix-Marseille Université, Marseille, France

The study area was located in the forest of Fénouane (Sidi M'barek, wilaya of Saïda, Algeria). This forest of the southern foothills of the Tell Atlas Mountain can be compared to others semi-arid fresh bioclimatic stages of Algeria with recurrent fires and difficulties to reafforestation. In this region, the average fire frequency is close to 1 fire every 25 years. The study area (2,537 ha) presents an altitude close to 850 m above sea level and is characterized by a Mediterranean climate. At the early stage of succession (*i.e.* following forest fires), plant communities are dominated by herbaceous and fast growing species such as *Pistacia lentiscus*, *Stipa tenacissima* and *Cistus villosus*. At the later successional stage (*i.e.* with no fire for at least 20 years), close forest is dominated by a tree stratum of *Pinus halepensis* and *Tetraclinis articulata*.

In this forest, 25 sampling plots (400 m^2) were selected taking into account the year of the last fire. To assure five replicates of each "time since fire", 25 sampling plots (400 m^2) were selected along a chronosequence of 2, 4, 8, 13 and 20 years since fire. Soils were sampled at the top 5 cm and their physico-chemical properties (water holding capacity and moisture content at sampling time, contents in organic carbon, total nitrogen, ammonium, nitrate, inorganic phosphorus and CaCO3, pH) and microbial properties (basal respiration, microbial biomass, enzymatic activities) were analysed. Our results showed lowest soil moisture content (2%) in the soils unburned since 2 years. The destruction of arboreous vegetation and it substitution two years after the fire by an herbaceous stratum are hypothesized to increase evapotranspiration and thus soil drought. Similar organic carbon contents in the recently burned soils and unburned soil since 20 years suggested inputs of charred material from carbonized vegetation. In comparison with soils unburned since 20 years, FDA hydrolase and phosphomonoestreases activities remained weaker two years after the last fire in spite of the recovery of microbial biomass. These results are coherent with those of Boerner and Brinkman (2003) who have shown a decrease in the short term of hydrolytic activities due to an alteration of the quality of the organic matter. However, concomitant increase in total nitrogen content to black carbon also suggested inputs of inorganic nitrogen of fresh organic matter. These inputs of available resources, probably originated from grazing sheep, have stimulated the recovery 8 years after fire of microbial biomass and some enzyme activities (FDA hydrolase and phenol-oxydase). Indeed, fire is frequently used by breeders to promote the development of grasslands and thus allow the grazing of sheep.

Either traditional or intensive grazing is then practiced for 5 years with, in average, 20 or 150 sheep per hectare respectively (DSA, 2010). The stimulation of microbial growth and activities may have generated a priming effect of the black carbon and soil organic matter leading, by adding-on to a strong erosion of surface soil, to extremely low organic carbon contents 8 years after the last fire. This study showed that the post-fire reconstitution of both soil physico-chemical characteristics and microbial properties depend on climatic conditions, especially drought, of the intensity of erosion processes and on the grazing use of the burned ecosystems. Our results suggested that the soils burned since 10 years ago may be particularly vulnerable to new fires. However addition of exogenous organic matter (compost, manure…) 8 years after a fire could be envisaged to reduce soil erosion, compensate the losses in carbon content and create a litter layer that would limit the evaporation.

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*Author for all correspondence

METAGENOMIC ANALYSIS OF MICROBIAL CONSORTIUM ISOLATED FROM PERMANENTLY CONTAMINATED AREAS OF THE FORMER WOOD TREATING PLANT

Agnieszka Piotrowska-Cyplik* (1)*;* Jakub Czarny (2); Roman Marecik (3); Łukasz Chrzanowski (4); Zuzanna Szczepaniak (1); Justyna Staninska (3); Agnieszka Wolniewicz (5); Paweł Cyplik (3)

(1) Institute of Food Technology of Plant Origin, Poznań University of Life Sciences, Poznań, Poland;
(2) Institute of Forensic Genetics, Al. Mickiewicza 3/4, 85-071 Bydgoszcz, Poland
(3) Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences, Wojska Polskiego 48, 60-627 Poznań, Poland;
(4) Institute of Chemical Technology and Engineering, Poznań University of Technology, Pl. M. Skłodowskiej-Curie, Poznań, Poland;
(5) PROTE Technologies for our Environment, Ltd. Dziadoszańska, Poznań Poland.

Wood treating plant in Solec Kujawski (Poland) was built in 1876 and for over one hundred years the plant produced mainly saturated sleepers and poles. As an industrially used, degraded area its surface is completely disturbed by the numerous traces of various demolition and earthworks in the form of semi-heaps and pits and trenches after liquidated underground installations. The sand of the depth of approximately 2 m is characterized by a horizontal stratification (a plain transformed aeolitically and fluvially) and it is fine sand, silty in some places, while deeper mid-sand, silty sand and sand with gravel have oblique stratification, specific to the construction of a outwash plain.

The initial presence of impurities outside the quartz sand was not significant (a good fluvial-aeolian selection), but in the last 100 years for example, the share of organic matter has significantly increased as a result of economic activity and the contamination of ground soil and water. The isolation of the microorganisms and metagenomic analysis of the gene encoding 16S rRNA-based V4 hypervariable region of the 16S rRNA gene was performed on such degraded soil. To amplify the selected region-specific primers 515F and 806R were used. Sequencing was conducted using MiSeq sequencer (Illumina, USA). An automatic analysis of the data was carried out on the camera MiSeq using software MiSeq Reporter (IAS) v2.4 protocol 16S metagenomics. Metagenomic analysis allowed the identification of 252 species of bacteria, of which the dominant ones are as follows: *Pseudomonas azotoformans* 30.39%, Enterobacter amnigenus 6.58%, Pseudomonas syncyanea 5.59%, *Pseudomonas tremae* 4.21%, *Achromobacter piechaudii* 4.20%, *Stenotrophomonas retroflexus* 3.43%, *Pseudomonas poae* 2.73 %, Other 42,78%.

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*Author for all correspondence

AEROBIC DEGRADATION OF ORGANIC SOLID WASTE AND MOLECULAR ANALYSIS OF MICROBIAL COMMUNITIES

Laura Martínez-Montiel (1); Luis R. Tovar-Gálvez (2);
Francisco J. Fernández-Perrino (1); Gerardo Saucedo-Castañeda* (1)

(1) Metropolitan Autonomous University, Iztapalapa Campus, Biotechnology Dept., Mexico City.
(2) Interdisciplinary Research Centre for Environment and Development, CIIEMAD-IPN, México City.

During aerobic degradation of organic solid waste (OSW), digestibility and hydrolysis of raw material depend on the early appearance of hydrolytic enzymes. Microbial communities have not been extensively studied because of the experimental difficulties in the designing of culture conditions to be used for the culturing of microorganisms. The aim of this work was to determine the profile of CO_2 production rate during aerobic digestion of OSW under controlled conditions and analyze the microbial communities using molecular biology techniques such as PCR of 16S rDNA, internal transcribed spacer (ITS) and denaturing gradient gel electrophoresis (DGGE).

Three assays were carried out, a mixture of OSW (83%), sawdust as bulking material (4%), paper (3%), pruning waste (2%), added (8%, M+C) or not added (M-C) of mature compost. The source of compost was the composting plant of Bordo Poniente in Mexico City. Approximately 100 g of the fermenting material was introduced in tubular reactors and incubated at 35°C and 70 % moisture with an air flow of 1mL/min. On line monitoring of CO_2 production was carried out by an infrared detector using a respirometer during 120 hours. Evolution of pH, and cellulolytic, pectinolytic and xylanolytic activities were determined. DNA extraction was carried out by an ultrasonic method combined with bead beater and enzymatic lysis. DNA quality was obtained using NanoDrop 2000. Gene 16SrDNA was amplified using 1492R and 8F primers. The polymerase chain reaction (PCR) products were visualized in agarose gel (1%).

Maximum CO_2 production rate was observed at 20 h in the case of M+C (8 mg CO_2*g^{-1} initial dry matter*h^{-1}). For the assay M-C the same observation was detected at 41 h. After this time, the CO_2 production rate decreased to values near to zero probably due to the consumption of easily degradable substrates. Pectinase, xylanase and cellulase activities were observed from the beginning of the process, decrease of hydrolytic activities can be related to the decline of pH. In another set of experiments addition of pectine, cellulose and xylan to the microbial consortium enhance production of hydrolitic enzymes. DNA used in amplification was of high quality and purity (Abs 260/280 was 1.82-2.06). PCR products were in high amount and ready to DGGE analysis. During the first hours of aerobic degradation of OSW, easily assimilable sugars were rapidly consumed, which can be associated to an increase in the rate of production of CO_2. A decline of pH is observed. It is worth noticing that hydrolytic enzyme activities are observed during the first stage of the process, showing a potential of degradation of biopolymer in an advanced stage of the process.

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*Author for all correspondence

ESTUDIO METAGENÓMICO Y METABÓLICO DE DOS ZOOGLEAS DE INTERÉS BIOTECNOLÓGICO

Jaime García-Mena* (1); ***Verónica L. Cano-García*** (1); Selvasankar Murugesan (1); Alberto Piña-Escobedo (1); Héctor Mario Poggi-Varaldo (1); Elvira Ríos Leal (1); Carlos Hoyo Vadillo (1); Bulmaro Cisneros Vega (1)

(1) Cinvestav- IPN, México DF, México;

En México, los búlgaros y los tibicos, son dos zoogleas diferentes que representan una simbiosis microbiana, compleja y dinámica; son gránulos gelatinosos, de 1-3 cm de diámetro y consisten en una mezcla de microorganismos agrupados de manera organizada de bacterias acido lácticas, levaduras y bacterias acido acéticas. La microflora de los gránulos se mantiene unida gracias a una matriz de proteína y a un material de exopolisacárido denominado kefirano en los búlgaros y dextrana en los tibicos. Estas zoogleas se utilizan para producir bebidas fermentadas, parcialmente alcohólica a partir de leche para el caso de los búlgaros y de piloncillo para el caso de los tibicos. Por sus propiedades, este tipo de zoogleas pueden tener aplicación ambiental o bien servir como modelo para el estudio de la comunicación célula-célula en comunidades mixtas de procariontes y eucariontes.

El objetivo del trabajo fue comparar mediante una caracterización metagenómica y metabólica el crecimiento de los gránulos de búlgaros y tibicos aclimatados a medios de cultivo simple, lactosa para búlgaros y sacarosa para tibicos, con aquellos crecidos en su medio de cultivo natural, leche para búlgaros y piloncillo para tibicos. Para el estudio metagenómico, se extrajo DNA de gránulos aclimatados a cuatro condiciones, se prepararon genotecas de 16S rDNA por PCR y se realizó secuenciamiento masivo en equipo Ion Torrent. Para el estudio metabólico se exploró el consumo de substrato por HPLC y producción de metabolitos etanol, ácido láctico y ácido acético por cromatografía de gases.

Se logró identificar cuatro diferentes *fila* en los búlgaros crecidos en leche y en lactosa, Bacteroidetes, Firmicutes, Proteobacteria y Fusobacterias y tres en los tibicos crecidos en piloncillos y sacarosa, Firmicutes, Proteobacteria y Bacteroidetes. Siendo Bacteroidetes el *filum* dominante en el caso de los búlgaros y Firmicutes en el caso de los tibicos. Los resultados del secuenciamiento muestran también una alta diversidad en la composición microbiana en los cuatro tratamientos siendo *Prevotella* el género dominante en los búlgaros crecidos en leche y lactosa, *Lactobacillus* en tibicos crecidos en piloncillo y *Clostridium* en los tibicos crecidos en sacarosa. Para el caso de los metabolitos, los estudios caracterizan el consumo del substrato y la producción de los metabolitos como se espera para una comunidad compleja. Financiamiento Cinvestav y CONACyT 163235 INFR-2011-01 para JGM.

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*Author for all correspondence

CINÉTICAS DE CRECIMIENTO DE MICROORGANISMOS DEGRADADORES DE COMPUESTOS ORGÁNICOS VOLÁTILES (COV'S) EN FASE DE ADAPTACIÓN

Gladis Rodríguez-H. (1); Eloy Conde-Barajas (2); Ma. Guadalupe Olvera-Torres (1); Jorge L. Corona-Carrillo (1); José L. Hernández-López* (1).

(1) Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Grupo de Bioelectroquímica y Nanotecnología, Querétaro, Qro., México.
(2) Instituto Tecnológico de Celaya, Departamento de Ingeniería Ambiental, Celaya, Guanajuato, México.

Los compuestos orgánicos volátiles (COV's) son hidrocarburos los cuales tienen presiones de vapor por arriba de 0.1 mm Hg en condiciones estándar (20 °C y 760 mm Hg). Estas moléculas tienen como efectos negativos el agotamiento del ozono estratosférico, la potenciación del efecto invernadero global, la acumulación y persistencia en el ambiente y la formación fotoquímica de ozono troposférico. Hoy en día existe variedad de tecnologías térmicas, fisicoquímicas y biológicas que ayudan a la mitigación de la contaminación. El uso de tratamientos biológicos ha logrado que los expertos pongan más atención en estas tecnologías por sus altas eficiencias de remoción y su bajo costo. Para lograr de manera exitosa este tipo de tratamientos es necesario estudiar la adaptación de los microorganismos expuestos a los contaminantes de interés y para ello se requieren llevar a cabo mediciones de cinéticas de crecimiento que indiquen si el tratamiento es favorable o no desde el punto de vista biológico. En este estudio se realizó la adaptación de un hongo tipo levadura para lograr remover contaminantes orgánicos volátiles, utilizando el thinner como contaminante modelo por contener moléculas como el tolueno, etanol, isopropanol, 2-butoxi 1-etanol, acetona, isobutanol y xilenos.

Se realizaron inoculaciones del microorganismo de estudio en un sistema conformado por thinner y medio mineral, el cual carece de carbono en su composición. Así, el thinner, es la única fuente de carbono que tiene la levadura como sustrato. Estos experimentos tuvieron una duración de 7 días a una temperatura de 24°C, presentando un comportamiento típico de una cinética microbiana. Las cinéticas se realizaron tomando 100 µL de muestra del sistema en 900 µL de solución de NaCl 0.85%, ejecutando diluciones de 10^1 hasta 10^9 para determinar las células sobre mililitro (Cél/mL) durante los 7 días de experimentación

Mediante la CG-MS se llevó a cabo el monitoreo del contaminante presentando al día 3 un 99% de remoción de Tolueno, mientras que xileos etanol, isopropanol, 2-butoxi 1-etanol, acetona, isobutanol feron removidos en 100%. Por lo tanto el hongo de tipo Levadura, resulta ser una especie microbiana adecuada para la degradación de COV's.

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*Autor de correspondencia.

ANÁLISIS DE RESPIROMETRÍA EN LA PRODUCCION DE CONIDIOS DE *Isaria fumosorosea* EN CULTIVO EN SOPORTE SÓLIDO

Alejandro Angel-Cuapio (1); Ernesto Favela-Torres (1); Isabelle Perraud-Gaime *(2);* Octavio Loera*(1).

(1) UAM-Iztapalapa, Departamento de Biotecnología, México DF, México;
(2) Aix Marseille Université, Marseille, France

Los micoinsecticidas basados en conidios de hongos entomopatógenos (HE) son alternativas amigables con el ambiente y su uso mitiga la contaminación por plaguicidas químicos, para controlar o reducir los insectos plaga en el sector agrícola, además son inocuos para el ser humano y otros mamíferos. La produccción de conidios de HE a gran escala se realiza mediante el cultivo en soporte sólido (CSS). Por otro lado, el lirio acuático es una plaga en los cuerpos de agua y conviene tener usos para su aprovechamiento. El objetivo de este trabajo fue evaluar el uso de lirio acuático (LA) como texturizante en CSS con arroz (A) como sustrato principal, en la producción de conidios usando la cepa de *Isaria fumosorosea* ARSEF3302. Se utilizaron 10 gramos de materia sólida inicial (gmsi), en mezclas de A-LA (%) en diferentes proporciones (100-0, 90-10, 80-20 y 70-30), se inoculó con $1x10^6$ conidios por gmsi manteniendo en 40% la humedad inicial (a 28ºC por 8 días). Se determinó la porosidad (%) en cada mezcla de A-LA, estimando el espacio vacío con aceite mineral. El crecimiento se monitoreó a través de respirometría (cuantificación del CO_2). La viabilidad (%) de los conidios se calculó por la formación de unidades formadoras de colonias (UFC).

Se encontró un incremento hasta del 50% en la producción de conidios al aumentar la porosidad del medio de cultivo, con un valor óptimo de porosidad (entre 34 y 36%), con la máxima producción de conidios ($6x10^9$ con $gmsi^{-1}$) o bien, expresado por gramo de arroz seco inicial ($7x10^9$ con $gasi^{-1}$). Por los resultados de respirometría, la cepa ARSEF3302 presentó un tiempo lag (T_{lag}) variable entre 43 y 52 h, y una tasa específica de CO_2 ($\square CO_2$) promedio de 0.07 h^{-1}. Hubo un consumo más rápido del sustrato en la mezcla A-LA 90-10, con una tasa de producción de CO_2 (VCO_2) de 1.4 mg $CO_2 \cdot gasi^{-1} d^{-1}$ y una producción de CO_2 acumulada (PCO_2) de 160 mg $CO_2 \cdot gasi^{-1}$, esto fue más lento en la mezcla A-LA 70-30 (VCO_2 =0.5 mg $CO_2 \cdot gasi^{-1} d^{-1}$ y PCO_2 =50 mg $CO_2 \cdot gasi^{-1}$). Además, no se alteró la calidad de los conidios en ningún tratamiento, en todos los casos se obtuvo una viabilidad mayor del 80 %, que es deseable en lotes que se aplican en el campo.

Los resultados revelan que el lirio acuático funciona como texturizante en cultivos en soporte sólido, incrementando la producción de conidios de *I. fumosorosea* ARSEF3302, sin alterar la viabilidad, lo cual es un factor importante de considerar en la producción a gran escala.

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*Autor de correspondencia.

ISOLATION AND SCREENING OF SURFACTANT-PRODUCING BACTERIA AND HYDROCARBONOCLASTIC BACTERIA FROM CHRONICALLY PETROLEUM CONTAMINATED SOILS

Gilberto Morales-Guzmán* (1); Alejandro Alarcón (1); Luis G. Torres-Bustillos (2), María R. Mendoza-López (2); María del C. Rivera-Cruz (1); Ronald Ferrera-Cerrato (1) (3); Ramón Arteaga-Garibay (4)

(1), (2), (4) Colegio de Postgraduados. Montecillo, Estado de México
(2) UPIBI-Instituto Politécnico Nacional, Av Acueducto s/n Colonia Barrio la Laguna Ticoman
(3) Colegio de Postgraduados, Campus Tabasco. Periférico Carlos A. Molina s/n km 3.5. H. Cárdenas, Tabasco, México.
(4) Colección de Microorganismos del Centro Nacional de Recursos Genéticos, Centro Nacional de Recursos Genéticos INIFAP. Tepatitlán de Morelos, Jalisco, México.

Biosurfactants are organic compounds with high surface activity that are produced by several microorganisms, and this production is a desirable property for microorganisms able to degrade petroleum hydrocarbons (hydrocarbonoclastic). The aim of this research was to isolate surfactant-producing bacteria from chronically petroleum-contaminated soils collected at Huimanguillo and Cardenas, Tabasco (Mexico).

Soil samples were characterized as control (150 mg weathered TPH kg^{-1}), moderately contaminated (50 000 mg kg^{-1}), and highly contaminated (150 000 mg kg^{-1}). Bacteria were isolated by using a mineral solid medium enriched with diesel as a single carbon source, and selected on the basis of colonial morphology and appearance. Each bacterial strain was cultivated in flasks with 30 mL of liquid medium during 10 days at 28 ºC and 200 rpm. From these bacterial cultures an aliquot was taken to inoculate test tubes with 3 mL of liquid medium enriched with diesel (2 mL). Bacterial growth was allowed until an emulsion was observed and compared to that obtained with a Tween 80 control.

Regardless soil contamination, 25 bacterial strains were selected, and only 12 of them showed positive activity as emulsifiers. The strains C5S3, C7S3 and LPS1 showed the highest emulsifying activity, which were isolated from either highly contaminated or control soil. These bacterial strains were Gram-negative cocci and diplococci. Our results demonstrated the presence of diesel-emulsifying bacteria from chronically contaminated soils, and may potentially have hydrocarbonoclastic activity.

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*Author for all correspondence.

FORESTRY ECOSYSTEMS: A SOURCE FOR ISOLATING BACTERIAL STRAINS FOR SOIL BIOREMEDIATION PURPOSES*

Vivian F. Quiroz-Sarmiento, Herminia A. Hernández-Ortega, Juan J. Almaraz-Suarez, Ronald Ferrera-Cerrato*, Alejandro Alarcón

Área de Microbiología Colegio de Postgraduados Campus Montecillo. Carretera México-Texcoco Km 36.5, Montecillo, Edo de México. C.P. 56230. Montecillo, Estado de México.

Bioremediation of petroleum hydrocarbons is unfortunately needed for detoxifying contaminated soils, by using microorganisms isolated from several microbial niches such as soil or rhizosphere soil. The goal of this study consisted on isolating hydrocarbonoclastic bacteria from a forest ecosystem.

Sixteen rhizosphere soil samples taken from vegetated areas dominated by *Pinus*, *Cupressus*, *Abies religiosa*, or alpine prairie at the Tlaloc mountain, Texcoco, Estado de Mexico. Bacteria were isolated by utilizing nutrient agar (total bacteria –TB-), Rennie medium (N-fixing free living bacteria –NFB-), modified Rennie medium enriched with either diesel (N-fixing free living hydrocarbonoclastic bacteria -NFHB-) or diesel plus nitrogen (N-assimilating hydrocarbonoclastic bacteria -NAHB). After isolation, bacteria were tested for their tolerance to diesel or crude oil applied in liquid culture medium. The most tolerant bacteria were macroscopically (shape, elevation, edge, and texture) and microscopically (gram staining) characterized, and molecularly identified (PCR amplification of the 16s rDNA gen).

The highest number of bacterial colony forming units was achieved in samples collected from *Abies religiosa* vegetation. Regardless the dominant vegetation, a total of 28 hydrocarbonoclastic bacteria were obtained. By performing the tolerance test against diesel or crude oil, four bacterial strains were selected for further evaluation of their growth kinetics under phenanthrene-contaminated liquid medium during seven days. The strains identified as *Pseudomonas* sp. and *Ralstonia* sp., were the most tolerant to this contaminant. Forestry ecosystems represent an important source of microbial genetic resources to isolate and select hydrocarbonoclastic bacteria for further bioremediation purposes.

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*Author for all correspondence.

EVOLUTION OF MICROBIAL COMMUNITIES IN SOILS TREATED WITH PENDIMETHALIN

Paloma Pinilla; Pilar García-Gonzalo; Mª Carmen Lobo*

IMIDRA. Finca "El Encin" A-2, Km 38,2 28800 Alcalá de Henares (Madrid), Spain.

The use of pesticides in agriculture is a common practice that can produce adverse effect on soil and groundwater quality depending compound characteristics and its behavior in the soil. Pesticides can be adsorbed by the soil colloidal particles which could lead to inactivation of the product and can move through the soil with the risk of groundwater pollution. Pesticides can also suffer different chemical transformations in the soil inducing the degradation of the product or its transformation into other compounds than can be sometimes more persistent and more toxic than the original product.

Pendimethalin (N-(1-ethylpropil)-2, 6-dinitro-3, 4-xylidine) is a herbicide often used for pre-emergence or early post-emergence control of weeds in cotton, wheat, maize, tomato and onion crops. Belong to group of dinitroanilide herbicides. dinitroanilide is sensitive to photodegradation and relatively volatile. Due to its hydrophobic nature, the persistence of this herbicide in soil represents an environmental hazard.

The main objective of this study was to evaluate the effect of the pendimethalin application on microbial communities in an agricultural soil. The soil comes from a plant nursery. Soil samples were taken from a pine plot (*Pynus pinea*) treated with pendimethalin. Samples were taken before application of herbicide and 9, 44 and 81 days after application.

For each sample cultivable bacterial counts and DNA extraction was conducted in triplicate. The universal bacterial primers, targeting 16s rDNA V3-V5 variable region, 341F-GC and 907R were used. For DGGE analysis, PCR products generated from each sample were separated on 6% polyacrylamide gels with a denaturing gradient ranging from 30 % to 70%. Gels were stained with SYBR Gold and gel images were analyzed using GeneTools software. Bacterial diversity was compared through Richness and Shannon-Weaver indices and cluster analysis was conducted to determinate any differences in the microbial community over time. Bacterial communities structure in the pendimethalin and untreated soils are discussed.

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*Author for all correspondence.

AISLAMIENTO Y CARACTERIZACIÓN DE BACTERIAS METALORREDUCTORAS PROVENIENTES DEL JALE MINERO "EL FRAILE", GUERRERO

Emmanuel Alvizo* (1), Enrique Morett (1), Katy Juárez (1)

(1) IBT-UNAM, Cuernavaca, Morelos.

La minería es fundamental para la obtención de materias primas además representa un aporte fuerte a la economía de diversos países, sin embargo la actividad minera y sus desechos tóxicos traen consigo fuertes problemas ambientales. En México es posible encontrar gran cantidad de sitios contaminados debido a la actividad minera, el municipio de Taxco en el estado de Guerrero, ha sido considerado como uno de los distritos mineros más importantes de México debido a la extracción de metales como la plata. Actualmente existen residuos producto de la actividad minera (Jales) que se generaron y se confinaron inadecuadamente hace muchos años. Estos residuos, generan factores de riesgo para el ambiente y para diversos organismos incluyendo al humano, por ello es importante llevar acabo la adecuada deposición de estos contaminantes. La biorremediación es una alternativa viable que puede ser empleada para remover contaminantes específicos del suelo mediante el uso de microorganismos. Actualmente ha surgido el interés de aislar bacterias capaces de oxidar y reducir elementos tóxicos presentes en los residuos mineros como los metales pesados. En este trabajo se aislaron dos cepas bacterianas provenientes de los jales mineros de "El Fraile" que debido a la exposición a metales pesados por tiempos muy largos, y a su diversidad metabólica tienen las características necesarias que les permiten desarrollarse y reducir altas concentraciones de metales pesados, para el aislamiento de estas cepas bacterianas se utilizaron distintos medios de cultivo adicionados con diferentes concentraciones de metales pesados así como distintos donadores de electrones, la identificación se llevó a cabo por medio de PCR amplificando el gen que codifica para el ARNr 16S, además de esto se realizaron cinéticas de reducción para distintos metales por medio de métodos colorimétricos

Una de las cepas bacterianas aisladas presenta una alta identidad con *Shewanella putrefaciens* y presenta la capacidad de transformar los óxidos de fierro insolubles a magnetita. La otra cepa bacteriana aislada está relacionada filogenéticamente con *Klebsiella oxitoca,* la cual tiene la capacidad de reducir tanto Fe(III) a Fe(II) así como Cr(VI) a Cr(III) y la propiedad de formar un hidrogel en un medio de cultivo en el que emplea citrato férrico como aceptor de electrones y lactato como donador de electrones, las características de ambas cepas bacterianas tienen gran potencial en el uso biotecnológico así como en la biorremediación de sitios contaminados por metales pesados como los jales mineros.

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*Author for all correspondence.

AISLAMIENTO Y ANÁLISIS CUALITATIVO DE BIOMASA MICROBIANA FÚNGICA DEGRADADORA DE HIDROCARBUROS NATIVA DE UN CENOTE DE QUINTANA ROO

Manuel A. Lizardi-Jiménez (1); Ricardo Gómez-Reyes (2);
Angélica Jiménez-González (1); Sergio A. Medina-Moreno (1)

(1)Universidad Politécnica de Pachuca, Ingeniería en Biotecnología, Pachuca, México.
(2)Universidad Politécnica de Quintana Roo, Ingeniería en biotecnología, Cancún, Quintana Roo

En trabajos previos se describió una correlación entre las temporadas de turismo y el aumento de contaminantes hidrocarbonados en los cenotes contaminados del Estado de Quintana Roo, debido posiblemente al afluente del parque vehicular durante las temporadas de turismo alto y las fugas de combustible de los transportes terrestres y el desgaste de las vías públicas de transito que ocasionan un reencarpetamiento permanente durante los posteriores meses a las temporadas altas. Se aisló *ex situ* en biorreactores *airlift*, la biomasa microbiana de un cenote contaminado por Fenantreno y Naftaleno, ubicado en Cancún, Quintana Roo, coordenadas: 21º9'41"N - 86º52'23"W. La adición de diésel demostró la capacidad hidrocarbonoclasta del consorcio. En un estudio previo se logró identificar distintas especies de bacterias como: *Pseudomonas, Vibrio, Diplococcus* y *Enterobacter.* Sin embargo, no se lograron reunir resultados sobre hongos. Los objetivos de este trabajo se enfocaron en el aislamiento de la biomasa fúngica del consorcio y la aproximación de una breve explicación cualitativa de los mecanismos de consumo de hidrocarburo.

Inicialmente se añadió 13gL^{-1} de diésel a Biorreactores *airlift* de 1L con medio mineral+cloranfenicol y la biomasa fúngica, cultivada previamente en medios PDA y DSA. Se midió la absorbancia a 403nm demostrando los valores, una tendencia logarítmica con un R^2=0.8 durante los primeros cuatro días. La dinámica de crecimiento microbiano se analizó utilizando tres modelos: *Gompertz*, de *Von Bertalanffy* y logístico; los puntos obtenidas por simulación presentan coherencia con los puntos obtenidas experimentalmente. En las curvas de crecimiento pueden observarse la fase de crecimiento y fase estacionaria de microorganismos. Para todos los casos, *Von Bertalanffy* fue el modelo que se ajustó mejor a los valores reales de con $\sigma \leq 0.4$ promedio.

Las pruebas de placa – medio mineral recubiertas con diésel y, los análisis microscópicos permitieron resaltar que dicha biomasa microbiana fúngica produce proteínas de bajo peso molecular denominadas hidrofobinas para el consumo de los crudos usados. El crecimiento microbiológico fúngico demostró que el consorcio es un cultivo mixto – aún no definido- Un breve análisis a la morfología de la biomasa microbiana fúngica propone que el consorcio está constituido al menos por hongos filamentosos del genero *Cladosporium*.

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*Author for all correspondence.

HIGH CH_4 CONSUMPTION RATES BY A METHANOTROPH NEW CLASS, *Sphingobacterium* sp. CZ-UAM; KINETIC CHARACTERIZATION USING COPPER AND NH_4

Hugo S. Morales (1), N. Cristal Zúñiga Peña (1), Marcia G. Morales Ibarría (1), Sergio Revah M. (1)

(1) Universidad Autónoma Metropolitana-Cuajimalpa. Av. Vasco de Quiroga 4871, Cuajimalpa, Santa Fe, CP 05348 Ciudad de México, D.F.

Methane (CH_4) is the second most important greenhouse gas after CO_2, with a global warming potential between 25-72. When CH_4 is below of the low explosive limit (5% in air) biological treatments are the best option to degrade it. The main drawback of CH_4 elimination is the low solubility (Henry coefficient = 30).

The objective was to isolate and characterize a methanotroph with high CH_4 consumption rates, the methanotrophic activity was stimulated by Cu and NH_4 addition; morphological studies were done using different microscopy techniques.

The microorganism *Sphingobacterium* sp. CZ-UAM was isolated and identified by molecular techniques. It was deposited at the Collection of Microbial Strains in CINVESTAV (CDBB-B-1879); the sequence size was 1400 pb and it was submitted to the GenBank database (KJ411920). The microcosms assays were done in serological bottles of 125 mL with 10% of CH_4 in the headspace; copper concentrations were 10, 25, 50, 75 and 100 µg L^{-1}. For ammonia experiments concentrations of 50, 250, 500, 750 and 1000 ppm were assayed. A Multi-Gas Detector Dräger was used to determine ammonia concentration; the CH_4 and CO_2 were quantified by GC-TCD. The microscopy studies included transmission electron (TEM), scanning electron (SEM) and red-nile epi-fluorescence microscopy to detect sphingolipids-like compounds.

In the copper assays, the best specific degradation rate was obtained at copper content of 50 µg L^{-1} reaching values of 167 $mg_{CH4}g_x^{-1}h^{-1}$, very high specific methanotrophic activities between 283 and 325 $mg_{CH4}g_x^{-1}h^{-1}$ were observed at NH_4^+ concentrations between 250 and 750 ppm; under these conditions the average CO_2 yield was about 0.3.

TEM images shown a halo surrounding the cell wall of *Sphingobacterium* sp. CZ-UAM, SEM pictures shown cells size 3 or 4 times lower than other methanotrophs. Epi-fluorescence was positive with orange-red fluorescent colors, giving evidence of sphingolipids-like compounds; TEM images also support their presence around the cell.

Sphingobacterium sp. has CH_4 consumption rates four times higher than values reported for any methanotroph. This is the first report of CH_4 consumption at different copper concentration and NH_4^+ presence by this bacterium. Never before has been reported the presence of own surfactants in a methanotrophic organism.

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*Author for all correspondence.

EVALUACIÓN DEL CONSORCIO LB5 ANTE INCREMENTOS DE LA CONCENTRACIÓN DE PERCLOROETILENO

Luz Bretón-Deval (1); Elvira Rios-Leal(1); Juvencio Galíndez-Mayer (2); Simona Rossetti (3); Héctor M. Poggi-Varaldo* (1)

(1) CINVESTAV- IPN, México DF, México;
(2) ENCB-IPN, México DF, México ;
(3) IRSA-CNR, Roma, Italia.

La biodegradación del PCE se lleva a cabo en su mayoría de forma anaerobia mediante un proceso conocido como deshalogenación reductiva en la cual un cloro se va remplazando por un hidrógeno, generándose productos menos clorados como el TCE, DCE y CV. En este proceso el PCE sirve como aceptor de electrones durante la oxidación de un compuesto rico energéticamente como el hidrógeno o un sustrato orgánico. La deshalogenación también puede llevarse a cabo mediante cometabolismo, catalizada por coenzimas reductoras. Investigaciones sugieren que concentraciones superiores a 80 mg/L de PCE, resultan tóxicas a los consorcios utilizados en biorremediación. Durante el proceso de biorremediación los consorcios pueden ser sometidos a concentraciones fluctuantes y/o altas de PCE.

El objetivo de esta investigación fue evaluar al consorcio deshalogenante residente en biorreactores anaerobios de lecho fluidizado mediante técnicas moleculares y fisicoquímicas a lo largo de incrementos sustanciales de la concentración de PCE. Se implementaron cuatro reactores de lecho fluidizado, dos de ellos tenían un filtro con limaduras de hierro metálico (ZVI). Todos los biorreactores fueron alimentados con concentraciones crecientes de PCE (80, 165, 278, 393 mg/L). Se utilizó un consorcio metanogénico alimentado a 1 g DQO/L.

El consorcio deshalogenante presentó una alta remoción de contaminante aunque conforme se incrementó la concentración de PCE, algunas actividades metabólicas del consocio se vieron afectadas negativamente. Se observó un incremento significativo del tiempo de aclimatación del consorcio a cada incremento de concentración de PCE en la alimentación a los reactores. Después de la presión selectiva de las altas concentraciones de PCE resultó en un consorcio (denominado consorcio deshalogenante LB5) rico en bacterias deshalogenantes con los siguientes géneros: *Dehalobacter spp., Desulfurospirillum spp., Desulfitobacterium spp.,* and *Dehalococcoides spp.* y *Methanosarcina spp.* El filtro de ZVI fue otro factor selectivo, los reactores que tenían filtro mostraron el doble de *Dehalococcoides,* probablemente debido al incremento en la liberación de hidrógeno. La evaluación de la tasa específica para el gen 16S DHC mostró un incremento directamente proporcional a la concentración creciente de PCE, lo que sugiere que las enzimas deshalogenantes pudieran ser estimuladas y/o inducidas con los compuestos clorados. Se concluye que situaciones de stress pueden especializar a un consorcio dirigiendo su maquinaria metabólica en orden de mejorar el proceso de biorremediación.

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*Author for all correspondence.

MICROBIAL COMMUNITY ANALYSIS OF A FULL SCALE WINERY WASTEWATER TREATMENT SYSTEM

Agustina Ziliani (1); Guadalupe Paolino (2); Gustavo Ochoa (2); ***Angela Cabezas**** (1)

(1) Biotechnology Department, Universidad ORT Uruguay, Montevideo, Uruguay;
(2) Bodega Juanicó, Montevideo, Uruguay;

Wine industry is a major global industry with 252 million hectolitres produced in 2012. Winery wastewater can originate at various stages during the wine production process as well as from bottling and cleaning of barrels, fermentation tanks and additional equipment as well as during maintenance of cellar hygiene. Biological wastewater treatment of industrial wastewaters represent an ecofriendly approach to meet increasing regulation constrains for disposal of industrial wastes. These processes rely on the interaction of diverse, complex, and highly dynamic microbial communities. Understanding the factors which determine community assembly will greatly inform our understanding of process stability and aid in the maintenance and adaptation to new conditions. Particularly, winery wastewater present some peculiarities which might difficult their biological treatment such as large seasonal fluctuations in volume and composition and high variability of organic loads, which typically assume concentrations of 1,000–20,000 mgCOD/L, depending on the harvest load and processing activities. To our knowledge very few attempts have been made to understand the microbiology involved in these systems.

Here we studied the microbial community of two treatment units of a full scale winery wastewater treatment system: an activated sludge reactor (LEA) and a facultative lagoon (LAP). Samples were taken periodically including vintage and nonvintage periods in which the COD varied from 250 mg/L to 25176 mg/L. DNA was extracted and the microbial community was studied using T-RFLP and 454-pyrosequencing.

Different microbial communities were detected in each unit and only one T-RF was present in all samples analyzed. In the activated sludge, a clear change in the microbial community was detected in samples taken after the harvest start. A less diverse community was developed during the vintage period probably due to changes in the effluent concentration and composition. The microbial community in the facultative lagoon was highly variable and also changed after the harvest start. Pyrosequencing data showed the predominance of the phylum *Chloroflexi* (48%), *Proteobacteria* (14%) and *Bacteroidetes* (11%) in the LEA. *Chloroflexi* members might be related to floc formation in activated sludge reactors, however, an excess of these filamentous bacteria might also cause sedimentation problems. Even though *Chloroflexi* was also the predominant phylum (23,5%) in the LAP, a predominance of *Planctomycetes* (22%), *Proteobacteria* (10%) and *Verrucomicrobia* (10%) were also detected.

In conclusion, microbial communities changed when the wine harvest started and controlling *Chloroflexi* populations might improve sedimentation in the activated sludge.

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*Author for all correspondence

ISOLATION AND CHARACTERIZATION OF URUGUAYAN NATIVE STRAINS OF *Bacillus thuringiensis*

Alfonso García-Pintos (1); Natalia Scioscia (1); Carlos Sanguinetti (1); Lorena Betancor (1); ***Angela Cabezas**** (1)

(1) Biotechnology Department, Universidad ORT Uruguay, Montevideo, Uruguay;

In the last decade the agricultural sector has experienced an exponential growth in Uruguay with a concomitant increase in the use of chemical insecticides for plague control. The irrational use of insecticides might cause serious environmental and health problems. The need for environmentally friendly alternatives for plague control has therefore become imperative. *Bacillus thuringiensis* (Bt) is one of the most relevant bacteria used as an alternative to chemical insecticides, specially due to their capacity to produce entomopathogenic crystal proteins (CRY). However, the identification of *B. thuringiensis* using the 16S rRNA gene is not possible due to high sequence similarity with other *Bacillus* species within the *Bacillus cereus sensu lato* group.

In the present work we developed a rapid screening method for the identification of *B. thuringiensis* based on the PCR amplification with three primer sets described previously (Noguera and Ibarra, 2010) which detect 100% of the described Cry genes. Positive strains were characterized using REP-PCR, cry gene content, 16S rRNA gene sequence and soluble protein profiles.

Colony PCR was performed for 51 isolated strains with the three primer sets. 8 strains amplified with at least one of the primer sets. After 16S rRNA gene sequence, 3 of the strains were discarded as members of the *B. cereus sensu lato* group indicating false positive results when using primer set 3. REP PCR was performed for five strains and two positive controls, *B. thuringiensis serovar israelensis* (Bti) and *B. thuringiensis serovar kurstaki* (Btk). Three different DNA fingerprints were obtained for the isolated strains. Cry genes 1, 2, 4, 9 and 11 were not present in the native isolates so the presence of other Cry genes is being tested. Analysis of soluble protein fraction demonstrated unique patterns for the isolates where two of them shared soluble protein profile that differed from the Bti and Btk controls. Major bands and profile of the controls was correspondent to previously reported data (Porcar, 2013). Our results demonstrated that protein profile changed before and after sporulation. Phase contrast microscope analysis showed that two of the native strains contained spherical protein crystals.

In conclusion, we were able to identify 4 strains as *B. thuringiensis* with a fast screening method with three different REP-PCR profiles.

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*Author for all correspondence

CARACTERIZACIÓN DE MICROORGANISMOS ASOCIADOS AL DETERIORO DE LOS MURALES DEL PALACIO DE ATETELCO, TEOTIHUACAN

Yunuen Martínez-Calixto (1), Cesar H. Hernández Rodríguez (1), Roxana Olvera Ramírez* (1)

(1) ENCB-IPN, México DF, México;

El biodeterioro de la propiedad cultural es el daño físico o químico efectuado principalmente por microorganismos en monumentos o edificios que pertenecen al patrimonio cultural. Frecuentemente se les observa formando comunidades o biopelículas de bacterias, algas, hongos y líquenes dentro y sobre del sustrato. En Teotihuacán, los murales están asociados a los diferentes elementos arquitectónicos están constituidos por tezontle, cal, hematita, azurita, malaquita y negro carbón. Considerando las características físicas y químicas de este ambiente creado por el hombre, se espera que la comunidad microbiana de estos murales esté conformada básicamente por organismos quimiolitotróficos-autótrofos, fotoautótrofos y/o fijadores de nitrógeno, heterótrofos fijadores de nitrógeno y heterótrofos.

El objetivo principal de este trabajo fue determinar los microorganismos causantes del biodeterioro de los murales del Palacio de Atetelco, Teotihuacan. El muestreo se realizó en dos temporadas: en la de lluvias y en la de sequía. Tomando cinco muestras del mural “Biznaga” en cada temporada, tres muestras del Patio Rojo en la temporada de secas y dos extras en la temporada de lluvias, con y sin signos evidentes de biodeterioro con ayuda de un hisopo húmedo estéril. De cada muestra se realizaron diluciones seriadas 10^{-1} a 10^{-3} tomando 100 µL para inocular por dispersión en diferentes medios de cultivo. Se cuantificaron las unidades formadoras de colonias (UFC) en cada medio sólido. Se obtuvieron aislados con base a sus diferencias morfológicas y se incubaron a 30°C en un intervalo de 3 a 5 días. Se hizo la caracterización microscópica. La extracción del DNA se realizó empleando la técnica propuesta por Hoffman y Winston en 1987. La identificación de los aislamientos se realizó por el análisis filogenético y de similitud del gen 16S rRNA y 18S rRNA.

Se encontró que la comunidad microbiana presente está formada por los siguientes gremios: fotoautótrofos (63% de cianobacterias y 37% de microalgas) y heterótrofos (29% de gram negativos, 18% de levaduras y 11% de gram positivos). Con base en los resultados el biofilm colectado en la temporada de sequía se encuentra en un biodeterioro avanzado de los 24 morfotipos aislados 50% corresponde a levaduras, 33% a Gram positivas y 17% a Gram negativos. Mientras que en la temporada de lluvias de los 77 aislados el 56% corresponde a microorganismos fotoautótrofos, 30% de Gram negativas, 5% de Gram positivas y 9% de levaduras.

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*Autor de correspondencia

ACTIVIDAD ANTIMICROBIANA DE EXTRACTOS METANÓLICOS DE *Leptolyngbya cebennensis*

Raúl Alcalde-Vázquez (1); Nora B. Medina-Jaritz (1);
Margarita Pineda-López (2); Roxana Olvera-Ramírez* (1)

(1) ENCB-IPN. Laboratorio de Fisiología Vegetal. México DF, México.
(2) ENCB-IPN. Laboratorio de Genética Microbiana. México DF, México.

Los productos naturales microbianos son el origen de la mayoría de los antibióticos en el mercado de hoy en día. Sin embargo, el uso indiscriminado de los antibióticos ha provocado que diversos microorganismos patógenos sean resistentes a éstos, limitando su eficiencia. Es por ello que la investigación se está enfocando hacia fuentes alternativas que no sean tóxicas para los seres humanos: los antimicrobianos naturales, que indudablemente tienen un papel importante en la protección contra infecciones. Esta nueva dirección en la investigación ha sido el tema de muchos estudios del efecto antimicrobiano de varios organismos incluyendo a las cianobacterias dado que han demostrado ser productores de compuestos activamente biológicos (algunos tóxicos), con aplicaciones potenciales en biomedicina, y en salud ambiental.

El objetivo del presente trabajo fue evaluar la actividad antimicrobiana de extractos metanólicos de la cianobacteria *Leptolyngbya cebennensis* sobre cinco cepas microbianas: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Salmonella typhi* ATCC 6534, *Escherichia coli* ATCC 10536 y *Pseudomonas eruginosa* ATCC 9027.

El extracto metanólico se obtuvo a partir de 2.0 g de biomasa de *L. cebennensis* cultivada en medio BG11. La extracción consistió en añadir 60mL de metanol y 2mL de ácido acético por cada gramo de biomasa; se dejó 24h en reposo y se evaporó a sequedad. El residuo seco se resuspendió en 3mL de metanol. Con el extracto se impregnaron discos de papel filtro de 6cm de diámetro y se pusieron en contacto con cada cepa sembrada en medio Müller Hinton por la técnica de dispersión en superficie; se incluyeron también dos controles en discos separados: uno con metanol y el otro con medio BG11. Después de 24h de incubación a 37°C se midieron los halos de inhibición formados alrededor de los discos.

El efecto antimicrobiano del extracto metanólico se observó significativamente en todas las cepas probadas en el ensayo siendo *B. subtilis* la más sensible y *S. aureus* la más resistente. En ninguno de los controles se observo inhibición del crecimiento de las cepas por lo que dicho efecto se debe únicamente al(os) metabolito(s) de *L. cebennensis*. Los resultados de éste trabajo pueden ser la base para posteriores estudios en los que se busque purificar y caracterizar el metabolito responsable de la actividad mostrada, para proponer terapias alternativas en el tratamiento de algunas infecciones.

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*Autor de toda la correspondencia.

DETECCIÓN DE BACTERIAS BENÉFICAS (FOTOSINTÉTICAS) EN LA FRACCIÓN LÍQUIDA DE BORDO PONIENTE ETAPA IV

María T. Núñez-Cardona* (1); María E. Gutiérrez-Castillo (2); Luis R. Tovar Gálvez (3)

(1) UAM-XOCHIMILCO México DF, México;
(2) CIIEMAD-IPN, México DF, México;
(3) CIIEMAD-IPN, México DF, México.

El estudio de las bacterias fotosintéticas se ha realizado desde finales del siglo XVIII, especialmente de aquéllas que habitan en ambientes acuáticos. Estos microorganismos son anaerobios y anoxigénicos y para realizar la fotosíntesis cuentan con bacterioclorofilas (a,b,c,d,e y g) además de pigmentos accesorios, han sido incluidas dentro de los microorganismos benéficos por la gran cantidad de compuestos que se pueden obtener a partir de éstas, además de que no se conocen especies patógenas.

El objetivo del presente trabajo fue detectar, aislar y cultivar bacterias fotótrofas a partir de muestras de la fracción líquida (lixiviados) resultante de la degradación de los residuos orgánicos del Bordo Poniente (Etapa IV) y que son colectados en cárcamos. Las muestras de la fracción líquida colectadas se utilizaron para inocular frascos de vidrio conteniendo medio de cultivo líquido cuya composición es la siguiente: 1000 mL agua destilada, 5.0g KH_2PO_4; 3.0g $MgSO4{\bullet}7H_2O$, 0.4 g NH_4Cl, 0.05 g $CaCl_2{\bullet}2H_2O$; 0.4 g de NaCl, 1.0 succinato; vitamina B_{12} (2.0 mg/100 mL agua destilada). 1.0 $FeCl_3$ (1.6 g/1000 ml distilled water) y 1.0 ml solución de van Niel. Los cultivos líquidos fueron incubados con luz incandescente (1800 lux), a temperatura ambiente y condiciones anaerobias. Para el análisis de los pigmentos fotosintéticos *in vivo* se utilizó glicerol y también fueron extraídos con metanol para analizarlos por espectrofotometría (luz ultravioleta).

Los análisis *in vivo* revelaron la presencia de bacterioclorofila *a* por los máximos de absorción: 375-379, 588-589, 802-804, 856-858 nm y de carotenos: 456, 494-496 y 594 nm. Los pigmentos extraídos en metanol mostraron máximos de absorción a 364 y 771 nm que corresponden a la bacterioclorofila *a*; los máximos: 428, 452, 467, 473, 461-468 nm, son característicos de los carotenos. La presencia de bacterioclorofila *a* en los cultivos líquidos pone en evidencia que en la fracción líquida de los cárcamos del Bordo Poniente crecen microorganismo benéficos como las bacterias fotosintéticas, las cuales pueden ser utilizadas en procesos biotecnológicos como son la producción de herbicidas, hormonas y factores de crecimiento para plantas (Ej. ácidos indolacético y aminolevulínico) así como otros compuestos que son utilizados para la salud humana (Ej. vitamina B_{12}, antibióticos, antioxidantes) y del ambiente (Ej. biorremediación del agua).

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*Author for all correspondence

DETERMINACIÓN DE LA DIVERSIDAD FUNCIONAL DE FITOBACTERIAS AISLADAS DE PLANTAS DE *Lemna gibba* Y SU POTENCIAL ECOLÓGICO EN EL ECOSISTEMA DE XOCHIMILCO

Orlando Ortega-Acosta; Aída V. Rodríguez-Tovar; Eugenia López-López; Angélica Rodríguez-Dorantes*

Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México, D.F., México

Recientemente se tienen estudios relacionados con la rizosfera de plantas acuáticas, orientados a su potencial para la remediación de sitios contaminados, en particular con la remoción de metales a través de la interacción planta acuática-microorganismo. El presente trabajo tuvo como objetivo comparar la diversidad funcional de fitobacterias aisladas de *Lemna gibba* con su contexto ecológico, considerando la calidad de agua de tres sitios ubicados en los canales de la zona, bajo dos temporadas.

El área de estudio seleccionada, se localizó dentro del sistema lacustre de Xochimilco, para la colecta de las plantas acuáticas de *Lemna gibba* de tres zonas distintas con características particulares, las cuales fueron: La Zona Turística, Zona Chinampera y la Zona Urbana. Se realizó la colecta de plantas de *L. gibba* durante el periodo de sequias en el mes de mayo y de lluvias en el mes de agosto; se tomaron muestras de agua de cada sitio de muestreo así como también muestras de biomasa de *L. gibba* de las que se aislaron y seleccionaron las fitobacterias con morfologías coloniales diferentes. Los aislados se caracterizaron por su producción de sideróforos, solubilización de fosfato, actividad de ACC deaminasa y producción de fitohormonas.

La diversidad funcional de las fitobacterias aisladas de las plantas de *Lemna gibba* provenientes de los diferentes sitios de estudio colectadas en la temporada de sequía, evidenció diferencias en la naturaleza de las fitobacterias aisladas, ya que no todas ellas, presentaron los cuatro atributos determinados: el 57.89% de los aislados presentaron dos atributos, el 26.31% un atributo, el 10.5% tres atributos y el 5.30% los cuatro atributos. Para la temporada de lluvias, el 42.85% de las fitobacterias aisladas presentaron un solo atributo, el 35.71% tres atributos, el 14.28% dos atributos y el 7.16% los cuatro atributos. Las características ecológicas de cada sitio de estudio, determinaron la diversidad de los organismos aislados, en particular con respecto a la producción de ácido indol acético, mostrando un número considerable de aislados que presentaron alta producción en ambas temporadas, independientemente del origen de las plantas colectadas. Dado que en la actualidad el sistema lacustre de Xochimilco presenta serios problemas de contaminación, la evaluación y caracterización de bacterias que presenten atributos promotores del crecimiento vegetal; permitirá proponerlas como alternativa para el desarrollo sostenible de cualquier ecosistema sin causar daño al medio ambiente y entender su papel funcional en este ecosistema.

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*Autor de correspondencia

SELECCIÓN DE BACTERIAS HIDROCARBUROCLASTAS AISLADAS DE LA RIZÓSFERA DE MANGLARES DEL ESTADO DE CAMPECHE, MÉXICO

Miguel A. Ramírez-Elías* (1,5); Ronald Ferrera-Cerrato (1); Alejandro Alarcón (1); Juan J. Almaraz (1); Luz E. de-Bashan (2); Fernando J. Esparza-García (3); Oscar García-Barradas (4)

(1) Área de Microbiología. Postgrado de Edafología. Colegio de Postgraduados. Carretera México Texcoco km 36.5. Montecillo 56230, Estado de México, México.
(2)Grupo de Microbiología Ambiental, Centro de Investigaciones Biológicas del Noroeste (CIBNOR). Avenida I.P.N. 195, Col. Playa Palo de Santa Rita Sur. La Paz, B.C. S. 23096, México.
(3) Laboratorio de Ecología Bioquímica. CINVESTAV. México, D.F.
(4)Unidad de Servicios de Apoyo en Resolución Analítica (SARA).Universidad Veracruzana. Xalapa, Veracruz, México.
(5) Facultad de Química. Dependencia Académica de Ciencias Químicas y Petrolera. Universidad Autónoma del Carmen. Calle 56 # 4. Esquina Avenida Concordia. Col. Benito Juárez.C.P.24180. Cd. del Carmen, Campeche, México. Tel.: 01 938 38 26514. mramirez.unacar@gmail.com

El presente estudio seleccionó cualitativa y cuantitativamente e identificó filogenéticamente bacterias hidrocarbonoclastas del petróleo y diésel de cuatro especies de manglar del estado de Campeche.

La selección cualitativa se realizó por medio de ensayos de tolerancia de hidrocarburos de petróleo crudo y diésel, utilizando como variable principal la turbidez de la escala de Macfarland, mientras que la selección cuantitativa se realizó utilizando cromatografía de gases-masas (GC-MS).

De un total de 86 bacterias hidrocarburoclastas identificadas filogenéticamente, se seleccionaron las 10 mejores que sobresalieron por su porcentaje degradación de petróleo crudo y diésel, destacando bacterias del tipo *Pseudomonas, Rhodococcus, Stenotrophomonas, Serratia, Providencia* y *Acinetobacter.* En la selección cualitativa, las bacterias hidrocarbonoclastas de petróleo y diésel presentaron diferencias con respecto a la turbidez de sus testigos de petróleo (T_P) y diésel (T_D), respectivamente. El mayor porcentaje de bacterias hidrocarbonoclastas fue obtenido a partir de aislamientos de la rizósfera de mangle blanco en comparación con las especies de manglares restantes. La mejor bacteria hidrocarbonoclasta de petróleo crudo fue la cepa MARW 27 (*Pseudomonas sp.*) con 88% de degradación, mientras que la mejor bacteria hidrocarbonoclasta de diésel fue MARW 25 (*Rhodococcus erythropolis*) con 94% de degradación. Estas bacterias pertenecieron al grupo de bacterias hidrocarbonoclastas fijadoras de nitrógeno aisladas de la rizósfera de mangle blanco.

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*Author for all correspondence

IDENTIFICACIÓN FISIOLÓGICA DE UNA *Nocardia* SPP, DE ACUERDO A SU CAPACIDAD DE FIJACIÓN DE NITRÓGENO ATMOSFÉRICO Y DEGRADACIÓN DE HIDROCARBUROS DERIVADOS DEL PETRÓLEO

Gustavo Medina-Mendoza (1), Aldo Sánchez-Farías (1), Carlos Cruz-Mondragón (1), Teresa Rodríguez-Casasola (1), Elvira Ríos Leal (1), Fernando José Esparza-García* (1)

(1) Dept. Biotechnology and Bioengineering, CINVESTAV – IPN, México D.F.

La biodegradación del petróleo es la capacidad metabólica que tienen los microorganismos para utilizar los hidrocarburos como fuente de carbono y energía para su crecimiento celular. Existen diversas bacterias que pueden utilizar los hidrocarburos para su metabolismo en condiciones aeróbicas. Los actinomicetos son bacterias Gram-positivas y se caracterizan por formar filamentos miceliares ramificados semejantes al de los hongos, son quimioautótrofos y aerobios. En el presente trabajo se aisló una *Nocardia* de muestras de suelo contaminado con hidrocarburos, se incubo en medio mineral con queroseno y manitol como fuentes de carbono a 30°C y 150rpm por 0, 4, 8, 12 ,24 ,48, 72, y 96hrs.

Se determinó el porcentaje de emulsión por el método de Cooper D.G. A las 0 y 96h de incubación se realizó extracción liquido-liquido con n-Hexano del caldo de cultivo, para su posterior análisis en un Cromatografo de Gases con detector FID y columna FAMEWAX, utilizados para la determinación del porcentaje de degradación de queroseno. La determinación de la actividad nitrogenasa se realizó por el método de reducción de acetileno, y en viales estériles de 144ml con 25ml de medio de Rennie sin fuente de nitrógeno, se taparon e incubaron a 30°C durante 0, 3, 6, 9,12, 24, 48, 72 y 96h, fueron sellados a presión y posteriormente se les extrajo 10ml de aire y se cambió por 10ml de acetileno. Las muestras se analizaron en un Cromatografo de Gases con detector FID y columna carbowax 15% porapack, para la determinar la reducción de acetileno a etileno.

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*Author for all correspondence

9- FENANTROL COMO SUBPRODUCTO DE LA BIOTRANSFORMACIÓN DEL FENANTRENO POR *Mucor rouxii* IM80

Rosalina Magallón-Sánchez (1); Teresa Rodríguez-Casasola (1); Elvira Ríos Leal (1); Gustavo Medina-Mendoza(1); Carlos Cruz Mondragón (1); Fernando José Esparza-García* (1).

(1) Dept. Biotechnology and Bioengineering, CINVESTAV – IPN, México D.F.

En la biotransformación de hidrocarburos aromáticos policíclicos que es catalizada por monooxigenasas, que incluyen la eliminación de hidrógenos e inserción de oxigeno, puede inducir una posterior transformación con apertura de los anillos aromáticos.
Los hidrocarburos aromáticos policíclicos (HAPs) y otros contaminantes ambientales tóxicos se han transformado biológicamente y en forma exitosa por monooxigenasas y epóxido hidrolasas estas se han aislado de hongos, bacterias y células vegetales.

En ensayos realizados bajo condiciones controladas: en matraces Erlenmeyer con 25 ml de medio YPG se inocularon con $7.5x10^7$ esporas/ ml de *Mucor rouxii* IM80 incubándose por 24 hrs a 30° C y 150 RPM. Después de 24 hrs de crecimiento se adicionó fenantreno disuelto en metanol a una concentración 50 ppm de medio, se incubo a 72 y 96 hrs. Al término de esta incubación se cosechó el micelio por filtración, se efectuó la extracción líquido-líquido del hidrocarburo en el micelio y en el caldo de cultivo con 50 ml de cloroformo.

Los extractos obtenidos del caldo de cultivo se concentraron a sequedad y el residuo se disolvió con 3 ml de metanol HPLC. Los extractos se analizaron por HPLC-UV- DAD y CG-MS, por medio de las cuales se confirmó la presencia de fenantrol entre uno de los productos de biotransformacion, además de determinarse la presencia de grupos hidrxilo por TLC.

El primer subproducto, conforme aumenta la incubación disminuye su concentración y aparecen otros subproductos más polares que aumentan su concentración conforme aumenta el tiempo de incubación, esto es indicativo del desarrollo de una vía metabólica de biotrasformación.

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*Author for all correspondence

BIODEGRADACIÓN DE HIDROCARBUROS DEL PETRÓLEO POR ACTINOMICETOS

Anabel Patiño-Hernández (1); Josefina Barrera-Cortés (1);
Rosa Olivia Cañizares Villanueva (1), Elvira Ríos-Leal (1);
Teresa Rodríguez Casasola (1); Carlos Cruz Mondragón;
Fernando José Esparza-García* (1)

(1) CINVESTAV-IPN, México DF. México

La biorremediación es una tecnología que utiliza el potencial metabólico de los microorganismos para limpiar ambientes contaminados. La diversidad metabólica de los actinomicetos y sus características de crecimiento tales como su rápida colonización de sustratos, micelio invasivo y en algunos casos la producción de biotensodepresores les confiere una alta capacidad para degradar hidrocarburos.

Cuatro cepas de actinomicetos fueron aisladas de un suelo contaminado de la ex refinería “18 de Marzo” con el objetivo de estudiar su capacidad de biodegradación. Las cuatro cepas fueron identificadas taxonómicamente como pertenecientes a los géneros *Nocardia* una cepa, dos cepas de *Streptomices,* y una cepa de *Microbispora*. Para determinar su capacidad de degradación, estas cepas fueron cultivadas en matraces Erlenmeyer de 125mL con 25mL medio de Rennie modificado con queroseno al 1% v/v como única fuente de carbono, se incubaron a 150 rpm y 30ºC durante 120h para su posterior análisis por cromatografía de gases e investigar en el caldo de cultivo la posible producción de biotensodepresores.

Los resultados indicaron que todas las cepas fueron capaces de degradar queroseno pero a diferentes niveles; *Nocardia* 52.67%, *Streptomices (1)* 26.94%, *Streptomices (2)* 45.19% y en *Microbispora* 50.0% de degradación. Así mismo se identificó en el caldo de cultivo con queroseno la presencia y degradación de naftaleno, acenafteno y fenantreno. Por otro lado se observo una disminución de la tensión superficial con un aumento de la capacidad emulsificante en las primeras 12 horas de crecimiento.

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*Author for all correspondence

ISOLATION AND CHARACTERIZATION OF BACTERIA REDUCING OF Cr(VI) WITH POTENTIAL APPLICATION IN BIOREMEDIATION

Paloma Lara; Enrique Morett; ***Katy Juárez****

Instituto de Biotecnología, UNAM, Av. Universidad 2001, Chamilpa, Cuernavaca, Morelos. CP 62210.
katy@ibt.unam.mx

Hexavalent chromium Cr(VI), is a widely used form of the element chromium, and is employed in a variety of industries, from pigment and dye production to steel welding. Hexavalent chromium is a toxic compound that has the potential to cause serious health issues, especially with long-term exposure in high concentrations. The uncontrolled Cr(VI) industrial wastes and their improper disposal have resulted in an anthropogenic pollution of various environments including soils and aquifers. Diverse bacteria have developed several strategies to resist high concentrations of Cr(VI), mainly through chromate efflux and Cr(VI) reduction to Cr(III), which is highly insoluble, and thousand-fold less toxic and unable to permeate biological membranes. These microorganisms are phylogenetic and metabolically diverse and they perform the Cr(VI) reduction as part of its miscellaneous metabolic process. The present study was aimed at the characterization the mechanisms of Cr (VI) resistance and reduction in *Klebsiella pneumoniae* ChroAq1, a strain isolated from a long-term contaminated aquifer in León Guanajuato, México.

Initially, the identification of the strain ChroAq1 was performed by sequencing the DNA 16S, *gyrA*, *rpoB* and *parC* genes. Recently we obtained the complete genome of ChroAq1 using Ilumina GAIX platform, it was automatically annotated and then analyzed to identify putative genes involved in resistance and reduction. This microorganism resists concentrations about 1.8mM of Cr(VI) in anaerobic conditions and 22mM in aerobic conditions. This level of resistance is remarkable in comparison with other microorganisms reported like *Pantoea agglomerans* SP1 that resists 100µM in aerobic conditions. *K. pneumoniae* ChroAq1 showed the ability to reduce Cr(VI) in anaerobic growth conditions but not aerobically. Complete reduction of Cr(VI) was achieved within 336h and white-grayish Cr(III) precipitate was visible at the bottom of the tube. The cell-free extract of *K. pneumoniae* ChroAq1 showed a chromate-reductase activity with NADH as electron donor. Cell fractionation allowed us to conclude that the NADH-dependent chromate-reductase activity is in the soluble cell fraction. The sequence genome analysis showed the presence of the gen ChrA, which encode a chromate pump, considered the major chromate resistance determinant.

On other hand, genes probably involved in the reduction processes were detected and its role in reduction processes is being studied. In conclusion, *K. pneumoniae* ChroAq1 shows a remarkable resistance and the capability to reduce Cr(VI) thereby greatly interest by its potential applications. The chromate reductase activity present in this strain can accept both with NADH and NADPH as electron donors and this activity was associated with the soluble fraction in both cases, then it could be either cytoplasmic or periplasmic.

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*Author for all correspondence

POLYURETHANE DEGRADING MICROORGANISMS: CHARACTERIZATION OF THEIR BIOLOGICAL ACTIVITY OVER THE POLYMER

Herminia Loza-Tavera*(1); Martín Vargas-Suárez (1); Alba Savín-Gámez (1); Joyce Álvarez-Barragán (1); Daniel Ochoa-Gutiérrez (1); Ricardo González-Hernández (1)

(1) Facultad de Química, Departamento de Bioquímica, UNAM. México D.F. México

Polyurethanes (PUs), the sixth most commonly used plastic material, are compounds synthesized from polyols and isocyanates, generating urethane bonds. Their production, which started in the 50's, is continuously increasing because of their wide versatility, based on the different types of PUs that can be synthesized. They are used for cushioning foams, thermal insulation foams, surface coatings, printing rollers, car bumps, etc. Therefore, after their useful life, their accumulation in land-filling dumps is huge, because they are not easily degraded. Recently, several microorganisms able to attack PUs have been identified.

In our laboratory, we have isolated a bacterial strain, *Alicycliphilus* sp BQ1, several bacterial consortia, and filamentous fungi strains able to attack PU. These organisms were isolated from the environment by using a selective media containing different types of PU varnishes, as the only carbon source. The biological activity of these microorganisms over distinct PUs is being studied by using different approaches. Fourier-transformed infrared spectroscopy (FTIR) has shown that in liquid PUs different types of chemical groups are attacked by bacterial and fungal biological activities. Some fungal strains are able to attack solid PU foams, reducing more than 60% their weight. After demonstrating the changes PUs suffered by these microorganisms activity, some of them have been indentified and the conditions in which they are able to better degrade PU, established. Moreover, we are studying the enzymes responsible for PU biodegradation, by purifying and identifying the proteins involved, and the genes that encode them by generating transposon mutants unable to grow in PU. Our main goal is to define the metabolic pathway(s) involved in PUs biodegradation by bacteria and fungi, and to design biotechnological systems for increased degradation of these polymers.

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*Author for all correspondence

METAGENOMES FROM HIGH TEMPERATURE AND ACIDIC ENVIRONMENTS REVEAL NOVEL ARCHAEAL COMMUNITIES FROM A MEXICAN THERMAL FIELD

Luis E. Servín-Garcidueñas (1); Esperanza Martínez-Romero* (1)

(1) Center for Genomic Sciences, National University of Mexico. Department of Ecological Genomics. Cuernavaca, Morelos, Mexico.

Los Azufres field is located in the Mexican Volcanic Axis and contains a high number of geothermal features with microbial communities that had remained unanalyzed. Microbial communities from habitats with acidic and thermal conditions generally contain a limited diversity and therefore their study is facilitated by metagenomics. We describe the diversity of microbial communities that thrive under extreme geochemical conditions by using high throughput sequencing of environmental DNA. The metagenome from an acidic hot spring revealed an archaeal-dominated community with mainly DNA from a single *Sulfolobales* population.

The metagenomic assembly (437 Mb) and its analysis revealed a composite genome for the *Sulfolobales* archaeon AZ1 that represents a novel candidate genus from the phylum Crenarchaeota. The complete genomes of the *Sulfolobales* Mexican Rudivirus SMR1 and the *Sulfolobales* Mexican Fusellovirus SMF1 from novel archaeal viruses were obtained. Further metagenomic efforts (729 Mb) revealed other crenarchaeotes from the order *Thermoproteales*. The metagenome from a microbial community deposited at the edge of a thermal and acidic fumarole (5 Gb) identified an archaeal community dominated by euryarchaeotes corresponding to the "ABC-plasmas" from the order *Thermoplasmatales*. Further metagenomic assemblies uncovered the near complete genome of a novel candidate species of the phylum Parvarchaeota that contains enigmatic and ultra-small archaeal members. This study allowed the reconstruction of almost complete genomes from novel archaeal lineages inhabiting Mexican thermal features. The generated set of predicted genes would yield clues on the genetic mechanisms allowing microbial survival under extreme conditions.

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*Author for all correspondence

SYNTHETIC ELECTROMICROBIOLOGY: BIOCOMMODITIES, BIOENERGY, BIOLECTRONICS, AND BIOREMEDIATION

Derek R. Lovley*)

Department of Microbiology, University of Massachusetts, Amherst, USA.

Electromicrobiology is a rapidly emerging field of study that investigates microbial electron exchange with electrodes, minerals, and other organisms, as well as electronic properties of microorganisms with applications in bioelectronics. The ability of microorganisms to directly exchange electrons with electrodes has led to novel applications. For example, microbial electrosynthesis is an artificial form of photosynthesis in which electrical energy derived from solar technologies is used to feed electrons to microbial biofilms on electrodes. The microbes use the electrons to reduce carbon dioxide to multi-carbon organic compounds that are excreted from the cells. Producing commodity chemicals and transportation fuels with microbial electrosynthesis is much more efficient and potentially more environmentally sustainable than biocommodity strategies that rely on biological photosynthesis.

Providing electrodes as a permanent, inexhaustible electron acceptor in anaerobic environments can accelerate the degradation of organic contaminants. When electrodes are poised at low potentials they can promote microbial reductive dechlorination and reductive precipitation of metal contaminants.

Direct interspecies electron transfer (DIET), is a recently discovered form of extracellular electron transfer in which microorganisms exchange electrons to cooperatively degrade organic compounds under anaerobic conditions. DIET appears to be an important mechanism for electron exchange in some wastewater digesters converting organic wastes to methane, a proven bioenergy strategy. Artificially enhancing DIET can accelerate methane production.

The microbial nanowires of *Geobacter* species, which contribute to microbe-electrode and microbe-microbe electron exchange are one of the most surprising discoveries in electromicrobiology. Although proteins typical function as insulators, the *Geobacter* wires, which are modified type IV pili, conduct electrons along their length via metallic-like conductivity. The possibility of metallic-like conductivity in a biological protein is a paradigm shift, differing dramatically from typical biological electron transfer, which is accomplished via electron hopping/tunneling between discrete redox-active proteins. Recently elucidated structural insights into biological metallic-like conductivity are leading to new synthetic biology strategies for enhancing microbial electrical connections in a growing diversity of applications, including biocomputers.

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*Author for all correspondence

Chapter 12.
Molecular Biology Applications to Environmental Problems

MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF THE GENE IN CHARGE OF THE ALKANE DEGRADATION IN *Bacillus Licheniformis*

Carlos Monroy-Cervantes* (1); Gabriel Pineda-Flores (2); Rosa Salgado-Brito (1)

(1) Simon Bolivar University, México DF, México;
(2) Mexican Cleaner Production Centre- IPN, México DF, México

Oil is a very complex mixture of hydrocarbons; these compounds can cause severe problems to the environment; like the interruption of gases exchange to the soil or the interruption of light into the ocean. Also the problems related with the deposition of solid waste paraffin´s, transportation and prosecution of oil are a serious issue to the industry because of the costs for maintenance as well as the use of chemicals to clean the pipelines.

Many bacteria strains had been reported to be able to degrade alkanes; however, just a few ones have the ability to degrade high molecular weight alkanes. In a previous work, a strain of *Bacillus licheniformis* capable of degrading high molecular weight alkanes between 11 and 42 carbons was isolated and identified. Nevertheless it was necessary to identify the gene that is responsibly of expressing the protein in charge of degrade this high molecular weight alkanes, and determine if this strain contains a similar gene to those that have been described.

The present study had the goal to identify the gene that expresses the alkane-degrading protein contained in *Bacillus licheniformis.* The gene identification was realized from a *Bacillus licheniformis* culture, grown in M9 medium with a concentration of 1 % of paraffin, where it was obtained microbial growth in order to perform the extraction and purification of the genomic DNA. Once the genomic material was obtained, the gene was amplified by PCR using specific primers that could select the alk gene's sequence within the *Bacillus licheniformis* genome. The amplified products were put in an agarose gel at 1.5% in a buffer solution tris-acetate EDTA 1X for an electrophoresis analysis and then visualized with a transilluminator. The amplified product was sequenced and analyzed with the use of available bioinformatics programs.

The results showed that the gene contained in *Bacillus licheniformis* and which is in charge of expressing the degrading-protein of high molecular alkanes, belongs to the alkB genes family. The local alignment analysis (BLAST), showed that the sequence amplified in this work has a 100 % of identity with the gene reported in *Pseudomonas oleovorans.* This is the first time that an alkB gene is described in a *Bacillus licheniformis* strain. This suggests that this strain of *Bacillus licheniformis* may have biotechnological importance for environmental remediation of contaminated sites with oil or being used to resolve problems related with the deposition of paraffin in pipelines.

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*Author for all correspondence.

THE GENETIC DIVERSITY OF AIRBORNE BACTERIA FROM THE LOW ATMOSPHERE OF GREATER MEXICO CITY

Selvasankar Murugesan (1); Monserrat Jacinto-Montiel (1); Ashael A. Pérez-Muñoz (1); Alberto Piña-Escobedo (1); Carlos Hoyo-Vadillo (1); María Teresa Nuñez-Cardona (2); Jaime Garcia-Mena* (1)

(1) CINVESTAV- IPN, México DF, México;
(2) UAM-X, México DF, México;

Many forms of atmospheric pollution affect human health and the environment at different levels from local to global. Air pollution trends are strongly affected by atmospheric conditions such as temperature, pressure, and humidity, and by global circulation patterns. Biological material is present at all levels of the atmosphere in the form of bacteria, pollens, fungal spores, viruses, and many fragments from plants and animals. It is essential to explore these biological contaminants to understand the risks we face in our daily life.

We sampled the air of the low atmosphere at several points in the Metropolitan Area of Mexico City by 30 minutes of the gravity method on rich medium and blood-agar during the Winter, Spring, Summer and Autumn. Plates were incubated at 37° C during 16 h, and all colonies of each plate were recovered using 0.89% saline. The hemolysis type was recorded for the colonies growing on blood agar. A pooled suspension of the bacterial cultures was made and bacterial genomic DNA was extracted. Convenient were made by PCR, amplifying the V3 hyper variable region of 16S rDNA gene. These libraries were barcoded and sequenced using an ion semiconductor massive sequencer Ion Torrent. Metagenomic analysis was done using the QIIME software pipeline.

From the metagenomic analysis of the massively sequenced amplicon libraries, mainly three types of phylum such as *Actinobacteria*, *Firmicutes*, and *Proteobacteria* were identified. From these, *Proteobacteria* was more abundant in Spring than the other three seasons. At genus level we found the presence of some pathogens like *Streptococcus* sp, *Staphylococcus* sp and *Pseudomonas* sp. By single conventional sequening of isolated bacterias from LB agar and blood agar media, almost 77% of bacteria were *Bacillus* sp in all samples. The colonial phenotype on blood agar showed the presence of α, β, and γ hemolytic activities among the collected bacteria.

From this study we conclude that the bioaerosols of different sites in the Greater Mexico City has a high abundance of bacteria in the low atmosphere where several activities are developed by the inhabitants. Even though we isolated several hemolytic bacteria and some presumed pathogens, this fact is not health risk to healthy people. There is an equilibrium between our immune competence and the presence commensal microorganisms, which support us to maintain our health. This novel work opens a new world to explore more for further understanding the relationship of bioaerosols and our human microbiota.

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*Author for all correspondence.

EVALUATION OF CORRELATION BETWEEN *mcr*A TRANSCRIPTS & METHANOGENIC ACTIVITY IN TWO DIFFERENT ANAEROBIC REACTORS FED WITH ANIMAL MANURES & BREWERY WASTEWATER

Lorena Palacio Molina (1); Ricardo Oropeza Navarro (2);
Nagamani Balagurusamy* (1)

(1) Laboratorio de Biorremediación, Escuela de Ciencias Biológicas, Universidad Autónoma de Coahuila, México.
(2) Instituto de Biotecnología de la UNAM, Cuernavaca, Morelos, México

Anaerobic digestion technology is widely employed for the treatment of high strength organic wastes and at the same time aids in recovery of carbon in the form of methane. However several operational factors could affect the performance and an early detection could help us to prevent the ill-effects. Monitoring methanogenic activity will aid us in knowing the state of biodigesters. Methyl coenzyme M reductase (MCR) is the key functional enzyme in methane formation. Although methanogens are phylogenetically diverse, *mcrA* gene encoding the alpha subunit of the enzyme is unique. The aim of this study was to evaluate the correlation betweeen *mcrA* gene transcripts and methanogenic activity in two anaerobic reactors, each fed with animal manures and brewery wastewaters.

Methane was analyzed by gas chromatography in a PerkinElmer chromatograph. Both aceticlastic and hydrogenotrophic methanogens and *mcrA* transcripts of Metanobacteriales and Metanosarcinales orders were studied. Total RNA extraction was carried out using the ZR Soil/Fecal RNA MicroPrep (Zymo Research, EUA). Later, cDNA was generated using a RevertAid H Minus First Strand cDNA Synthesis (Fermentas Vilnus, Lithuania). Real time PCR reactions were carried out in a LightCycler 480 (Roche Diagnostics, Burgess Hill, United Kingdom) using the Maxima SYBR Green/ROX qPCR Master Mix (Fermentas Vilnus, Lithuania).

Results obtained from samples of anaerobic reactor fed with dairy cattle manure recorded that around 90% of the methanogen population belonged to the order Methanobacteriales. However, transcriptional activity showed a different tendency, and over 90% of *mcrA* transcripts corresponded to the order Methanosarcinales. A correlation of 0.997 between methanogenic activity and *mcrA* transcripts was observed. However, in the case of UASB reactors Methanosarcinales order predominated both in population and *mcr*A transcripts.

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*Author for all correspondence: bnagamani@uadec.edu.mx; bnagas@gmail.com

OBTENCIÓN DE TIROSINASA PARA BIORREMEDIACIÓN DE AGUAS CON COMPUESTOS FENÓLICOS Y METALES PESADOS

Miguel Á. Ramos-Valdovinos (1); Raquel Peña-Martínez (1);
Agustino Martínez- Antonio* (1)

(1) CINVESTAV-Unidad Irapuato, Guanajuato, México.

La presencia de metales pesados y compuestos fenólicos en cuerpos de agua, tales como ríos y lagos, se ha convertido en un problema de salud para los seres humanos que hacen uso de ella y los organismos que habitan estos ambientes contaminados. Ante esto, se han buscado alternativas para el tratamiento de aguas que contienen dichos metales, siendo la melanina una posible solución. La melanina es un polímero oscuro de alto peso molecular que le confiere al ser humano protección contra la radiación ultravioleta mientras que a los microorganismos los protege de altas temperaturas, radiación ultravioleta, agentes oxidantes y metales pesados. Por su composición química, es capaz de actuar como agente quelante, y se ha demostrado que puede absorber el 9 % de su masa en plomo, lo cual hace de ella una alternativa para el tratamiento de aguas contaminadas con metales pesados. La melanina es sintetizada a partir de la L-tirosina por medio de la enzima tirosinasa (E.C.1.4.18.1) mediante una serie de reacciones enzimáticas y no enzimáticas. La tirosinasa es a su vez una enzima de gran interés, ya que por su capacidad de reaccionar con fenoles ha sido utilizada para el tratamiento de aguas contaminadas con compuestos fenólicos. Otra de sus aplicaciones es en la síntesis de levodopa (L-DOPA), medicamento utilizado en el tratamiento de la enfermedad de Parkinson. En este trabajo se produjo melanina en *E. coli* y se clonó el gen *melA* en un vector de expresión para aislar la proteína tirosinasa, y ser utilizadas en la remoción de metales pesados y compuestos fenólicos presentes en agua.

Se realizaron dos construcciones genéticas, una para la producción de melanina y la otra para aislar la enzima tirosinasa. En el caso de la construcción genética responsable de la producción de melanina, ésta se diseñó de tal manera que la bacteria sobreproduciera tirosina y al aumentar el precursor se incremente la producción de melanina. Dicha construcción consta de los genes *aroH*, *tyrA* y *melA*. Los genes *aroH* y *tyrA*, involucrados en el aumento de producción de tirosina fueron aislados a partir de la cepa *E. coli MG1655*; mientras que el gen *melA* para la producción de melanina se aisló de la cepa *Rhizobium etli*. Para aislar la enzima tirosinasa, se realizó una construcción únicamente con el gen *melA*, el cual se clonó en el vector de expresión pColdI.

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*Autor de correspondencia.

THE BIOTECHNOLOGICAL POTENTIAL OF SUBMERGENCE SENSING AND SIGNALLING PATHWAYS TO IMPROVE PLANT BIOMASS FOR BIOETHANOL PRODUCTION

Julián M. Peña-Castro (1); Lizeth Núñez-Lopez (1,2);
Blanca E. Barrera-Figueroa*** (1)

(1) Laboratorio de Biotecnología Vegetal, (2) División de Estudios de Posgrado, Instituto de Biotecnología, Universidad del Papaloapan, Tuxtepec, Oaxaca, México.

Saccharification is the chemical or enzymatic hydrolysis of starch and cellulose to produce glucose and xylose. These monosaccharides are substrates for ethanol-producing microorganisms. In order to improve plants as raw materials for saccharification, genetic factors and biochemical pathways controlling the accumulation or the structure of plant carbohydrates must be identified.

Carbohydrate consumption and signaling are crucial for plant survival during flooding stress. Excess of water excludes oxygen from plant cells, forcing an adjustment from aerobic to anaerobic metabolism. Plants must finely control the consumption of starch because when this reserve is depleted underwater, an energy crisis kills the cells. This response is mediated by transcription factors from the *Ethylene Response Factors* (*ERFs)* gene family. Up to date, the best studied ERF is *SUB1A* from rice.

SUB1A is responsible for plant tolerance to submergence stress by means of a tight control on starch consumption and sugar fermentation in the plant. *SUB1A* orthologues in *Arabidopsis* are also the main sensor of oxygen concentrations *in planta*.

In this work, we employed transgenic prototypes of the model plant *Arabidopsis thaliana* to analyze the potential of *SUB1A* to improve biomass saccharification potential.

We found that plants overexpressing *SUB1A*, when compared to Wild-type, have a delayed flowering time, an enhanced lipid catabolism, produce more leaves, offer less resistance to rupture and accumulate more starch. All these characteristics are desirable traits in plant biomass for bioethanol production.

In order to find a probable molecular mechanism for this phenotype, we searched in previously reported microarrays for key genes involved in starch degradation (SEX4 and GWD1), juvenile transition (SPL3, SPL5) and flowering time (FUL, SOC1). We followed by qPCR these transcripts but found no expression changes, indicating that starch accumulation is not orchestrated by previously known molecular mechanisms.

Its position at the crossroads of energy-capture reactions and plant-polymer build-up makes *SUB1A* an interesting target for manipulation of sensing and control of carbon flux in plant breeding for improvement of saccharification potential.

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*Author for all correspondence.

GENOME WIDE ANALYSIS OF THE EXPRESSION AND REGULATION OF GENES INVOLVED IN ELECTRON TRANSFER MECHANISM IN *G. sulfurreducens*

Getzabeth González*; Aurora Labastida; Emanuel Salazar; Verónica Jímenez; Leticia Vega; Maricela Olvera; Sonia Dávila; Enrique Morett; Katy Juárez

Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, UNAM, Campus Morelos. Avenida Universidad 2001, Cuernavaca Morelos. México C.P. 62210. getzabet@ibt.unam.mx

Geobacter sulfurreducens is an anaerobic bacterium that participates in biogeochemical cycles of different elements such Fe and Mn. It is involved in the oxidation of organic matter in sediments and soils and it is capable of transferring electrons from a variety of substrates to electrodes, producing electricity or transforming heavy metals to insoluble forms in contaminated soils[1]. These capabilities are made possible by electron transfer mechanism in which an oxide-reductive process is carried out. Although significant progress has been made in the understanding the genetic regulation of electron transfer, in *G. sulfurreducens* only few regulators have been discovered and even less is known about regulatory mechanisms directed by non-coding RNAs (ncRNAs)[2]. In order to identify ncRNAs involved in electron transfer mechanism and obtain a complete transcriptional picture of electron transfer mechanism, two RNA sequencing approaches were employed using Illumina GAIIX platform. In the first one, *G. sulfurreducens* transcriptome was analyzed in two different growth conditions, both using acetate as electron donor and employing either fumarate or soluble Fe(III) as electron acceptors.

Based in transcriptome data, we identified 50 novel ncRNA candidates in intergenic regions included 10 ncRNAs that were only expressed when soluble Fe(III) is electron acceptor. The expression of 12 ncRNA were verified and validated by Northern blot analysis and by qRT-PCR. In addition, computational methods were employed to reveal likely putative mRNA targets for the confirmed ncRNAs in order to determine interactions and function within the regulatory networks.

Differential expression analysis detected significant differential expression in 301 genes of these, 42 genes involved in regulatory functions were up regulated when soluble Fe (III) was used as electron acceptor. In the second approach, we employed a method aimed to the enrichment of mRNAs 5'PPP for the global mapping of transcriptional starts sites (TSSs). This method detected 3013 TSSs distributed along the genome and in some cases; we observed genes with more than one TSSs and also in antisense position. In conclusion, the combination of RNAseq data with Genome-wide identification of transcription start sites provides us a complete transcriptional landscape of *G. sulfurreducens* and a unique tool for the search for novel ncRNAs with potential regulatory function.

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*Author for all correspondence.

FUNCTIONAL METAGENOMIC ANALYSIS OF THE BIOREMEDIATION OF A HYDROCARBON-POLLUTED SOIL WITH A MIXED MICROBIAL CONSORTIUM

German Zafra (1); Angel E. Absalon (1); Todd D. Taylor (2);
Diana V. Cortés-Espinosa* (1)

(1) CIBA-TLAXCALA, IPN, Tlaxcala, México;
(2) RIKEN-IMS, Yokohama, Japan.

Microbial degradation constitutes the main form of degradation of hydrocarbons (HC) in soils. Metagenomic sequencing of soil microbial communities can provide insights regarding the metabolic pathways and specific enzymes involved in the HC degradation process in soil, especially for Polycyclic Aromatic Hydrocarbons (PAHs). In this study, we conducted a functional metagenomic analysis during the bioremediation of a HC-contaminated soil with a degrading microbial consortium at microcosm, to better comprehend the specific metabolic profiles and degradation pathways associated with each stage of the process and the differences between treated and non-treated soils. Microcosms systems were prepared using a non-sterile heavy crude oil-contaminated soil, inoculated with a fungal/bacterial degrading consortium (C1) and biostimulated with sterile corn stover, or only bioestimulated (non-inoculated control) for 30 days under aerobic conditions. Paired-end whole genome sequencing was performed using an Illumina Miseq 2x250 platform. Metagenomic unassembled reads were quality checked and annotated in MG-RAST using the M5nr database. Results were visualized and analyzed with the STAMP V2.0.3 software.

Global gene abundance based on KEGG orthology showed a dominance of metabolism pathways in all datasets, especially for carbohydrate, aminoacid and energy metabolism. Annotation of metagenomes focusing on the HC and aromatic compounds degradation revealed that peripheral pathways for aromatic degradation genes dominated in both inoculated and uninoculated microcosms datasets, while metabolism of central aromatic intermediaries were also found in relative high levels. Significantly higher abundance levels of HC and PAH degradation genes were observed in inoculated (C1) microcosms in comparison with non-inoculated systems, particularly for central aromatic intermediary pathway as protocatechuate 4,5-dioxygenase, p-hydroxibenzoate hydroxylase, gentisate 1,2 dioxygenase and catechol 1,2 dioxygenase genes. In contrast, thioesterase and transporter genes were significantly higher in control microcosms.

We concluded that using a degrading microbial consortium in HC-contaminated soils will produce appreciable changes on the functional profiles of inoculated and non-inoculated soils with a bias towards central aromatic hydrocarbon and PAH-intermediary degradation pathways, both favoring HC metabolism and mineralization.

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*Author for all correspondence.

CONTROL DE LA EXPRESION DE LOS GENES INVOLUCRADOS EN LA GENERACIÓN DE BIOELECTRICIDAD POR REGULADORES GLOBALES EN *Geobacter sulfurreducens*

José A. Hernández-Eligio*; Ángel Andrade; Leticia Olvera; Sonia Dávila; Enrique Morett; Katy Juárez-López

IBT-UNAM, Cuernavaca Morelos, Méx.

Geobacter sulfurreducens es un bacteria del subsuelo que tiene la capacidad de acoplar su respiración anaerobia a la reducción de metales. Además, puede generar bioelectricidad a partir de la oxidación de compuestos orgánicos, transfiriendo los electrones resultantes a electrodos (1). En esta bacteria, la reducción de metales y generación de bioelectricidad requieren de la participación de diversos citocromos y del pili, y la regulación de su expresión se encuentran estrictamente regulada. Se han identificado varios reguladores globales implicados en dicha regulación entre los cuales están dos homólogos de la familia CRP-FNR (FLP1 y FLP2), IHF (Integration Host Factor) y el sistema de dos componentes PilR/PilS (2 y 3). Estudiar los mecanismos de regulación de los genes codificantes para los citocromos y el pili, nos ayudará a comprender mejor los procesos de generación de bioelectricidad y reducción de metales. En este trabajo, mediante experimentos de fosfotransferencia, retardo de la movilidad en gel (EMSA), fusiones transcripcionales, mutagénesis puntual, deleciones génicas y reverso transcripción y PCR en tiempo real (RT-qPCR) demostramos que la expresión de los genes que codifican para varios citocromos y el pili (*pilA*) son regulados transcripcionalmente por PilS/PilR, IHF, FLP1 y FLP2.

Con ensayos de fosfotransferencia *in vitro* confirmamos que PilR es fosforilado por PilS, siendo el regulador de respuesta par de PilS. Mediante fusiones *pilA*::*luxABCDE*, encontramos que PilR activa la expresión de *pilA*; sin embargo, una versión no fosforilable de PilR (D53N) activa al doble la fusión comparado a la cepa silvestre. A través de experimentos EMSA, revelamos que PilR se une al promotor de *pilA*, específicamente en una región donde se localizan 2 posible sitios de unión. Por otra parte, mutaciones en los genes que codifican para las subunidades del complejo IHF (ihf□1, ihf□2, ihf□1 e ihf□2) resultan en diversos fenotipos en el crecimiento, reducción de Fe(III), biogénesis del pili y afectan la presencia de varios citocromos. De manera interesante, usando RT-qPCR y experimentos EMSA identificamos varios genes relacionados con los fenotipos de las mutantes que son activados o reprimidos directamente por IHF. Finalmente, con fusiones *pilA::luxABCDE* y experimentos EMSA demostramos que FLP1 y FLP2 reprimen la expresión de *pilA,* al unirse sobre su región promotora. Con todos nuestros resultados, sugerimos un modelo de regulación donde IHF, PilS/PilR, FLP1 y FLP2 controlan la biogénesis del pili, a través de *pilA*; y que IHF es además un factor clave en el control de los genes involucrados en el proceso de generación de bioelectricidad.

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*Author for all correspondence.

INCREMENTO DE LA EXPRESIÓN EXTRACELULAR DE CELULASAS BACTERIANAS EN *Saccharomyces cerevisiae* MEDIANTE LA EDICIÓN DEL VECTOR PYEX-S1 PARA LA PRODUCCIÓN DE ETANOL CELULÓSICO

Francisco J. Ríos-Fránquez(1); Ana C. Ramos-Valdivia (1);
Héctor M. Poggi-Varaldo (1); Jaime García-Mena (2); Alfredo Martínez-Jiménez (3);
Teresa Ponce-Noyola* (1)

(1) Departamento de Biotecnología y Bioingeniería, Cinvestav- IPN, México DF, México;
(2) Departamento de Genética y Biología Molecular, Cinvestav- IPN, México DF, México;
(3) Instituto de Biotecnología, UNAM, Cuernavaca Morelos, México.

El bioetanol es uno de los principales biocombustibles actualmente utilizado en vehículos automotores. En México el bioetanol se puede obtener mediante la fermentación de residuos agrícolas sacarificados, sin embargo esta tecnología es costosa y requiere ser optimizada para hacerla rentable. Una estrategia para optimizar la producción del etanol celulósico es la sacarificación y fermentación simultánea de los residuos agrícolas con levaduras productoras de celulasas recombinantes.

En nuestro grupo de trabajo hemos estudiado las holocelulasas de *Cellulomonas flavigena* para sacarificar residuos agrícolas. Esta bacteria expresa todas sus celulasas de forma extracelular a excepción de su β-glucosidasa que permanece en el espacio periplásmico, haciendo que los sacarificados obtenidos con concentrados enzimáticos extracelulares de esta bacteria sean ricos en celobiosa.

Por lo anterior, en el presente trabajo sepropone sacarificar y fermentar simultáneamente el bagazo de caña con concentrados enzimáticos extracelulares de *C. flavigena* y una cepa de *Saccharomyces cerevisiae* productora de la β-glucosidasa recombinante.

Se identificó y secuenció el gen *bgl*A que codifica para la β-glucosidasa de *C. flavigena* y se expresó en *Saccharomyces cerevisiae* mediante el uso del vector pYEX-S1, sin embargo, los niveles de actividad extracelular de β-glucosidasa y producción de etanol en celobiosa con esta recombinante fueron muy bajos.

Para aumentar la expresión de la β-glucosidasa de *C. flavigena*en *S. cerevisiae*, se optimizó el gen *bgl*A mediante la adaptación de los codones del gen para los de uso preferente en la levadura. Aunado a ésto, mediante un análisis de expresión *in silico* del gen *bgl*A con el plásmido pYEX-S1, se observaron problemas para exportar la proteína recombinante al exterior de la célula, debido a un error en la secuencia señal del vector. Para superar esta limitante, se diseñaron olgonucleótidos con los que se linealizará el vector por PCR inversa, corrigiendo las secuencia y obteniendo el vector lineal que se utilizará para construir el vector de expresión para la β-glucosidasa mediante la tecnología In-Fusion®.

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*Author for all correspondence.

ESTANDARIZACIÓN DE LA AMPLIFICACIÓN DE REGIONES MICROSATÉLITE PARA UNA POBLACIÓN DE *Pinus montezumae*

Lizbeth V. Vázquez-Hernández (1); Nora B. Medina-Jaritz* (2);
Raúl Alcalde-Vázquez, (2) y Carlos F. Vargas-Mendoza (1).

(1) ENCB-IPN. Laboratorio de Variación Biológica y Evolución. México DF, México.
(2) ENCB-IPN. Laboratorio de Fisiología Vegetal. México DF, México.

La Genética de poblaciones es el área de la Biología que se enfoca en los genes de las especies que conforman una población y pretende describir sus niveles de variación genética en términos de fuerzas evolutivas. Entre sus herramientas están los marcadores genéticos que permiten valorar tanto la estructura como la variación genética de las poblaciones de una manera cuantificable. En este sentido, el empleo de marcadores microsatélite resulta muy útil; además, se pueden emplear en el estudio de sistemas de apareamiento, mapeo genético, fitomejoramiento, identificación de variedades y en general para la conservación de las especies debido a que constituyen una medida objetiva de la variación y no están sujetos a las condiciones ambientales.

El género *Pinus* es uno de los más representados en México y Norteamérica, tienen gran importancia económica y ecológica por lo que el estudio y la preservación de este grupo son muy importantes.

El objetivo del presente trabajo fue estandarizar la reacción en cadena de la polimerasa (PCR) para lograr la amplificación de siete regiones microsatélites polimórficos nucleares derivados de *P. taeda* (PtTX2037, PtTX2123, PtTX2146, PtTX3013, PtTX3020, PtTX3025 y PtTX3029) en ejemplares de *P. montezumae* provenientes de un bosque fragmentado del estado de Hidalgo.

Para lograr la estandarización se realizaron varias PCR en las que se probaron variantes de manera independiente. Las variantes probadas fueron: la concentración de DNA (2 ng/µL; 4 ng/µL y 6 ng/µL); la concentración de cloruro de magnesio (4mM; 6mM y 8mM); la concentración de iniciadores (0.001 µg/µL; 0.002 µg/µL y 0.004 µg/µL) y la concentración de Taq DNA polimerasa (0.04 U/µL y 0.1 U/µL); las temperatura óptima de alineamiento también se verificó para cada uno de los iniciadores en un intervalo de ± 2°C la Tm.

Los mejores resultados de amplificación se obtuvieron con las siguientes condiciones de reacción: la concentración de DNA 4 ng/µL; la concentración de cloruro de magnesio 4mM; la concentración de iniciadores 0.002 µg/µL y la concentración de Taq DNA polimerasa 0.1 U/µL; las temperatura óptima correspondió a la Tm de cada PAR de iniciadores. Estos avances permitirán analizar la estructura y variabilidad genética de la población de *P. montezumae* del bosque fragmentado en estudio.

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*Autor de correspondencia.

DYNAMIC CHANGES IN THE MICROBIAL COMMUNITY COMPOSITION IN AN ELECTROBIOCHEMICAL SLURRY REACTOR

Beni Camacho-Pérez (1); Héctor M. Poggi-Varaldo (1); Selva Sankar (2); Alberto Piña-Escobedo (3); Jaime García-Mena* (3)

(1) Dept. Biotechnology and Bioengineering, CINVESTAV-IPN
(2) Dept. of Pharmacology, CINVESTAV-IPN
(3) Dept. of Genetics and Molecular Biology, CINVESTAV - IPN

Lindane has been widely used as an insecticide to protect crops, and also in human health and veterinary medicine to control insect-borne diseases. Nowadays, its use has been banned, since it is considered one of the priority organic pollutants under the Stockholm Convention on persistent organic pollutants. Bioremediation is the application of biological treatment to cleanup of hazardous chemicals. It requires the control and manipulation of microbial processes in reactors or *in situ* treatment. On the other hand an electrobiochemical slurry reactor (EBCR) is a device that uses bacteria as catalysts to oxidize organic matter and recalcitrant pollutants in contaminated soils and generate electrical energy. So far, few research works have studied the changes in microbial populations during the bioremediation process in EBCR. Therefore, the goal of this research was to examine the changes of the bacterial community harbored in the EBCR with respect time.

1The EBCR consisted of a Plexiglass cylinder approximately 6 cm in diameter per 8 cm in length, fitted with two anodes and two cathodes. The anodes were graphite discs (5cm D x 0.5 cm) whereas the cathodes were of Toray carbon cloth, the cathodes were in contact with atmospheric air. The EBCR was inoculated with a sulfate reducing inoculum acclimated to lindane, and loaded with soil polluted with 100 mg lindane/kg. The metagenomic analyisis was performed by amplification of the V3 hyper variable region of 16S rDNA gene. Sequencing was conducted using the Ion Torrent Personal Genome Machine.

Metagenomic analysis allowed the identification of 18 *phyla* for the electrobiochemical slurry reactor, of which the dominant *phyla* were *Actinobacteria* (6.35%), *Bacteroidetes* (17%), *Firmicutes* (46.4%), *Proteobacteria* (17.9%), and *Synergistetes* (7.16%) . On the other hand, the microbial community of the control EBCR (without generation of electricity) exhibited 16 phyla, of which the dominant phyla were *Actinobacteria* (4.22%), *Bacteroidete*s (12.33%), *Firmicutes* (57.5%), and *Synergistetes* (7.22%).

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*Author for all correspondence.

ENZYMATIC SACCHARIFICATION IMPROVEMENT OF *Arabidopsis thaliana* OVEREXPRESSING RICE *SUB1A* AND *SUB1C* TRANSCRIPTION FACTORS.

Lizeth Núñez-López (1,2); Blanca E. Barrera-Figueroa (1); Andrés Aguirre-Cruz (3), ***Julián M. Peña-Castro**** (1)

(1) Laboratorio de Biotecnología de Plantas,
(2) División de Estudio de Posgrado,
(3) Taller de Alimentos, Instituto de Biotecnología, Universidad del Papaloapan, Tuxtepec, Oaxaca, México.

The economy of modern society depends on fossil fuels such as gasoline and diesel. Currently, scientists are looking for alternatives to replace them with biofuels like biodiesel or bioethanol. Plant biotechnology is an important tool to improve plants as raw material since the first step in bioethanol production is the chemical or enzymatic saccharification of starch and cellulose to sugar monomers for microbial fermentation.

SUB1A and *SUB1C* genes are involved in controlling flooding response in rice. They keep a balance between degradation and consumption of starch during stress. We hypothesize that taking *SUB1A* and *SUB1C* out of abiotic stress context could allow plants to accumulate starch and improve their saccharification potential. Thus, in this work, we explored saccharification improvement of *Arabidopsis thaliana* leaves by overexpressing *SUB1A* or *SUB1C* under 35S promoter (OxSUB1A and OxSUB1C).

Free sugar was quantified with DNS. Saccharification yield of cellulosic material and starch was quantified by hydrolysis with Accellerase® cellulases alone (celullose) or together with amylase/amyloglucosidase (cellulose and starch). Each sampling point represented at least 5 individuals and 3 to 6 independent experiments.

It was observed that at the end of the day, and 7d after WT flowering (25d old plants), OXSUB1A had 35±6% more free sugars, 20±8% more glucose from cellulose and 80±8% (SE) more starch than WT. OxSUB1C plants had no positive changes and some lines even showed lowered yields. We then characterized accumulation kinetics in a diurnal cycle and found that OxSUB1A accumulated more starch (80-100%) than WT at all sampling points.

Next, we followed starch accumulation from 2 weeks to 5 weeks after germination. Using I_2 staining and enzymes we observed that starch accumulation is higher in young plants and tends to fade with age (170±23% at 2 wk, 98%±22 at 3 wk, 63%±14 at 4 wk and -1.8% ± 10 at 5 wk). To analyze if *Arabidopsis* cell wall had any structural changes, leaves of WT and transgenic plants were subjected to a hardness test on a texturometer. OxSUB1A were significantly weaker than WT and OxSUB1C, resisting a force of 2.34±0.26, 3.47±0.35 and 3.14±0.24 N respectively.

Using *Arabidopsis* as a functional prototype, we demonstrated that *SUB1A* is a genetic tool to improve free sugars content, cellulose and starch saccharification and to diminish hardness. All these are traits sought in plants to be used as raw materials for the production of bioethanol.

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*Author for all correspondence.

GENOMIC AND FUNCTIONAL PROTEOMIC ANALYSES OF METABOLIC PATHWAYS LEADING TO ETHANOL VS H_2 FERMENTATION IN CELLULOLYTIC CLOSTRIDIA FOR CONSOLIDATED BIOPROCESSING

Richard Sparling* (1); David B. Levin (2)

(1) Department of Microbiology, University of Manitoba, Winnipeg, MB, Canada
(2) Deaprtment of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada

Cellulose is the most abundant biopolymer in the world. Waste cellulosic biomass derived from forestry, agriculture, and municipal sources are potential feedstocks for the synthesis of biofuels (and other value-added bioproducts) that could displace fossil fuel consumption and reduce greenhouse gas emissions. However, the crystallinity of cellulose, as well as its imbedding with lignin and hemi-cellulose into plant fibers, makes it difficult to ferment directly into biofuels (for example ethanol or hydrogen). Current technologies require extensive pretreatment to liberate the sugar polymers (cellulose, hemi-cellulose) followed by enzymatic saccharification of these polymers releasing monosaccharides that can be fermented using more conventional fermentation technologies. This approach is time consuming and rather expensive. Consolidated bioprocessing is an alternative process being explored. This process combines cellulase production, substrate hydrolysis and fermentation in a single step using cellulolytic fermentative microorganisms, mainly form the genus *Clostridium* and other related genera.

Development of industrially-robust strains requires a comprehensive understanding of the relationships between genome content, gene and gene product expression, enzyme activity levels, as well as carbon and electron flow through metabolic pathways relative to end-product synthesis patterns. While genome sequence data are necessary to understand carbon flow, the current state of gene annotation of these organisms can lead to misinterpretation. By themselves, genomic data are also insufficient to provide insight into how varied growth conditions such as carbon source and loading, pH, and end-product accumulation influence carbon and electron flow to various metabolic end-products. This presentation will provide an overview of comparative genomics between hydrogen and ethanol producing strains, and how functional genome sciences, including proteomics and transcriptomics has provided added insights into the pathways involved in central metabolism in cellulosic clostridia. Through these studies, non-conventional variations of the Embden-Meyerhof-Parnas pathway of glycolysis and of the pentose phosphate pathway have been observed in different lignocellulosic clostridia including *Clostridium thermocellum*, *C. stercorarium* and *C. termitidis*. The importance of pyrophosphate as an energy currency is also emerging from these studies. These insights may guide genetic engineering, and complement bioprocess engineering, to increase the efficiency of conversion of lignocellulosic biomass to biofuels (ethanol, hydrogen)

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*Author for all correspondence

IMPACTO Y APLICACIONES DE LA REVOLUCIÓN GENÓMICA

Xavier Soberón-Mainero*

Instituto Nacional de Medicina Genómica, México.

A partir del lanzamiento del proyecto del Genoma Humano, al final del siglo pasado, se ha producido una transformación radical en la capacidad de indagación de la ciencia biológica experimental. Por otra parte, las innovaciones trascendentes en ciencia son las que generan impacto en la forma de vida de las personas a gran escala. Eso sucedió con las tecnologías de la información y lo que sigue es, sin duda, la era digital de la biología. Dentro de 25 años las tecnologías "ómicas" serán el eje de las innovaciones que transformarán la salud de millones de personas y la forma de hacer negocios en agricultura y otros sectores productivos.

La construcción de modelos integradores que expliquen cómo funcionan los sistemas biológicos, desde el DNA y las proteínas hasta el cerebro en su enorme complejidad, serán esenciales para la humanidad en las próximas dos décadas. Caracterizar y entender la genómica del humano, los animales, las plantas y los microorganismos plantea grandes retos para cada país, sin embargo constituye la base de la siguiente gran revolución industrial y biológica.

El genoma humano, cuyo desciframiento es el eje de la revolución genómica, constituye tres enciclopedias: biología, historia y medicina. ¿Cómo hemos avanzado en nuestra comprensión de la información en esos tres grandes rubros? Dentro de la evidente complejidad del sistema biológico humano se han abierto grandes nuevas avenidas y el camino ha estado lleno de sorpresas. El impresionante avance tecnológico ha permitido el abordaje paralelo de información referente a miles o millones de moléculas informacionales en cada experimento. Tal avalancha de datos ha demandado el uso de enfoques bioinformáticos sofisticados y el desarrollo de una Bilogía de Sistemas, para su análisis.

En la comprensión de la biología destacan la publicación reciente de los primeros hallazgos de los grandes proyectos ENCODE (sobre la función de los diversos elementos del genoma humano) y el del Proteoma Humano. Se abren preguntas sobre la extensión y el significado de las variantes estructurales y de nuevos tipos de RNAs no codificantes. Además, se ha hecho posible el análisis de los microorganismos que colonizan al organismo humano, hoy referido como "microbioma", revelando una diversidad y una dinámica hasta ahora totalmente desconocidas.

El genoma como libro de historia se nutre de los grandes proyectos del HapMap y de los 1000 genomas, más los diversos proyectos regionales que han alimentado las bases de datos de diversidad genética humana (la dbSNP contiene más de 40 millones de polimorfismos de un solo nucleótido). Con esta información se analiza la historia evolutiva humana, desde su salida de África hasta los más recientes movimientos poblacionales, incluyendo la secuenciación de DNA de fósiles cada vez más antiguos.

La aplicación médica del conocimiento del genoma humano empieza a ser evidente, después de una década de aparente rezago, a partir de miles de enfermedades asociadas a genes específicos (estudios de GWAS) y de enorme sensibilidad y profundidad de los estudios moleculares hoy disponibles.

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*Author for all correspondence

Chapter 13.
Control and Modelling and Environmental Processes

ÍNDICE DE MEDICIÓN AMBIENTAL (IMAVA), METODOLOGÍA Y SEMAFORIZACIÓN PARA ÁREAS DE VALOR AMBIENTAL (AVA's) EN LA CIUDAD DE MÉXICO

Fernando De Lorenz-Santos (1, 2); Salvador Mitre-Carlos (3);
Martha Ledesma-Flores (1); Alejandra Vargas-Espinoza de los Monteros (3);
Gustavo Calvillo-Gandulfo (1)

(1) TESE, Div. de Contaduría y Admción., Posgrado en Gestión Adm., Edo. Mex, México.
(2) UNITEC-Cuitlahuac, Dirección de Ingenierías, D.F., México.
(3) FACULTAD DE INGENIERÍA, UNAM, Div. Ciencias Basicas.

Los servicios ambientales (SA) que dan las barrancas, contribuyen a la recarga del manto acuífero y son refugio para especies silvestres. Por ello, se generaron 2 Índices IMAVA´s complementarios, para medir el nivel de impacto de los SA que proporcionan las Barrancas. Éstos fueron analizados y resultaron en un paquete de indicadores para medir cada uno de los SA, que permitan su medición cualitativa y cuantitativa con el Índice de Medición de Áreas de Valor Ambiental (IMAVA), y se proporcione un valor numérico a dichos indicadores dando seguimiento a través del tiempo. Para ello, se realizó un análisis que permitió elegir el método de la FAO.

El **IMAVA 1** informa el nivel de impacto de la barranca por cada uno de los SA y se conforma "**IMAVA 1=BHSU (B=**Valoración de los SA de Biodiversidad, **H=**Valoración de los SA de Hidrología y Suelos, **S=**Valoración de los SA Sociales, **U=**Valoración de los SA Urbanos)". El **IMAVA 2** es la suma de las medias ponderadas de cada uno de los SA que lo integran, generando un valor numérico similar al IMECA, y se conforma "**IMAVA2=β1*SA1+β2*SA2+β3*SA3+β4*SA4** (SA1=Valoración SA de Flora y Fauna, SA2=Valoración de los SA de Hidrología y Suelos, SA3=Valoración de los SA Urbanos, SA4=Valoración de los SA Sociales, β1=Media ponderada del nivel de importancia de SA1, β2=Media ponderada del nivel de importancia de SA2, β3=Media ponderada del nivel de importancia de SA3, β4=Media ponderada del nivel de importancia de SA4).

En la escala de valoración de los SA, se consideró cada uno de los indicadores, así como los resultados en relación a la importancia y nivel de impacto. Se seleccionaron los indicadores que obtuvieron mayor nominación en dicha reunión, considerando el nivel de impacto y los SA que ofrecen las barrancas. La determinación de las ponderaciones se obtuvo por especialidad, asignando un porcentaje de importancia y relevancia a cada uno de los SA para su representatividad en el IMAVA. Se concluye que la medición y evaluación del impacto ambiental es un instrumento preventivo de gestión ambiental. En la consideración de Indicadores de gestión y desempeño por monitoreo del desarrollo de Programas de Manejo para la recuperación o reforestación de las barrancas, se recomienda emplear los que tengan mayor relevancia en cada proyecto, con la finalidad de homologar procedimientos, acciones de seguimiento, facilitar la comparación y simplificar el trabajo de control.

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*Author for all correspondence

PARAMETRIC MODELLING FOR THE DESCRIPTION OF THE SULFATE REDUCTION AND BIOMASS PRODUCTION IN A UASB REACTOR

Selene M. García-Solares (1); Isaac Chairez-Oria (1);
Eréndira A. Carrasco-Cándido (1); ***Claudia Guerrero-Barajas*** *(1)

(1) Unidad Profesional Interdisciplinaria de Biotecnología. Instituto Politécnico Nacional (UPIBI-I.P.N). Av. Acueducto s/n Col. Barrio la Laguna Ticomán, México DF, México;

The anaerobic biological process of sulfate reduction (SR) consists of a series of oxidation- reduction reactions that produce hydrogen sulfide (H_2S) and HCO_3^-. The H_2S concentration has been reported as an inhibitory factor in the SR. This concentration is related to the chemical equilibrium of the SR. In a pH range of 4-6, the prevailing sulfur species is the non dissociated H_2S. In a pH range of 8-12 the prevailing sulfide species is the dissociated form of H_2S: HS^-. The SR is used to eliminate some contaminants in residual waters such as organic matter, chlorinated compounds and some metals.
Thus, the objective of this work was to design a mathematical model that describes the relationship between the sulfate reduction and the H_2S production, at low pH, i.e. 4, using a mixture of volatile fatty acids (VFA) as substrate and 12 g/L of sulfate (SO_4^{-2}).

Based on the results obtained from the kinetic experiments conducted in a UASB reactor (Up flow anaerobic sludge blanket reactor), which was inoculated with a sulfidogenic sludge tolerant to high sulfide concentrations even higher than the ones reported as toxic to many more known microorganisms.
At the end of the kinetic experiments 99% of sulfate reduction was obtained coupled to 80% of substrate consumption and a yield of 4 g/L of H_2S at a final pH of 5. From these results, a mathematical model was proposed for describing the relationship between the sulfate reduction, the H_2S production and the produced biomass. The evolution of H_2S over time presented a similar behavior to the proposed model based on the Monod microorganisms growth model.

A parametric identification algorithm was proposed to adjust the proposed model. The time derivative of the model variables was solved by a robust differentiator. The parameters were identified using a Hammerstein-Wienner parametric identification algorithm. The correlation between experimental data and modeling results was 98%.

The sulfate reduction reaction constant was 0.13 h^{-1} meanwhile the H_2S production reaction constant was 0.28 h^{-1}

In conclusion, the results obtained in the present work demonstrated that experimental data obtained for sulfate reduction presented a good correlation with the proposed model for sulfate consumption and sulfide production rates (according to the theoretical stoichiometry for the sulfate reduction reaction).

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*Author for all correspondence

A MATHEMATICAL MODEL BASED ON THE TAFEL EQUATION TO PREDICT THE MICROBIAL FUEL CELL PERFORMANCE

Giovanni Hernández-Flores (1); Juvencio Galíndez-Mayer (2); Omar Solorza-Feria (1); Elvira Ríos-Leal (1); María T. Ponce-Noyola (1); Fernando J. Esparza-García (1); Tatiana Romero-Castañón (3); Héctor M. Poggi-Varaldo*(1)

(1) CINVESTAV - IPN, México DF, México;
(2) ENCB-IPN, México DF, México;
(3) IIE, Cuernavaca, Morelos, México.

The anodic material constitutes the surface area where the microorganisms yield electrons in a microbial fuel cell (*MFC*). The anodic material properties such as high electrical conductivity, long time stability, biocompatibility, low resistance, low cost, and a high specific surface area play an important role to improve the MFC performance.

It was observed that by increasing the anodic specific surface area (A'_s), the volumetric power increases, meanwhile the internal resistance (R_{int}), decreases. In order to scaling up *MFC*s, using a defined anodic material it is of paramount important to predict the behavior of the *MFC* performance.

The goals of this work were: (*i*) to develop a mathematical model based on Tafel equation and (*ii*) to evaluate three anodic materials on the MFC performance to fit the mathematical model. The mathematical model relates the maximum volumetric power ($P_{V,max}$) as well as the R_{int} in a *MFC* to the A'_s. Another anodic properties such as conductance (*C*) or electrolytic conductivity (σ) of the material were incorporated in the developed mathematical model.

The experimental single chamber *MFC* consisted of a horizontal cylinder built in Plexiglas 80 mm long and 57 mm internal diameter. The graphitic anodes used to fill the MFC were a graphite rod (*GR*), triangles of graphite (*GT*) and graphite flakes (*GF*) where their A'_s were 7.3, 931and 1302 m^2/m^3, respectively. The results were: $P_{V,max}$ of 1326, 2108 and 3052 mW/m^3 for *GR, GT* and *GF,* respectively, whereas the R_{int} were 795, 410 and 273 Ω for *GR, GT* and *GF,* respectively. There was a correspondence of either the decrease of R_{int} or the increase of $P_{V,max}$ by increasing the log A'_s of the graphite anodic materials. Based on Tafel equation we developed 18 mathematical models, 9 for the $P_{V,max}$ and 9 for R_{int}. Among these models, according to statistical information (R^2, P-value, F-test or t-tests, analysis of residuals and confidence intervals) on the fitting models tested in our work, we consider the best fitting models as $P_{V,max} = a_0' + a_1' * \log A'_s$ and $R_{int} = b_0' + b_1' * \log A'_s$. Other variables such as σ and *C* or their logarithms, did not contribute significantly in the fitting model.

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*Author for all correspondence

ANÁLISIS ESTADÍSTICO Y MODELACIÓN DE LA SORCIÓN MULTICOMPONENTE DE METALES PESADOS EN AGUA EMPLEANDO CARBÓN DE HUESO

Didilia I. Mendoza-Castillo (1); ***Adrián Bonilla-Petriciolet *** (1);
Juan Jáuregui-Rincón (2)

(1) Instituto Tecnológico de Aguascalientes, Depto. de Ingeniería Química, Av. López Mateos 1801, Fracc. Bona Gens Ote., C.P. 20256, Aguascalientes, México. Tel. (52) 4499105002 ext. 127, Fax. (52) 4499700423. e-mail: petriciolet@hotmail.com;
(2) Universidad Autónoma de Aguascalientes, Depto. de Ingeniería Bioquímica, Aguascalientes, México.

El proceso de sorción empleando carbón de hueso (CH) es una alternativa efectiva y económica para el tratamiento de aguas residuales. A la fecha, dicho material ha sido empleado en la remoción de algunos metales pesados y los resultados obtenidos indican que este sorbente tiene un amplio potencial para la remoción de estas especies contaminantes. No obstante, la mayoría de los trabajos reportados se limitan a la remoción de metales pesados en sistemas monocomponentes pese a que en la práctica las aguas residuales industriales contienen más de un metal. Debido a lo anterior, en este trabajo se estudió la sorción simultánea de iones de Cd^{2+}, Ni^{2+} y Zn^{2+} en solución acuosa empleando CH con el objetivo de identificar y cuantificar los efectos de competencia causados por la presencia de co-iones en la misma solución.

Para el análisis de datos, se aplicaron herramientas estadísticas basadas en un diseño experimental tipo Taguchi y el concepto de señal-ruido (S/R) a fin de facilitar la interpretación de los datos de equilibrio.

Los resultados obtenidos indican que existen efectos de sorción antagónicos significativos, derivados de la competencia de estos iones metálicos que están presentes en la solución multicomponente. De forma global, se ha establecido que el Ni^{2+} tiene el mayor efecto antagónico sobre la remoción de los otros metales, mientras que el Zn^{2+} es el metal con menor efecto de competencia durante el proceso de remoción multicomponente empleando CH. La capacidad de sorción incrementa de acuerdo al siguiente orden: $Cd^{2+} < Zn^{2+} << Ni^{2+}$. El origen de este comportamiento se relaciona con las propiedades fisicoquímicas de los diferentes cationes presentes en la solución. Por otra parte, cabe destacar que con las herramientas utilizadas en este trabajo es posible visualizar detalladamente el efecto de la concentración de los diferentes cationes sobre la remoción multicomponente de los mismos mediante gráficas de la relación S/R. Este enfoque ofrece varias ventajas ya que en la práctica los datos de equilibrio no pueden ser representados gráficamente mediante isotermas de sorción convencionales cuando el sistema multicomponente posee más de dos contaminantes. En síntesis, este estudio introduce la aplicación de herramientas estadísticas alternativas para analizar procesos de sorción en sistemas multicomponentes de forma confiable y sencilla.

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*Autor de correspondencia

SYNGAS TO ETHANOL: A SIMULATION APPROACH

J.C.C. Miranda* (1); G.H.S.F. Ponce (1); M. Alves (1); M.R.W. Maciel (1); R. Maciel F. (1)

(1) University of Campinas, School of Chemical Enginnering.

The chemical route to directly produce higher alcohols, among them ethanol, from syngas ($CO+H_2$) is gaining space in the last decade as a possible route to produce synthetic fuels and additives. Here we simulate a case of study using the kinetic data of K-Co-MoS_2/C catalyst to produce ethanol and propanol from syngas.

The process was divided in three parts: reaction section, separation section and recycle, recycling only pure syngas in order to make possible optimize downstream processes. After sensitivity analysis and optimization the results showed that from 100 kmol/h (1069.8 kg/h) of syngas input are generated 432.91kg/h (99.4 %) ethanol and 92.72 (99.7 %) propanol.

The process showed itself favorable presenting, due the exothermic reaction, a net energy consumption of -959.36 kW/h (cooling).

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*Author for all correspondence

CONTROL ROBUSTO PARA UN CULTIVO CONTINUO DE *Spirulina maxima*

A.E. Rodríguez (1); J.A. Torres-Muñoz* (1); A.R. Domínguez-Bocanegra (2)

(1) CINVESTAV- IPN, México DF, México
(2) TESE, Div. Ing. Qca. yBqca., Edo. deMéx, México.

En este trabajo se presenta un algoritmo no lineal con acción proporcional e integral (PI) para el control de una planta fotosintética. Los modelos matemáticos de plantas fotosintéticas presentan una fuerte sensibilidad en los parámetros, tales que el control de dichos procesos es una tarea difícil de cumplir. Por ejemplo, las leyes de control óptimo o control linealizante pueden presentar problemas de robustez paramétrica ya que suponen el conocimiento perfecto y completo del bio-proceso. El objetivo de control es asegurar el seguimiento asintótico de un modelo de referencia el cual representa el comportamiento que se desea obtener.

El control es robusto en el sentido de que la planta fotosintética puede estar sujeta a perturbaciones paramétricas o a dinámicas no modeladas. La convergencia está basada en el análisis de la dinámica del error entre la salida de la planta fotosintética (sic. concentración de biomasa) y la salida del modelo de referencia por medio de funciones de Lyapunov que inducen de manera natural una acción integral de retroalimentación de dicho error.

Al margen de la complejidad de los bioprocesos debida a sus dinámicas no lineales, estos tienen la bondad de presentar amplios rangos de funcionamiento donde las dinámicas no modeladas se reflejan como perturbaciones que alcanzan valores estacionarios constantes en tiempos relativamente cortos, lo anterior favorece el uso de acciones de control de tipo integral. Se presenta un estudio en simulación para un modelo obtenido de resultados experimentales de un cultivo continuo de *Spirulinamaxima*.

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*Author for all correspondence

MODELING, SIMULATION AND CONTROL OF REACTOR SYSTEM TO REMOVE SULFATES AND HEXAVALENT CHROMIUM

Vicente Peña-Caballero* (1); Elvira Ríos-Leal (2); M. Isabel Neria-González (3); Luis B. Flores-Cotera (2); Ricardo Aguilar-López (2)

(1) UNCA, Oaxaca, México;
(2) TESE, Div. Ing. Qca. y Bqca., Edo. de Méx, México;
(3) CINVESTAV- IPN, México DF, México.

Hexavalent chromium, Cr(VI), is a common industrial chemical used in many processes. It is highly soluble and toxic to microorganisms, plants, and animals, entailing mutagenic than trivalent chromium (Cr(III)). Therefore, the reduction of Cr(VI) to Cr(III) is necessary.

In this work, a no-linear controller algorithm is proposed with the purpose of the feeding trajectory optimization during continuous fermentation of *D. alaskensis* 6SR (sulfate-reducing bacteria) to produce biogenic sulfide in order to create reduction of Cr(VI) to Cr(III). The feed rate profiles are evaluated based on a number of set points using the no-linear sulfate-reducing process and chromium reduction process (SRP-CRP) model (the mathematical model is in the form of ordinary differential equations). Simulation results for SRP-CRP model without control and with control on sulfate concentration, at the same feed rate for the SRP and CRP. It has been demonstrated that nonlinear control has effectiveness and robustness, in reducing sulfate in SRP from 2000 to 500 mg/L. For zero dynamic (i.e. uncontrolled variables), the lactate from 800 to 0 mg/L and biogenic sulfide from 300 to 450 mg/L. In CRP, chromium form 60 to <0.5 mg/L. Entire simulation time, the zero dynamic was stable.

With regulation of the concentration of residual sulfate in biological reactor (SRP) is achieved a high biogenic sulfide production (450 mg/L), in contrast without using a control in the SRP (200 mg/L).

Therefore, the simulation and experimental results suggest that the implementation of a controller on the reactor system (SRP-CRP) can be an alternative to the removal of sulfate and chromium.

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*Author for all correspondence

MODELADO MATEMÁTICO DE CINÉTICAS BIOQUÍMICAS

Ricardo Aguilar López *

CINVESTAV- IPN, México DF, México;

La presente plática tiene como objetivo analizar las características principales de las diferentes estrategias de modelado matemático aplicadas a la descripción de procesos de cinética bioquímica. Se analizarán las características principales de los denominados modelos cinéticos no estructurados, resaltando sus importantes limitaciones en aplicaciones a ingeniería de reactores y control de procesos, ejemplificando con el proceso de hidrólisis de carboximetilcelulosa por *Cellulomonas cellulans*, en donde la velocidad de crecimiento es descrita por diferentes modelos de inhibición por sustrato y por el proceso de sulfato reducción realizados por la bacteria *Desulfovibrio alaskénsis 6SR,* el cual se puede describir por modelos cinéticos de inhibición por producto.

Posteriormente se presentarán las características principales de los denominados modelos cinéticos no estructurados fenomenológicos y los modelos cinéticos estructurados y su respuesta dinámica en modelos de bio-reactores continuos de tanque agitado. Se analizará el caso del proceso ABE de generación de solventes por la bacterias de la especie *Clostridium beijerinckii.*

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*Author for all correspondence

Chapter 14.
Environmental Chemistry

GLYCEROL DERIVATIVES AS BIO-SOLVENTS FOR ORGANIC SYNTHESIS

Adi Wolfson* (1); Dorith Tavor (1)

(1) Green Processes Center, Chemical Engineering Department, Sami Shamoon College of Engineering, Bialik 56, Beer-Sheva, 84100, Israel, adiw@sce.ac.il.

In the past two decades a variety of green solvents have been proposed, including water, ionic liquids, fluorous solvents, and supercritical fluids. However, their implementation in industrial processes is still limited due to their tedious and non-sustainable synthesis, lack of experimental data and familiarity, as well as operational restrictions and high cost.

Several years ago we presented, for the first time, the use of glycerol-based solvents as alternative sustainable reaction mediums in both catalytic and non-catalytic organic synthesis. Glycerol is the main by-product from the conversion of oils and fats in oleochemical production. Moreover, in the past decade, its price has substantially decreased due to an increase in supply from the production and use of fatty acid derivatives in the food, cosmetics, and drugs industries and in biofuel synthesis, i.e., biodiesel. The renewable origin, beneficial physicochemical properties and reusability of glycerol-based solvents, enabled improved product yield and selectivity as well as easy product separation and catalyst recycling. Furthermore, their high boiling point and polarity make them perfect candidates for non-conventional heating and mixing techniques such as ultrasound- and microwave-assisted reactions. Finally, in some reactions, such as catalytic transfer-hydrogenation or transesterification, they can also be used simultaneously as both solvent and reactant.

In our ongoing efforts to design a viable protocol that will facilitate the acceptance of glycerol and its derivatives as sustainable solvents, pure glycerol and glycerol triacetate (triacetin) as well as various glycerol-triacetin mixtures were used as sustainable bio-solvents in four representative organic reactions: nucleophilic substitution of benzyl chloride to benzyl acetate, Suzuki-Miyaura cross-coupling of iodobenzene and phenylboronic acid, baker's yeast reduction of ketones, and transfer hydrogenation of olefins. It was found that reaction performance was affected by the glycerol to triacetin ratio, as the solubility of the substrates in the solvent determined product yield. Thereby, employing optimal glycerol to triacetin ratio resulted in maximum product yield. In addition, using glycerol-based solvents enabled easy and successful separation of the products and recycling of the catalysts.

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*Author for all correspondence

EVALUATION OF THE USE OF ACRYLIC AND SALICYLIC ACIDS AS β-OXIDATION INHIBITORS DURING MEDIUM CHAIN LENGTH POLYHYDROXYALKANOATES PRODUCTION BY *Cupriavidus necator* FROM CANOLA OIL IN PRESENCE OF TRITON X-100.

Víctor G. Ibarra-García (1); Alejandro Téllez-Jurado* (1);
Jorge N. Gracida-Rodríguez (2)

(1) UPP, Hidalgo, México;
(2) UAQ, Querétaro, México

Massive use of synthetic plastics, mainly derived from oil, has caused numerous and serious environmental problems which are mostly caused by their poor biodegradability which added to the decline in world oil reserves make it necessary to find new more eco-friendly alternatives to these plastics. Polyhydroxyalkanoates (PHA's) have come as a viable alternative; nevertheless their fragility has been a drawback for their generalized use. Thus, the aim of this research work was to evaluate the effect of adding acrylic and salicylic acids to fermentation broth during lab scale production of medium chain length PHA's mediated by *Cupriavidus necator* expecting a decrease in their melting point (177 °C of polyhydroxybutyrate as control) as an indicator of an increase in their elasticity in presence of Triton X-100 which has shown has an effect over PHA's melting point.

Fermentations were carried at an Applikon® 5 L bioreactor with a working volume of 3-3.5 L and consisted of 3 stages: 1st biomass adaptation, 2nd exponential biomass growth and 3rd PHA production. The first stage started when reactor was inoculated with 10% v/v of seed culture and lasted about 8 hours after which culture had past lag phase; exponential biomass growth stage started right after this time and was prolonged until a concentration of 15 $g \cdot L^{-1}$ of biomass was reached using an exponential feeding rate, this exponential feeding rate was determined from previous experiments and maintained a μ of 0.17, C/N ratio was of 7 in both first and second stages. 3rd phase started immediately after reaching aforementioned biomass concentration and lasted about 24 hours, the C/N ratio was 200 in this phase and the concentration of Triton X-100 was 0.43 $g \cdot L^{-1}$.
Inhibitors were added at the 3rd phase. Evaluated concentrations of inhibitors were determined after growing *C. necator* in a fermentation broth with canola oil as a sole carbon source. Polymer characterization by NMR and DSC was carried out.

Inhibition tests showed that the needed concentration of both acids to decrease cell growth at least a 75% was less than 5 mM. Melting point of obtained PHA's was notably decreased when comparing to PHB and NMR also showed that monomer composition differed from that of a PHB homopolymer. We can conclude that acrylic and salicylic acids have an effect on *C. necator* metabolism and that they can be used to obtain polymers more suitable to be used as packaging and wrapping while maintaining production costs low.

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*Author for all correspondence

MODIFICACIÓN QUÍMICA DE MATERIALES LIGNOCELULÓSICOS Y SU APLICACIÓN EN LA SORCIÓN DE METALES PESADOS EN SOLUCIONES ACUOSAS

Hilda E. Reynel-Avila (1); Didilia I. Mendoza-Castillo (1); Adrián Bonilla-Petriciolet*(1)

(1) Instituto Tecnológico de Aguascalientes, Aguascalientes, México.

La toxicidad de los metales pesados es inherente a su estructura atómica, además son bioacumulables, no biodegradables y causan enfermedades y desórdenes fisiológicos en los seres vivos. La presencia de este tipo de contaminantes en el agua es un problema ambiental relevante que hace necesario desarrollar soluciones aplicables para alcanzar una remoción efectiva de estos contaminantes. En este trabajo se evaluó a las cáscaras del coco, las cuales son residuos lignocelulósicos abundantes, para remover metales pesados tales como plomo, cadmio y zinc presentes en soluciones acuosas. Se determinó la capacidad de sorción de este material en su forma natural, así como modificado químicamente con ácido tartárico empleando tres concentraciones: 0.2, 1 y 2 M.

Los experimentos de sorción se realizaron en condiciones por lotes utilizando 0.05 g de sorbente y 10 mL de la solución metálica a las siguientes condiciones de operación: pH 5, 30 °C, con agitación constante a 150 rpm.

Los resultados indican que la cáscara de coco en su forma natural tiene una capacidad de sorción máxima de 3.0, 1.93 y 1.1 mg/g para el plomo, cadmio y zinc, respectivamente. Después de modificar químicamente este material, se observó que al aumentar la concentración del ácido tartárico, se incrementó la capacidad de sorción. En forma particular, se logró incrementar entre un 300 y 600% la capacidad de sorción para los tres metales, en comparación con el material en su forma natural. Posteriormente, se determinaron las isotermas de sorción de plomo, cadmio y zinc empleando el sorbente en su forma natural y el modificado con las mejores condiciones experimentales. Los datos obtenidos de estas isotermas de sorción fueron ajustados a diversos modelos clásicos de sorción. En conclusión, se puede establecer que este residuo agroindustrial puede ser utilizado como un sorbente alternativo para la remoción de contaminantes tóxicos y persistentes en el ambiente, el cual muestra capacidades de sorción competitivas comparadas con otros materiales de bajo costo.

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*Autor de correspondencia

INFLUENCIA DE DIFERENTESESPECIES METÁLICAS EN LAS PROPIEDADES DE ADSORCIÓN DEL CARBÓN DE HUESO EMPLEADO EN LA DEFLUORACIÓN DEL AGUA

Cintia K. Rojas-Mayorga (1); Adrián Bonilla-Petriciolet* (1)

(1) Instituto Tecnológico de Aguascalientes, Aguascalientes, México.

La defluoración del agua para uso y consumo humano es una temática relevante en el contexto de control de la contaminación ambiental y la protección a la salud. Se ha estimado que alrededor de 260 millones de personas a nivel mundial están expuestas al consumo de agua potable con concentraciones de fluoruros superiores a 1 mg/L, el cual es el valor límite establecido para prevenir el desarrollo de fluorosis dental y esqueletal. En particular, la adsorción es una de las tecnologías que ofrecen mayores ventajas para la remoción de fluoruros del agua principalmente en países en vía de desarrollo. Sin embargo, la aplicación de esta tecnología es altamente dependiente del adsorbente empleado. El carbón de hueso ha sido considerado como un adsorbente con amplio potencial para la remoción de fluoruros del agua para consumo humano. No obstante, el desempeño de remoción de este adsorbente puede ser mejorado a través de la incorporación de especies metálicas en la superficie del material debido a la alta afinidad electrónica del ión fluoruro. En este estudio se reporta el efecto del proceso de impregnación del carbón de hueso usando cloruro de aluminio, hierro y lantano, así como el desempeño de los materiales modificados en la adsorción de fluoruros del agua.

Los resultados indican que es posible obtener un incremento en la capacidad de adsorción de fluoruros del agua empleando el carbón de hueso modificado. Se estableció que los adsorbentes modificados con el cloruro de aluminio presentaron capacidades de adsorción sobresalientes, en comparación con las muestras modificadas con el cloruro de lantano y de hierro, generando un aumento del 219, 124 y 88%, respectivamente. Adicionalmente, la capacidad de adsorción máxima obtenida en este estudio supera aproximadamente en un 600% a la capacidad de adsorción de fluoruros reportada para los carbones de hueso comerciales. Finalmente, se puede decir que la modificación química del carbón de hueso con especies metálicas es una alternativa interesante para mejorar las propiedades de defluoración del carbón de hueso.

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*Author for all correspondence

DETERMINATION OF OXYGEN TRANSFER COEFFICIENT (k_La) IN A STIRRED TANK BIOREACTOR (STR) INOCULATED WITH *Sphingobacterium* sp.

Cenit Soto (1); Raúl Muñoz (2); Cristal Zúñiga Peña (1); Sergio Revah (1)

(1) UAM- Cuajimalpa, D.F. México
(2) Universidad de Valladolid, Valladolid, España

Methane (CH_4) is the second most important greenhouse gas; CH_4 concentration has increased since the beginning of the industrial era by 150% and possesses a global warming potential 25-72 higher than CO_2. Biological CH_4 treatment is difficult due toits low aqueous solubility (Henry coefficient = 30), which entails the need for new technologies and microorganisms supporting a cost-effective CH_4 elimination.

The objective of this work was to characterize a novel methanotroph *Sphingobacterium* sp. CZ-UAM (GenBankKJ411920) using a stirred tank reactor (STR). This bacterium has shown the ability to produce surfactant and consequently the oxygen transfer coefficients (k_La) were determined in a 3L STR to assess any potential effect of surfactant production on the mass transfer of O_2 and CH_4. The effects of $CuSO_4$ concentration (10 to 50 ug L^{-1}) on CH_4 biodegradation in were also evaluated.

A 3.5 L Applikon bioreactor equipped with 2 Rushton turbines was operated at 800, 600 and 400 rpm. k_La measurements were performed using both the gassing out and sulfite methodologies. The k_La using gassing out were 0.0178, 0.0098 and 0.0049 at 800, 600 and 400 rpm, respectively, and 0.0313, 0.020 and 0.014, using the sulfite method. Biodegradation experiments were subsequently carried out at an air flow of 0.42 L min^{-1}, with an average CH_4 load of 150-200 $gm^{-3}h^{-1}$ and a dilution rate of 0.12 d^{-1}. The STR was operated at 800 rpm during the first 60 days, with a biomass concentration of 1.88 g L^{-1} and EC and CO_2 production values of 20 and 19 $gm^{-3}h^{-1}$ (RE of 14%). An increase in copper concentration up to 50μg L^{-1} resulted in EC, CO_2 and RE values of 55 y 91 $gm^{-3}h^{-1}$ and 23%, respectively (22 days). Then, the stirring rate was changed to 600 and 400 rpm, resulting in lower values for the EC, CO_2 and RE (lasted 25 days).

These empirical k_La were used to estimate the maximum EC after copper addition at the same operating conditions entailed experimental EC values close to twice the theoretical values, which was attributed to the presence of surfactants enhancing CH_4 elimination. This was confirmed by static partition experiments.

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*Author for all correspondence

ACIDOGENIC DIGESTION OF MICROALGAL BIOMASS TOWARDS BUTYRIC ACID PRODUCTION

Serge R. Guiot* (1); Marvin Gruhn (2); Guillaume Bruant (1); Jean-Claude Frigon (1)

(1) Energy, Mining and Environment Portfolio, National Research Council Canada (NRC). Montreal, Canada, H4P 2R2;
(2) Brandenburg Technical University Faculty of Environmental Sciences and Process Engineering 03046 Cottbus, Germany.

Anaerobic digestion (AD), a well-known, reliable and proven process for the conversion of a range of organic wastes into methane, including algal residues, can be constrained to the hydrolysis and acidification steps, to produce a range of low-carbon chemicals, among which short-chain carboxylic acids (VFA, i.e. acetic, propionic and butyric acids), with a higher commercial value than methane, especially the butyric acid.

This study compared the digestion of the microalgal biomass *Scenedesmus sp*.-AMDD under mesophilic (35 °C) and thermophilic (55 °C) conditions. Two 6L digesters (CSTR) were inoculated with bovine manure and operated at a hydraulic residence time (HRT) of 15 days, an organic loading rate (OLR) of 2.3 g total volatile solids (TVS)/L.day, and at a pH of 4.5 to prevent methane formation. The 55 °C digester showed better VFA and butyrate production during the first month of operation but this lead faded away. After 7 months of operation, the VFA concentration in the effluent were at 6455 and 3700 mg/L at 35 and 55 °C, respectively. This represents 190 and 93 mg VFA/g TVS in, with 81 and 87% as butyrate, at 35 and 55°C, respectively. The decrease of the HRT to 10 days reduced significantly the hydrolysis and the the acidification of the algal biomass (by 36% and 64%, respectively).

Metagenomic analyses (high throughput sequencing) were performed on both digesters and inoculum (manure). A significant impact was observed on the bacterial diversity with less phyla identified (20 phyla in manure as opposed to only 7 at 35°C and 11 at 55°C). The Firmicutes became the ultra dominant phylum in both mesophilic (66% of the total population) and thermophilic (80%) conditions. Inside the phylum Firmicutes, at 35°C, two classes strongly increased: Bacilli (22% of the total population) with dominance of Lactobacilli (57%) and Clostridia (42%) with dominance of *Ruminococcaceae* (25%). At 55 °C, the genus *Thermohydrogenium* (family *Syntrophomonadaceae*; order *Clostridiales*; class *Clostridia*) (76% of the total population).

In conclusion, mesophilic conditions at an HRT of 15 days seem to be more favorable than thermophilic conditions for an acceptable level of digestion of *Scenedesmus.*

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*Author for all correspondence

Chapter 15.
Environmental Health

THE USES OF ESSENTIAL OIL OF *Eucalyptus Camendulensis* AGAINST PATHOGENIC MICROORGANISMS

Mehani Mouna* (1); Salhi Nesrine (2); Valeria Terzi (3); Héctor Segni Ladjel (1)

(1) Laboratory of Process Engineering, University of Kasdi Merbah, Ouargla, Algeria;
(2) Laboratory for Bioressources Saharan preservation and enhancement, University of kasdi Merbah, Ouargla, Algeria;
(3) Istituto Sperimentale per la cerealicoltura, Fiorenzuola, Italy.

Red River Gum (*Eucalyptus camaldulensis*) is a tree of the genus *Eucalyptus* widely distributed in Algeria and in the world. The value of its aromatic secondary metabolites offers new perspectives in the pharmaceutical industry. This strategy can contribute to the sustainable development of our country.

The aromatic plant harvested in the month of May 2010 is among the most abundant species in the region South of Algeria. These *Eucalyptus camendulensis*: has been selected for screening antibacterial. Preliminary tests performed on the essential oil of *Eucalyptus camendulensis* showed that this oil has antibacterial activity vis-à-vis the bacterial strains (*Enterococcus feacalis, Enterobacter cloaceai, Proteus microsilis, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa*) and antifungal (*Fusarium sporotrichioide and Fusarium graminearum)*. We used the dilution method on medium solid to determine the minimum inhibitory concentration (MIC). The culture medium used was nutrient broth Muller Hinton. The interaction between the bacteria and the essential oil is expressed by a zone of inhibition with diameters of MIC indirectly expression. We used the PDA medium to determine the fungi activity.

The extraction of the aromatic fraction (essentially oil- hydrolat) of the fresh aerian part of the Eucalyptus camendulensis was performed by hydrodistillation. The average essential oil yield is 0.99%.

The antimicrobial and fungal study of the essential oil and hydrosol showed a high inhibitory effect on the growth of pathogens.

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*Author for all correspondence

PERSPECTIVAS EN LA GESTIÓN DE PAÑALES DESECHABLES USADOS: UNA REVISIÓN

Perla X. Sotelo-Navarro (1); Sylvie J. Turpin-Marion (1); Rosa M. Espinosa-Valdemar (1); Alethia Vázquez-Morillas (1); Margarita Beltrán-Villavicencio (1); Héctor M. Poggi-Varaldo* (2)

(1) UAM-A, México DF, México
(2) CINVESTAV- IPN, México DF, México;

Los pañales desechables constituyen un porcentaje significativo de los residuos sólidos municipales, debido a que son utilizados en todos los estratos de la población. Dada su composición constituyen un reto para su manejo adecuado. En los países desarrollados pueden ingresar a los procesos de incineración y de recuperación de energía, sin embargo, en Latinoamérica se disponen en rellenos sanitarios y tiraderos a cielo abierto, sin tratamiento alguno. El diseño y composición de los pañales desechables ha cambiado considerablemente a través de los años. Sus componentes principales son celulosa, plásticos y un polímero súper absorbente. Esta composición ha dado pie a distintas propuestas de tratamiento y recuperación de los materiales, principalmente de los plásticos y de fracción orgánica biodegradable como lo es la celulosa. Desde los años 90 se han realizado a nivel laboratorio diversos estudios sobre el tratamiento y aprovechamiento de los pañales desechables y algunos de sus componentes, entre ellos: la producción de hongos comestibles, el proceso de composteo y recientemente el tratamiento anaerobio de la fracción orgánica biodegradable para la producción metano, sin embargo, sólo el composteo se ha implementado a gran escala.

Por otro lado, las características de los plásticos como materia prima y sus posibles patrones de reciclaje necesitan ser estudiados, así como nuevas alternativas más prometedoras y productivas de tratamiento de este tipo de residuos. Esta revisión describe los cambios en la composición, los efectos ambientales del uso y producción de los pañales, la legislación para su manejo y gestión cuando se transforman en residuos, los tratamientos que son comúnmente aplicados y las perspectivas para un mejor aprovechamiento de los mismos.

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*Author for all correspondence

USO DE *Bacillus* sp. COMO BIOCONTROL DEL HONGO PATÓGENO *Colletotrichum gloeosporioides* EN TOMATE DE ÁRBOL *Cyphomandra batacea*

Tifanny Robayo (1); Erika Rodríguez (1); Luisa Velásquez* (1)

(1) Universidad Autónoma de Colombia, Ing. Ambiental, Bogotá D.C., Colombia.

El biocontrol surge de la necesidad de mitigar y prever el impacto que se genera a partir de patógenos, el hongo patógeno *Colletotrichum gloeosporioides* es uno de estos, se considera causante de la enfermedad antracnosis la cual genera manchas de color oscuro en su tallo, hojas y frutos; esta afectación es visualizada en el tomate de árbol, fruto producido en Colombia. El impacto que se genera en materia ambiental y económica para los agricultores es muy alto ya que se busca solucionar esta problemática por medio de sustancias químicas xenobióticas como fungicidas, porque en muchas ocasiones no son efectivas generando perdidas hasta un 45% en la producción. Por este motivo, el objetivo planteado es implementar el mecanismo de biocontrol con Bacillus sp. a nivel de laboratorio para combatir el patógeno *Colletotrichum gloeosporioides* en el tomate de árbol *Cyphomandra batacea.*

El desarrollo se da a nivel de laboratorio, inicialmente se realizó el aislamiento, identificación y selección de las cepas que cumplían con las características propias del hongo en agar PDA (colonias de color naranja con un micelio más bien aplanada y color de fondo gris o blanco), posteriormente se realizaron diferentes diluciones de suelo con urea y sin urea en agar nutritivo (AN), luego se tomaron las cepas efectivas para su caracterización a nivel microscópico, resultando efectivas en la cepa con la disolución en la tierra con urea en 10^{-2}, 10^{-3} y sin urea 10^{-4}. Se realizo por último, la prueba de antagonismo donde se tomó una caja de petri y se dispuso cada microorganismo en lados opuestos, visualizando mayor crecimiento de la bacteria respecto al hongo. De tal manera, se comprobó la efectividad del *Bacillus sp*. por medio de las diferentes reacciones metabólicas, bioquímicas, mecánicas y/o físicas que desarrollo esta bacteria por medio de la competencia observada entre el patógeno y el biocontrolador.

Se concluyó, la efectividad del biocontrol con *Bacillus sp*. porque se pudo observar la competencia que se genera entre el patógeno y la bacteria, quien inhibe el crecimiento y desarrollo del hongo. Por lo tanto, se puede afirmar que la presencia del *Bacillus sp*. en tierra con alto contenido de úrea o de nitrógeno, favorece el desarrollo de este biocontrol y el hongo, al ser tratado de manera biológica con esta bacteria puede reducir el impacto ambiental de sustancias xenobióticas y las perdidas en la agricultura que se generan actualmente en Colombia.

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*Author for all correspondence

Chapter 16.
Environmental Nanotechnology

ISOLATION AND CHARACTERIZATION OF FLAVONOIDS FROM POINSETTIA (*Euphorbia pulcherrima*) FOR A GREEN SYNTHESIS OF CONDUCTIVE BIOPOLYMER NANOCOMPOSITES

Rafael Leyva-Huitrón (1); Francisco Díaz-Cedillo (1); Tomás A. Fregoso-Aguilar (1); Darío I. Téllez-Medina (1); Rafael Herrera-Bucio (2); Jorge A. Mendoza-Pérez*(1).

(1) ENCB-IPN, México DF, México; (2) IIQB-UMSNH, Morelia, Mich.

Flavonoids are important and widespread plant metabolites, occurring naturally and with a great number of applications; they are used as antioxidants in food preparations and natural colorants (dyes) but their role as a chemical resource for engineering materials is still under research. Presence of Aromatic π-bonds in the main structure of flavonoids leads to a high possibility for synthesis of conductive biopolymers, able to be used as constitutional units of nanocomposites. Green chemistry is considered the best option for chemical work in order to have environmental benefits..

The present work was first developed under classic chemical processing and reaction conditions but the main goal was the isolation of a naturally occurring material for later polymerization under green chemistry principles.Flavonoids naturally structured as Cyaniding glucosides(Cygs) are the most important dyes in Poinsettia and give to the leaves their typical red color in winter. They were selected because the aromatic π-bonds in the molecule are potentially useful addition sites. Poinsettia leaves were dried at 40° C and their extract was obtained by Soxhlet in non-acid conditions, using hexane for grease removal and methanol for Cygs extraction. Solvents were evaporated under low pressure conditions.

Extract yields were near 100 mg of flavonoid mixture per gram of dry weight. The specific eluents for the chromatographic work with the mixture were identified by dye mobility in filtering paper, using different solvents. Materials were purified on column chromatography using the eluentestablished from the filtering paper test. Both the raw and pure extracts were characterized with FTIR, $^{1}H/^{13}C$-NMRand uHPLC. Extracts were submitted to hydrolysis in the glycoside bond with p-toluensulfonic acid.Total energy of the flavonoid target molecule(an anthocyanidin)after the hydrolysis was calculated by an *ab initio*Hartree-Fock approximation, in order to perform a theoretical prediction of possible polymerization products while performing the green synthesis route that has been proposed. The dyes obtained are being tested under microwave and UV light assisted green chemistry synthesis to form biopolymers potentially useful as organic semiconductor materials. Electrical conductivity will be evaluated by probe measurements after fixing and drying the biopolymer over a support material and if the tests showpositive results the biopolymer will be fluid and deposited at nanostructured scale for nanocomposite processing.

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*Author for all correspondence

MECHANISMS INVOLVED DURING THE PRODUCTION OF PALLADIUM NANOPARTICLES BY *Geobacter sulfurreducens*

Aurora Pat-Espadas (1); Elías Razo-Flores (1); J. René Rangel-Mendez (1); Francisco J. Cervantes* (1)

(1) División de Ciencias Ambientales, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), Camino a la Presa San José 2055, Col. Lomas 4ª. Sección, C. P. 78216, San Luis Potosí, SLP, Mexico

Because of the increasing demand of the precious metal, palladium (Pd), its price has oscillated and its recovery has become a reason of concern since availability is limited. Special interest on nanoparticles production by biological synthesis using bacteria has been explored. Several strains have been reported to reduce Pd (II) to Pd (0), but information about mechanisms involved is limited. Therefore the aim of this study was (1) to describe mechanisms involved in the microbial reduction of Pd(II) to Pd(0) by *G. sulfurreducens*, (2) to investigate the effect of anthraquinone-2,6-disulfonate (AQDS) as redox mediator in the reduction process and (3) to develop a mathematical model capable of describing the processes.

Microbial reduction experiments were carried out with late-exponential cells of *G. sufurreducens* (800 mg/L cell dry weight (CDW)), using a minimum reaction media, under anaerobic conditions. Different concentrations of Pd(II) were tested: 25, 50 and 100 mg of Pd(II)/L and acetate (5mM) was used as electron donor. Experiments amended with 100 µM AQDS as redox mediators were performed in parallel as well as cell-free media as control experiments. All experimental treatments were set up in triplicate, sampled at specific intervals and incubated at 30° C for 24h.

Results demonstrated that *G. sulfurreducens* is capable to reduce Pd(II) to Pd(0) by two mechanisms. **i)** direct, which involves interactions of amide, sulfur, and phosphoryl groups associated to proteins from bacteria on palladium reduction reaction, which includes 3 steps: electrostatic interaction, ligand substitution and reduction and nucleation, and **ii)** quinone-mediated, which implies multi-heme c-type cytochromes participation. In addition AQDS played two important roles: enhanced the microbial reduction of Pd(II) by *G. sulfurreducens* and facilitated the reduction of solid species of palladium ($Pd(OH)_{2(s)}$). Furthermore models describe well both mechanisms, considering important elements such as speciation, delay factor, area of bacteria, and mass transfer considerations for direct and quinone-mediated microbial reduction of Pd(II). The obtained parameters also give information about the process impact on the cells and the influence of external factors on direct and quinone-mediated palladium reduction.

This work provides elements for a better understanding of direct and quinone-mediated palladium reduction by *G. sulfurreducens*, which could facilitate metal recovery with concomitant formation of valuable palladium nanoparticles in industrial processes.

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*Author for all correspondence

DESARROLLO DE UN APTASENSOR PARA DETERMINAR LA CALIDAD DEL AGUA POTABLE EN TIEMPO REAL

Elia K. Velázquez (1); Israel Morales-Reyes (2); Nikola Batina (2); Oscar Monroy-Hermosillo* (1)

(1) CBS, UAM-I, México D.F, México.
(2) CBI, UAM-I, México D.F, México.

Actualmente, los métodos que se usan en México para determinar la calidad microbiológica del agua (NMP y filtración por membrana, NOM 127) están basadas en métodos estandarizados internacionales (APHA) requieren de por lo menos 48 horas de incubación para detectar bacterias fecales y de 2 a 3 días para tipificarlas. Estos métodos no están al alcance del consumidor de agua potable, por lo que se propone proporcionar una tecnología que le permita determinar la calidad microbiológica del agua que consume.

El objetivo de este trabajo es desarrollar un biosensor que permita conocer la calidad microbiológica del agua en tiempo real mediante la nanobiotecnología, empleando aptámeros específicos (E1, E2 y E10) y nanotubos de carbono (NTC) para medir el efecto que tienen las bacterias (*Escherichia coli*) sobre la conductividad electrolítica del agua.

En electrodos de grafito modificados con NTC se observo el efecto de la inmovilización de los aptámeros por enlace químico sobre su superficie para la detección de bacterias *E.coli* suspendidas en agua. Con tiempos de contacto de 5 min se midió la conductividad de los electrodos modificados con y sin aptámeros (E-NTC y E-NTC-A) en una suspensión de *E.coli* ($23*10^5$ UFC/mL) y agua de la red de Iztapalapa (ARI). Los resultados muestran que hay una disminución en la conductividad electrolítica del ARI al comparar el blanco (0.36 mS/cm, agua sin bacterias), la suspensión bacteriana sin y con aptámeros (0.31 mS/cm y 0.24 mS/cm, respectivamente). Los resultados sugieren una mayor presencia de bacterias atrapadas en el electrodo modificado con aptámeros que sin ellos. Para corroborar esta suposición, se tomaron micrografías con microscopia electrónica de barrido (SEM) en todos los experimentos. La optimización del aptasensor se encuentra en proceso para incrementar la sensibilidad, calibración y selectividad.

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*Author for all correspondence

Chapter 17.
Miscellaneous

REMOVAL OF STARCH FROM STARCH SOLUTIONS BY TANGENTIAL FLOW FILTRATION

Cirilo Nolasco-Hipolito* *(1)*; Octavio Carvajal-Zarrabal (2); Souji Esaki (3); Samantha Siong Ling-Chee (1) and Maria Guadalupe Aguilar-Uscanga (4)

(1) Faculty of Resource Science and Technology, University Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, MALAYSIA. Tel.: +60 138-134160; e-mail: hcnolasco@frst.unimas.my
(2) Biochemical and Nutrition Chemistry Area, University of Veracruz, SS Juan Pablo II s/n, Boca del Río, CP 94294 Veracruz,México.
(3) Kurume National College of Technology, 1-1-1 Komorino, Kurume-shi, Fukuoka 830-8555 Japan.
(4) Instituto Tecnológico de Veracruz, Unidad de Investigación y Desarrollo en Alimentos (UNIDA), Av. Miguel A. de Quevedo 2779, Col. Formando Hogar, C.P. 91860 Veracruz, México.

The starch sago industry in Sarawakis is operated with improper discharge of effluent into the nearby rivers. Tangential flow filtration (TFF) is an attractive alternative for treatment of sago effluent. The aim of this study was to demonstrate that TFF could remove the sago starch from model suspensions (2% w/v) to simulate the effluent of sago starch producing companies.

Polysulfone membrane filter cassettes (0.45 µm) with membrane area of 0.1 m^2, 0.2 m^2 and 0.3 m^2 were used to apply TFF. The starch suspensions (50 L) were filtered and concentrated by TFF to a final volume of less than 15 L.

The use of high starch concentration resulted in the high degree of fouling that affected the membrane permeability. The backflushing technique was applied to revert this effect. Consequently, the processing time took only five hours for the treatment of 2% starch solution. Expanding the filtration area from 0.1 m^2 to 0.2 m^2, and to 0.3 m^2 improved the filtration efficiency in the TFF of starch suspensions. The time process decreased from 1 hour to 30 minutes; concomitantly the removal of water increased from 81% to 85.4% for the treatment 1% starch suspension. In contrast, the normalized permeate flux (NPF), specific volume (SV), normalized retentate flux (NRF), normalized instantaneous specific flux (NISF), transmembrane pressure (TMP) and water recovery (WR) decreased as increased the filtration area at fixed feed flow of 4.5 LPM. These parameters decreased with operational time due to membrane fouling, except TMP. However, backflushing the membranes decreased the TMP and enhanced their permeability. The permeate generated was free of total suspended solids (TSS), turbidity and chemical oxygen demand (COD) and were significant different (p<0.05) between before and after treatments. These results suggest that using a membrane area of 0.3 m^2 as well as the backflush system could improve the efficiency of the removal of starching TFF for *in-situ* studies.

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*Author for all correspondence

EVALUATION OF A CONSTRUCTED WETLAND ATTACHED TO MICROBIAL MATS WITH RECIRCULATION FOR THE IMPROVEMENT OF THE CONDITIONS OF CULTIVATION OF *Litopenaeus vannamei*

Otniel Tostado-García (1); Ignacio A. Pérez-Legaspi (1); Carlos I. Pérez-Rostro (1); Paula N. Robledo-Narváez (2); Luis A. Ortega-Clemente* (1)

(1) ITBOCA, DEPI. Boca del Río, Ver. Mexico;
(2) ITSTB, Depto. Inv., Tierra Blanca, Ver. México.

The shrimp farming industry is growing significantly, bringing large financial profits, but many ecological problems have been presenting because of this activity. Therefore, it is necessary to apply several actions with the aim of stopping the deterioration process in some regions by discharges of water from shrimp farming, offering alternatives for improvement in the process of cultivation. The present study evaluated the integrated treatment by a constructed wetlands attached to microbial mats with recirculation as biofiltration systems to scale to improve the conditions of cultivation of *Litopeneaus vannamei.*

The treatments were T1: Constructed wetland, T2: Constructed wetland/microbial mats, T3: microbial mats and T4: Control. The study was conducted for a period of 91 days. The measurement of the parameters of ammonium, nitrites, nitrates and phosphates was carried out weekly; pH, OD and temperature daily and the biometrics every 15 days.

The results show that the treatment T2 presented the lowest concentrations of ammonium, nitrites and phosphates (NH_3-N mg/L: 0. 42 ± 0. 34; NO_3-N mg/L: 0. 06 ± 0. 06; P mg/L: 1. 38 ± 1. 08, respectively). However, between treatments there was no significant statistical difference. With respect to the specific growth rate (% day), the treatment T3 (4. 19 ± 0. 34 % day) was more efficient in the process of growth of the organisms in comparison to other treatments (T1: 4. 18 ± 0. 01, T2: 3. 98 ± 0. 17, T4: 3. 73 ±0 . 09 % day). The multiple correlation analysis, shows that the ammonium presented a high positive correlation with phosphate, temperature, nitrates (0.9977, 0.9588 and 0.9514, respectively), and an intermedia positive correlation with nitrites (0.7439). On the other hand, correlation between the type of biofilter in farming systems presented slightly high correlations with parameters, temperature, ammonium (0.8918, 0.8289) and tights with phosphates and nitrates (0.7930 and 0.6518). In general, there was significant difference between treatments constructed wetland, microbial mat and the combination of the two. I.e., three biofiltration systems were efficient in the improvement of the conditions of cultivation of *Litopeneaus vannamei.*

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*Author for all correspondence

EFECTO DE *Azospirillum* EN EL CRECIMIENTO VEGETAL DE DOS HORTALIZAS, EN UN CULTIVO EN ALMÁCIGO SIN FERTILIZAR Y CON AGREGADO DE COMPOSTA

R. Esquivel-Cote* (1); O.G. Velasco-Becerril (1); F. Jiménez-Flores (2); I.E. García de Salamone (3); g. Tsuzuki-Reyes (1); R.M. Ramírez-Gama (1)

(1) Facultad de Química, Departamento de Biología, UNAM, México,
(2) JITOSAN S.P.R. de R.L., Xochimilco, DF, México, (3) Cátedra de Microbiología, FAUBA, Buenos Aires, Argentina.

Azospirillum representa uno de los géneros mejor caracterizados de rizobacterias promotoras de crecimiento vegetal. Su uso como biofertilizante de cultivos agrícolas y forestales es una biotecnología muy atractiva para sustituir el uso de agroquímicos en el campo y así reducir los riesgos de contaminación de suelo y agua. La lechuga (*Lactuca sativa*) y la espinaca (*Spinacia oleracea*) son las hortalizas de hoja de mayor consumo mundial. El efecto de *Azospirillum* en el crecimiento de estas plantas, así como de otras hortalizas, no ha sido hondamente documentado. El objetivo del presente trabajo fue evaluar el efecto de diferentes cepas de *Azospirillum* en el crecimiento de plantas de lechuga y espinaca cultivadas por almácigo sin la adición de fertilizantes químicos y con composta como sustrato.

Para este estudio se evaluaron la cepa AZm5 (*A. lipoferum*) aislada de suelos contaminados con aguas residuales (México) y la cepa Az39 (*A. brasilense*) ampliamente empleada en biofertilizantes comerciales de Argentina. Las plántulas se inocularon cuando presentaron las primeras dos hojas verdaderas. Se consideraron tres tiempos para los muestreos (inoculación, trasplante, cosecha). El trasplante de llevó a cabo en suelo con composta (1:1) sin esterilizar.

Las cepas de *Azospirillum* son capaces de colonizar las raíces de estas dos especies vegetales. Esto demuestra la capacidad competitiva y colonizadora de estas bacterias aun sobre un sustrato con alta carga microbiana. Asimismo incrementaron significativamente el contenido hídrico y la producción de biomasa en plantas de lechuga y espinaca después del trasplante sin el uso de fertilizantes químicos, con lo que concluimos que este sistema de cultivo es una biotecnología ambientalmente adecuada para la producción sustentable de estas hortalizas de hoja. El uso de trasplantes a partir del almácigo en el establecimiento de hortalizas es una práctica común, que permite producir plántulas de calidad consistente y proveer un establecimiento temprano de plantas en el campo. Se piensa que la inoculación con estas bacterias podría favorecer la aclimatación del trasplante, brindar protección frente a la falta de agua y estimular el desarrollo de raíces adventicias. Además, podría disminuir el uso de fertilizantes pues para lograr los incrementos productivos obtenidos mediante la inoculación sería necesario agregar hasta 150 kg ha^{-1} de estos agroquímicos y esto tiene el consiguiente riesgo para el ambiente.

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*Author for all correspondence, rosesquivel_cote@hotmail.com, Tel/Fax (55)56223763,

DYEING POTENTIAL OF COLORANT-BEARING PLANTS OF THE YUCATAN PENINSULA, MEXICO

Manuel J. Chan-Bacab (1), Patricia Sanmartín (2), Juan C. Camacho-Chab (1), ***Benjamín O. Ortega-Morales**** (1)

(1) Universidad Autónoma de Campeche, DEMAB, Campeche, México.
(2) Harvard University, School of Engineering and Applied Sciences, USA
(3) Universidad de Santiago de Compostela, Facultad de Farmacia, España

Synthetic dyes are still the primary option chosen to dye textiles, in spite of the potential health and environmental risks, as some of them have been reported to have carcinogenic and other cytotoxic effects. In some region this discovery has prompted a resurgence of the use of dyes derived from natural to replace, at least partially, synthetic dyes. However, contrary to dominant public opinion, natural dyes are neither systematically safer nor more ecologically sound than synthetic dyes. Adjunct agents known as mordants that help bond the molecules of the fabric being dyed and the colorant used to dye it are often toxic. There is scant published information describing the dyeing potential and toxicity of colorant-bearing plants and the dye uptake with respect to the mordant treatments. Thus, the objectives of our study were to: (1) carry out a preliminary plant inventory and ethnobotanical study of the traditional dyeing resources from this region (Yucatan peninsula), (2) extract the natural dyes from the four most used plant species, (3) assess the potential toxicity of these selected natural dye extracts through bioassays of *Artemia salina* and *Lactuca sativa*, and, lastly (4) dye ordinary cotton cloth (manta) with *Bixa orellana* dye extract (one of the four selected), analyze the fabric dyed and to examine the color properties.

In this study, the preliminary survey of Yucatecan flora based on a range of sources of information showed its resourceful potential to obtain natural dyes. From the 23 plants included in the inventory and ethnobotanical study, four were selected to yield natural dyes. Dyes from *Justicia spicigera*, *Bugainvillea glabra*, and *Rhoeo discolor* without mordant demonstrated to be adjectives, contrary to fabrics dyed with *Bixa orellana* dye without mordant, which showed an adherence to the fabric material. In all cases the yield of extracted material was better with the traditional heating method (decoction) than with from the microwave method. Moreover, 10% ethanol replacing distilled water proved to be more efficient. The dye baths without mordant only present few toxicity, the *J. spicigera* and *B. glabra* dyes being the most toxic. The *B. orellana* dye was less toxic, although its toxicity is increased when mordants are used. Consequently, caution should be taken when stating that natural-derived dyes are safe to the environment and to the manufacturer or end user, as many of them require the use of mordants that shows a level of toxicity. Regarding the color performances of the dyed fabrics, mordant influenced the depth of the shades, improving dyeing and color fastness, although it had no significant effect on color values. All dyed fabrics had similar color, with hue angle values indicating yellowish, orange-yellowish color.

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*Author for all correspondence

DESALINATION OF SEA WATER WITH AQUATIC LILY

Isela V. Arámburo-Miranda*; Emmanuel H. Ruelas-Ramírez

Universidad Mexiquense del Bicentenario, Unidad de Estudios Superiores Tultitlán Edo. de México

The quantity of water in the world is 97.5% salt water and only 0.32% is available for human consumption. Due to the shortage of potable water currently exists in very dry regions such as the Middle East and north of our country, has had the need to investigate and develop new technologies to get this resource from seawater. Some technologies such as distillation, electrodialysis, reverse osmosis and evaporation by direct freezing have been developed for this purpose. However, the investment costs of desalination plants are very high reaching up to $ 2100.00 per cubic meter.

The Plant adaptation to osmotic stress (halo-conditioning) is only carried out at salinity soils for horticultural crops and not floating aquatic plants. This adaptation is achieved by the application of H2O2 (hydrogen peroxide) which inhibit the SV (slow vacuolar channel), which allows the entry of sodium ions into the cytoplasm.

In Mexico the water hyacinth (*Eichhornia crassipes*) is identified as a problematic weed nonetheless has a significant capacity to absorb heavy metals in their root system as well as other inorganic salts and can be found in freshwater and sometimes in low salinity waters its growth is greatly influenced by the levels of nutrients in the water, especially the levels of nitrogen, phosphorus and potassium. The moisture content of the lily has an average value of 93-95%, which represents a high water content which is not utilized so far as a possible source of this resource.

Considering the previous, this research has like aim bring lily in salt water using hydrogen peroxide, to desalinate seawater and retrieve the contents of water inside, allowing a more efficient scheme to reduce costs seawater treatment and be friendlier to the environment.

The method used is a modification of the methods of adaptation with peroxide in land plants, by varying the concentration of peroxide in water. Once adapted lily is processed to recover the water inside and the salt content is determined. Until today have gotten favorable results in adapting the lily in seawater.

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*Author for all correspondence

Pseudomonas aeruginosa ISOLATED FROM CORN RHIZOSPHERE AND COMPOST IN THE STATE OF GUERRERO, MEXICO THAT PRODUCE BIOSURFACTANTS AND ARE RESISTANT TO HEAVY METALS

Jeiry Toribio-Jiménez (1), Miguel A. Rodríguez Barrera (1), José L. Aguirre-Noyola (1), Daniel Chávez-González (1), Joseph Guevara-Luna (1), Abigail González (2), Adolfo Román-Román (1), Yanet Romero-Ramírez *(1)

(1) Academic Unit of Science Chemistry Biology, Autonomous University of Guerrero, Mexico. AutonomousUniversity of Guerrero, Mexico. Av. Lázaro Cárdenas s/n. Ciudad Universitaria. Apdo Postal 39070, Chilpancingo, Guerrero. Email: yromero@uagro.mx . Phone: 01-747-4719310 ext. 4525. Fax: 01-747-47 25503.
(2) Department of Molecular Biology and Biotechnology, Institute of Biomedical Research. University National Autonomous of Mexico.

Biosurfactants are molecules produced by a wide range of microorganisms including bacteria, have broad biotechnological applications as they are compatible with the environment, resist high or low temperature, pH, salinity, and applied for decontamination of objects contaminated with heavy metals, hydrocarbons, pesticides, among other places. This study describes strains of *P. aeruginosa* autochthonous capable of producing mono and di-rhamnolipid biosurfactants isolated from roots of creole maize and composts.

Six strains of *P. aeruginosa* were isolated, four from creole maize and three from compost. The production of Biosurfactants were evaluated in each of the strains through the production and stability of foam on PPGAS liquid medium, hemolysis on blood agar plates, drop collapse assay, the IE24 and emulsification ability. Heavy metal resistance was also evaluated. We observed through TLC that all the strains were able to produce mono and di-rhamnolipid biosurfactants, the strains PAM8 and PAM9 isolated from maize were the best biosurfactant producers and their extracts showed antimicrobial activity against Gram-positive, the IE24 with most activity in cyclohexane, xylene, toluene, diesel, vegetal and mineral oil. We also observed that the strain AM8 showed a 28.3%, and the strain PAM9 a 20.3% of hydrophobicity cell capacity to hydrocarbons, furthermore were capable of growth on various carbon sources such as glucose, toluene, cyclohexane, petroleum and diesel. Regarding heavy metal resistance we observed that all strains showed the same profile: As5+>As3+>Zn2+>Pb2+>Fe3+>Cd2+>Cu2+>Cr6+ in concentrations of 20, 10, 10, 6, 4, 4, 2 and 2 mM respectively. In conclusion, we described the isolation and characterization of strains of *P. aeruginosa* from roots of creole maize and composts of state of Guerrero, Mexico capable of producing biosurfactants mono and di-rhamnolipid type and growth on diverse carbon sources in the presence of high concentrations of heavy metals.

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*Author for all correspondence

APPLICATION OF MICROASSAY METHOD FOR ENZYMATIC HYDROLYSIS OPTIMIZATION OF WHEAT STRAW

Carlos E. Molina-Guerrero* (1); Arturo Sánchez (2)

(1) Depto. de Ing. Amb., DICIVA, Universidad de Guanajuato, Irapuato, México. *e-mail:carlosmolina0@hotmail.com
(2) CINVESTAV- IPN, Unidad de Ingeniería Avanzada, Guadalajara, Jal. México.

Lignocellullosic materials can be considered as suitable feedstock for the production of bioethanol of second generation (2G) due to their availability worldwide, whose estimated production is around 10-15 billion tons per year. Enzymatic hydrolysis of cellulose to glucose is carried out by cellulase enzymes which are highly specific catalysts. Microassay methods represent a technical advance to select the most appropriate combinations since many different cellulase complexes can be evaluated rapidly. Furthermore, when reducing the volume assay, the amount of enzyme required for the tests is very little. Since recombinant enzymes obtained at lab-scale are often produced in small quantities, micro-assay methods are appropriate to evaluate the enzymatic performance of novel enzyme mixtures.

This work studies the combined effects of temperature, pH and enzyme-substrate ratio (E/S_R) on hydrolysis yield and specific reaction rate (S_{RV}) in microassay in order to maximize enzymatic hydrolysis of pretreated wheat straw (WS).The WS was pretreated by alkaline-peroxide. Enzymatic complex Accellerase 1500^{TM} was used for hydrolysis assays. Using response surface methodology, optimal parameter values were determined. A complete enzymatic kinetic of the hydrolysis reaction was obtained in 10 h. The optimal value of reducing sugars concentration (RS_C), given by the model, was 5.97 mg/ml and the corresponding yield was 61.73%. The maximum yield for the WS hydrolysis was 61.73% and was achieved at a temperature of 52.0°C, pH 4.6, and a E/S_R of 2.1 ml of Accellerase 1500^{TM}/g of cellulose. The S_{RV} was 4.80 U/mg and was obtained with the following conditions: pH 5.0, temperature of 48.5°C and an E/S_R of 0.19 ml/g. A quadratic polynomial equation for predicting the hydrolysis yield was developed. The confirmation experiment showed a final value for RS_C of 5.98 ± 0.81 mg/ml. This result indicates a % error of 0.33. The experimental results were in good agreement with predicted value.

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*Author for all correspondence

RESPUESTA DE LA APLICACIÓN DE BIOFERTILIZANTE LÍQUIDO Y FERTILIZANTE SOBRE EL RENDIMIENTO EN EL CULTIVO DE CEBADA

S. Islas* (1); R. Gómez (2); R.I. Beltrán (3); A. Jiménez.(1); C. Lucho (1,3)

(1) Universidad Politécnica de Pachuca. Laboratorio de Bioprocesos Ambientales. Ex-Hacienda de Santa Bárbara. Municipio de Zempoala, Hidalgo. C.P. 42830. E-mail.
(2) Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Pachuca, Hidalgo.
(3) Universidad Autónoma del Estado de Hidalgo. Mineral de la Reforma, Hidalgo.

Actualmente, la rentabilidad de la producción de granos a nivel mundial, se ha visto afectada por los incrementos en el costo de los fertilizantes químicos y otros insumos agrícolas. Una opción viable para incrementar la rentabilidad en la producción de cebada (*Hordeum vulgare*) y disminuir el impacto negativo en el ambiente por el uso de fertilizantes químicos, es la biotransformación de sustratos orgánicos locales en biofertilizantes que contengan nutrientes que estimulen el crecimiento de las plantas e incrementen la productividad del cultivo. Por tal motivo, el objetivo de este trabajo fue evaluar la efectividad biológica de las diferentes dosis de un biofertilizante obtenido por el proceso de la digestión anaerobia del estiércol de conejo, el cual fue aplicado en diferentes etapas de desarrollo de la cebada, así como en los suelos de las parcelas experimentales (1.2x3.0 m) ubicadas en el municipio de Almoloya, Hgo., durante el ciclo mayo-septiembre de 2013.

Para determinar el efecto del biofertilizante sobre el cultivo de cebada, se evaluó la altura de la planta, tamaño de la raíz, peso seco, número de hojas y tallo y rendimiento en las etapas de amacollo, encañe, floración y madurez y algunas propiedades del suelo al inicio y final del experimento. Para este estudio se utilizó un diseño de bloques completos al azar con doce tratamientos con tres repeticiones cada uno (n=36), incluyendo un tratamiento testigo a base de una mezcla de fertilizantes químicos.

El tratamiento de aplicación foliar en la etapa de amacollo fue el único que mostró un incremento significativo del 59.7% en el rendimiento de la cebada en comparación con el tratamiento control. Por otra parte los suelos tratados con biofertilizante presentaron una mayor concentración de ácidos húmicos ($p \geq 0.5$), en comparación con el suelo control. Mientras que los contenidos de materia orgánica, N, K y Cu presentaron un decremento en los suelos a los cuales se les adicionó biofertilizante, este comportamiento puede deberse a la presencia de microorganismos benéficos y a la concentración de nutrientes disponibles en el biofertilizante, lo cual estimuló positivamente los procesos microbianos llevados a cabo en el suelo, incrementando la disponibilidad de nutrientes que pueden ser asimilados por la planta. Estos resultados indican que la valorización de las excretas de conejo mediante el sistema propuesto es promisoria como biofertilizante para el cultivo de cebada y como agente estimulador de procesos microbianos en los suelos.

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*Author for all correspondence, slapiiv@hotmail.com

OBTENCION DE CELULOSA Y HEMICELULOSA A PARTIR DE LOS RESIDUOS SOLIDOS DEL PROCESO DE OBTENCION DE LA VAINILLA (*Vanilla planifolia*)

Ma. Ascención Ramírez-Coronel (1); Luis V. Rodríguez-Durán (1);
Ana K. Ortega- Hernández (1); Itzamná Baqueiro, (2);
Gerardo Saucedo-Castañeda * (1)

(1) UAM-I, Ciencias Biológicas y de la Salud, Biotecnología, México DF, México;
(2) UDLAP, Departamento de Ingeniería Química, Alimentos y Ambiental., San Andrés Cholula., Puebla, México.

México es uno de los 5 primeros productores de vainilla en el mundo. Esta producción ha sido alrededor de 100-200 toneladas anuales entre los estados de Veracruz, Oaxaca y Puebla. Este proceso genera residuos sólidos que pueden ser aprovechados. El objetivo de este trabajo fue evaluar el contenido de celulosa, hemicelulosa y lignina en el residuo sólido del proceso de obtención de vainilla. Así como vainillina residual y ácido vainillínico.

Se utilizó hidrólisis ácida seguida de hidrólisis alcalina. Se colocaron 5 g de vaina de vainilla molida en presencia de 95 ml H_2SO_4 al 1.5% v/v, por 24 h, seguido de tratamiento a presión (15 lbs, 118ºC por 30 min) y despresurización en 1 min, el extracto frío fue filtrado. El material insoluble en ácido fue secado a 60ºC, hidrolizado con NaOH en presencia de peróxido de hidrogeno (2.25% p/v, de NaOH y 0.75% de H_2O_2), en agitación a 150 rpm y a 55ºC, durante 1h. Al término, se filtró la suspensión y se secó el residuo a 60ºC. El sobrenadante fue acidificado a pH4.5 dejando en agitación 10-15 min. Hasta formar la lignina alcalina, posteriormente fue centrifugado a 3,800 rpm a 4 ºC por 20 min. El sobrenadante fue recuperado y se mezcló con 3 veces su volumen con etanol absoluto. Posteriormente fue centrifugado para recuperar la hemicelulosa, la cual fue congelada, liofilizada y pesada. Por otro lado se extrajeron la vainillina y ácido vainillínico residuales. A 1 g de vaina molida con 10ml de una mezcla de etanol: agua (80:20) en un microondas durante 5 min, 10 y 15 min a 60ºC a potencia variable (0-400 W). Los azúcares fueron analizados por HPLC sobre una columna Aminex HPX-87H a través de un detector de Índice de refracción, en tanto que los fenoles fueron analizados en HPLC sobre una columna C18 y en un detector de UV con arreglo de diodos.

La cantidad de celulosa, hemicelulosa, lignina fueron determinados gravimétricamente y correspondieron a 65.6%, 6% y 10.1% respectivamente, la diferencia está dada por los extractivos y cenizas. La concentración de vainillina fue de 0.85 mg/gms y de vaillínico de 0.08 mg/gms. Este trabajo muestra el potencial de utilización del residuo de la producción de vainilla para la obtención de prebióticos a partir de la fracción hemicelulósica, azúcares fermentables a partir de la fracción celulósica y aromas residuales para la industria cosmética y de alimentos.

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*Author for all correspondence

MONITORING *Bromelia hemisphaerica*, AN HISTORIC OVERVIEW ON A MULTIPURPOSE PLANT

María I. Cortés-Vázquez (1); Roberto Briones-Martínez***** (1)

(1) CEPROBI-IPN, Yautepec, Morelos, México;

Cultivation trials of the monocot *Bromelia hemisphaerica* (L) (Bromeliaceae), known as “timbirichi” in Mexico, were initiated almost forty years ago, with the main objective to obtain proteases and other biochemical products for industrial use. With an envisioned agronomic potential, a study was made on experimental plantations established on selected plots of flat ground and stony hillside fields. The selected area was located in a village within the surroundings of the Montenegro mountain range (18°49’31’’ N, 99°5’34’’ O, Morelos, Mexico). Here we report the analysis of a sequence of images showing the agronomic potential of this plantation and its capacity as a potential supplier of environmental services.

Simultaneous to the biochemical and technological studies carried on the cysteine proteases isolated from the fruit, it became apparent the potential usefulness of an indigenous plant in the improvement of the landscape and environment, based on the possibility of revegetation of an area with a long history of degradation, previously used in livestock activities.The visible evidence of the perceived changes in a period of almost forty years is based on photographs showing that in the first two decades the stony hillside plot underwent major changes in the population of trees and shrubs, thus obtaining a successful revegetation. It is concluded that the plantation of *Bromelia hemisphaerica,* a multipurpose plant, is a viable model for revegetation and sustainable use, especially in rural areas and unused rocky slopes of the dry forest in Mexico where the reduction of plant diversity has provoked a negative impact on the agro ecosystem. As a tank bromeliad, *Bromelia hemisphaerica* has minimal requirements for growth as a crop, its culture and exploitation gives an excellent alternative for local development and landscape management.

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*Author for all correspondence

PRODUCCIÓN DE ACETONA, BUTANOL Y ETANOL (ABE) A PARTIR DE PAJA DE TRIGO MEDIANTE BIOPROCESAMIENTO CONSOLIDADO

Adán Tapia-Pérez (1); Carlos E. Molina-Guerrero (1); Lorena Amaya Delgado (2); Arturo Sánchez (2); ***I. Valdez-Vazquez**** (1)

(1) Depto. de Ciencias Ambientales, DICIVA, Universidad de Guanajuato CIS, Guanajuato, México correo e: valdez_idania@yahoo.com
(2) CIATEJ
(3) Unidad de Ingeniería Avanzada, Centro de Investigación y Estudios Avanzados (CINVESTAV), Av. del Bosque 1145, Zapopan 45019, Jalisco, México.

La paja de trigo (PT) es uno de los subproductos agrícolas más abundantes en México, y tiene un gran potencial para su conversión en solventes (acetona, butanol y etanol, ABE) vía fermentación. La composición química de la PT (celulosa cristalina embebida en una matriz de hemicelulosa y lignina), la hace un material recalcitrante al ataque biológico. De forma convencional, la producción ABE a partir de PT requiere de las siguientes etapas: (*i*) separar la lignina, (*ii*) sacarificación de la celulosa, (*iii*) fermentación ABE, y (*iv*) destilación. Sin embargo, hasta el momento, esta plataforma convencional resulta costosa y con bajos rendimientos. De forma alternativa, en un bioproceso consolidado (BPC) se integran las 3 primeras etapas, lo que reduce los costos de operación y mejora los rendimientos.

Este trabajo tuvo como objetivo la producción ABE a partir de paja de trigo mediante un BPC utilizando un cultivo mixto silvestre (CMS) y un cultivo mixto definido artificialmente (CMA). Como sustrato se utilizó paja de trigo molida (2-3 mm). Se utilizó un diseño central compuesto para evaluar el efecto del pH (4.0-6.0) y % de PT (5-11%) sobre la producción ABE utilizando un CMS (obtenido de un digestor anaerobio). Después, se llevó a cabo la producción ABE con diferentes CMA integrados por 2 cepas de *Clostridium* y/o *Thermoanaerobacterium* obtenidas de la ATCC. Cada miembro del CMA fue crecido en condiciones anaerobias e inoculados en la fase exponencial a una relación 1:1. En ambos ensayos, las fermentaciones se realizaron a un volumen de 100 mL, 37°C/20 días. La cuantificación de solventes se realizó mediante GC-FID acoplado a un head space. Los ensayos se realizaron por triplicado, y los resultados fueron sujetos de un análisis de varianza con un nivel de significancia de 0.05.

Se encontró que el pH y el % de PT tuvieron un efecto significativo sobre la producción ABE ($P<0.05$). La máxima producción ABE con el CMS se logró a los 15 d con un total de 13.57 g/L, a un pH de 4.3 y 10.1 % de PT. Por otro lado, con el CMA la máxima producción ABE se logró a los 5 d con un total de 11.40 g/L, este CMA estuvo formado por 2 especies de *Clostridium*. Finalmente, los resultados demuestran la factibilidad de producir solventes a partir de sustratos celulósicos sin previo tratamiento, ya sea utilizando cultivos mixtos silvestres o definidos artificialmente. Sin embargo, el uso de estos últimos logró reducir la producción de 15 a 5 días de incubación.

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*Author for all correspondence

EVALUACIÓN DE LA ACTIVIDAD HOLOCELULOLÍTICA DE *Cellulomonas flavigena* PR-22 CRECIENDO EN BAGAZO DE CAÑA A DOS CONCENTRACIONES DIFERENTES.

Enrique González-Bautista (1); Ana C. Ramos-Valdivia (1); Eliseo Cristiani-Urbina (2); Teresa Ponce-Noyola* (1)

(1) CINVESTAV- IPN, México DF, México;
(2) ENCB-IPN, México DF, México;

En 2013, la producción de caña de azúcar en México llegó a ser más de 53 millones de toneladas, de las cuales sólo se obtiene el 11% de azúcar física real por lo que se genera una gran cantidad de bagazo de caña. Este residuo puede utilizarse como sustrato para la producción de holocelulasas, y a su vez para la producción de azúcares simples que se pueden usar en la producción de etanol de segunda generación.

En el proceso de sacarificación, las holocelulasas juegan un papel muy importante. *Cellulomonas flavigena* PR-22 es una mutante que produce holocelulasas resistentes a inhibición por producto final, sin embargo los niveles de producción aún no son competitivos comercialmente. Se ha reportado que la concentración de sustrato juega un papel importante en la producción de estas enzimas. Por lo anterior se probaron dos concentraciones diferentes de bagazo de caña como sustrato para la inducción del sistema holocelulolítico en *C. flavigena* PR-22.

Para el crecimiento de esta cepa se utilizaron 2 concentraciones diferentes de un mismo medio de cultivo (M10: 1% de bagazo y M25: 2.5% de bagazo). En el medio M25 los componentes se pusieron en una relación de 1 a 2.5 respecto a M10 exceptuando el NaCl. Se evaluó la actividad volumétrica y específica de celulasas y xilanasas y proteína total como crecimiento durante 72 h de cultivo.

Como resultado se obtuvo que a las 72 h, la actividad volumétrica de celulasas y xilanasas fue 45 y 40% mayor en el medio M10 respecto al medio M25. Mientras que la proteína total fue 26% mayor en el medio M25 respecto al medio M10.

El incremento en la proteína total en el medio M25 sugiere que este medio favoreció el crecimiento de *C. flavigena* PR-22 y no la producción de las holocelulasas. Por lo que el medio M10 es el más idóneo para la producción de enzimas que puedan ser usadas en el proceso de sacarificación de bagazo de caña.

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*Autor de correspondencia

ESTUDIO INTEGRAL DEL EFECTO DEL SECADO SOBRE LA MICROESTRUCTURA DE LA SEMILLA, RENDIMIENTO Y CALIDAD DEL ACEITE Y BIODIESEL DE SEMILLA DE CASTOR (*Ricinuscommunis*)

Mariana Lara-Serrano (1); S. Montserrat Gutiérrez-Martínez (1);
Jorge Chanona-Pérez (1); Jorge A. Mendoza-Pérez (1);
Georgina Calderón-Domínguez (1); ***María de Jesús Perea-Flores* (2).***

(1) ENCB-IPN, México DF, México;
(2) CNMN-IPN, México DF, México.

La semilla de castor (*Ricinuscommunis*) es un cultivo alternativo utilizado en la industria energética para producción de biodiesel. El objetivo de esta investigación fue estudiar el efecto del secado por lecho fluidizado sobre la microestructura, y como ésta beneficia la extracción del aceite de ricino y su influencia en la calidad del aceite y el biodiesel.

Las semillas de castor fueron secadas por lecho fluidizado de 80 a 110ºC durante 240 min (Condiciones A) y 120 a 50 min (Condiciones B); después se evaluó los cambios microestructurales en la cascara y endospermo mediante microscopia electrónica de barrido (MEB) y métodos mecánicos. El aceite fue extraído por maceración y soxhlet, y se determinó los rendimientos y eficiencias para determinar las mejores condiciones. La calidad del aceite se determinó por métodos analíticos y Resonancia Magnética Nuclear (RMN).El aceite de castor se sometió a una reacción de transesterificación para producir biodiesel, se calculó los rendimientos de conversión y se validó la reacción por medio de la Cromatografía de capa fina (CCF) y RMN.

Las cinéticas de secado sugieren que las semillas se deshidratan en el periodo de velocidad decreciente y que la estructura del material controla la difusión de agua. Los Deff fueron de $8.21x10^{-10}$ a $2.61x10^{-9}$ m^2/sy la energía de activación de 41.41 kJ/mol, dichos valores proporcionan criterios de selección de condiciones de secado. Los cambios microestructurales inducidos por el secado sobre la cascara fueron asociados a las pruebas de compresiónmecánica, donde la máxima fuerza para la fracturafue de 67.5 N para la nativa y 62 N para las secas; estos datos son útiles para procesos de descascarado. Las altas temperaturas de secado incrementaron la destrucción de las estructuras celulares en el endospermo, lo cual fue notorio en los rendimientos de extracción de aceite obtenido por el método de soxhlet para las Condiciones A (61a 64%) en comparación con las semillas sin tratamiento (53.2 %). Para las Condiciones B se obtuvo de 28 a 35% de aceite. La calidad del aceite para las temperaturas de 90 a 110ºC presentaron menor calidad en comparación con la de 80ºC, independientemente de la temperatura; la calidad de los aceites se ven afectados por el tiempo de secado. Los espectros de RMN confirmaron la presencia de los ácidos grasos principales del aceite de castor (ácido ricinoleico y linoleico). El % de conversión parcial (biodiesel) fue de 27 a 65% para las Condiciones A, y de 21 a 67% para las Condiciones B. Esta variación está dada por la calidad del aceite. La CCF corroboró la presencia del biodiesel, y mediante RMN se observó una modificación en la estructura química del ácido graso.En conclusión, esta investigación genera conocimiento básico y aplicado relacionado con un mejor aprovechamiento y explotación de la semilla de castor de manera sustentable en la industria energética.

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*Author for all correspondence

FATTY OIL AND SECONDARY METABOLITES COMPOSITION OF SOMATIC EMBRYO CULTURES OF *Jatropha curcas*: A BIODIESEL PLANT

Anamarel Medina-Hernández (1); Ileana Vera-Reyes (1); Elvira Ríos Leal (1); Teresa Ponce-Noyola (1); Carlos M. Cerda-García-Rojas (2); Gabriela Trejo-Tapia (3); ***Ana C. Ramos-Valdivia**** (1)

(1) Depto. de Biotecnología y Bioingeniería, CINVESTAV-IPN, México DF, México;
(2) Depto. de Química, CINVESTAV-IPN, México, D. F., México;
(3) Depto. de Biotecnología, CEPROBI-IPN, Yautepec, Morelos, México.

Jatropha curcas, a plant native from Mexico, is currently one of the most promoted crops for biodiesel production since its seeds have high oil content, it grows on poor soil and it does not conflict ethically or economically with food crop production. Likewise, its oil presents elevated cetane value, generally composed by highly-unsaturated fatty acids.

Somatic embryos (SE), arising from a somatic cell, allow clonal plant propagation via embryo germination. Additionally, they reproduce original seed characteristics and can be cultivated in liquid medium for further biotechnological studies. Therefore, SE cultures were induced from *J. curcas* shake-flask cell suspension cultures in a Murashige and Skoog medium containing growth regulators as naphthalene acetic, benzyladenine, and a sequential supply of different concentrations of sucrose and ascorbic acid.

Cultured SE (28 days old) in globular state and heart stage produced 4-12% of total lipids. The fatty acid composition was analyzed by GC-MS, showing that SE mainly produce the unsaturated fatty acids oleic acid in 53% and linoleic acid in 12%, together with saturated fatty acids such as palmitic acid (26%) and stearic acid (4%). Moreover, SE biomass contains the flavonoids vitexin, orientin, and kaempferol and some triterpenes, mainly oleanolic acid and lupeol.

Our results indicate that somatic embryos of *J. curcas* produce similar lipid profiles and metabolites than those found in the seeds and their production can be stimulated using *in vitro* culture conditions.

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*Author for all correspondence

A FIRST STEP IN THE SCALING UP OF THE ENZYMATIC HYDROLYSIS PROCESS OF WHEAT STRAW

Javier U. Hernández-Beltrán (1); Karla Cervantes-Quintero (1);
Héctor Hernández-Escoto* (1)

(1) UG-DCNE, Guanajuato, Guanajuato, México.

This work addresses the problem of settling down main process conditions in the way of scaling up an enzymatic hydrolysis process of dilute acid pretreated wheat straw.
In a first step, a surface response methodology was followed to determine effective values of pH, temperature (T) and the enzyme/wheat straw ratio (CE); the corresponding numerous experiments were carried out in a fast and economic system based on 1.5 mL micro-reactors. In a second step, to determine effective values of straw size, solid dilution ratio and processing time, experiments were carried out in a 0.5 L reactor (mechanically stirred) at controlled temperature.

An effective process conditions is {T, pH, CE} = {45 ºC, 4.5, (8/0.015) μL-Enzyme/g-Wheat}; higher temperatures and enzymatic dosages do not show a significant increment in the yield.

Analyzing the effect of the wheat size, in terms of the number of the screen can be seen that the unscreened wheat (#US) had a much lower yield than the ones of #16 and #40, and between these two, the #40 reaches a slightly higher yield. In terms of yield, the best result was obtained with #40 at a concentration of 10 %w/v.

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*Author for all correspondence

PINEAPPLE JUICE EXCELLENT CULTURE MEDIUM FOR GROW THE YEAST *Saccharomyces*

A.R. Domínguez-Bocanegra* *(1),* Ricardo Aguilar-López (1), Jorge Torres-Muñoz (1)

(1) CINVESTAV- IPN, México DF, México

Preliminary studies have shown that pineapple juice is an excellent substrate for growing the yeast *Saccharomyces cerevisiae*, as pineapple juice contains a high content of sugars, vitamins and amino acids. The aim of this study was to use pineapple juice as substrate for *Saccharomyces cerevisiae* biomass and ethanol production. This ethanol would first generation is now of paramount importance given energy reforms in our country.

To obtain pineapple juice (PJ) a pineapple hair about 2 kilos was flock and ground in a blender for 5 minutes it was sterilized by filtration and no nutrient was added, allowed to cool and stand for 24 hours. *Saccharomyces cerevisiae* cells were grown in YM medium (1% glucose, 0.3% yeast extract, 0.3% malt extract, 0.5% casein peptone) for 24 hours at constant temperature of 28 °C and stirrer speed 150 rpm and these cells were used as inoculum for our fermentation. Cultures were grown aseptically in 500 mL flasks containing 350 mL of PJ or YM medium and 10% inoculum v/v in exponential growth phase, the cultures was incubated at a constant temperature of 28°C and stirrer speed of 150 rpm. Samples were taken every two hours and reducing sugars were quantified according Miller, 1959, dry weight, cell number, optical density and percentage of ethanol using a gas chromatograph.

The results obtained indicate that pineapple juice turned out to be an excellent substrate for *Saccharomyces cerevisiae* growth. A maximum growth of $78x10^6$ cell per milliliter at 20 hours was achieved with a substrate consumption of 15% .

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*Author for all correspondence

SHIP BREAKING OR SCUTTLING? A REVIEW OF ENVIRONMENTAL, ECONOMIC AND FORENSIC ISSUES FOR DECISION SUPPORT

Devault D.A. (1)*, Beilvert B. (2, 3), Pascaline H. (1)

(1) Université des Antilles et de la Guyane, EA 929 AIHP-GEODE, groupe Biospheres, Campus de Schœlcher, Schœlcher Cedex, France
(2) Ingemar EURL5, boulevard Vincent Gâche F-44262 Nantes cedex 2
(3) Université de Nantes, BP 81307, Chemin de la Censive du Tertre, 44313 Nantes Cedex, France

In a globalized world, the world trade fleet plays a pivotal role in limiting transport fees. But the management of obsolete ships is an acute problem, because of prevalence of scrap yard situated in developing countries. Such ship breaking facilities are known for their workers' poor work conditions and safety -and environmental impact. Paradoxically, dismantlement is to be paid for by the shipowners in accordance with international conventions therefore it pays to sell ships destined for ship breaking in scorn of this. Then, scuttling could be seen as an alternative to scrapping –the aim of the present review is to compare the benefit/cost to scrap or to scuttle.

On one hand, scrapping provides employment and raw materials –but at what environmental and health price! In the other hand, scuttling provide fisheries and diving tourism opportunities –but needs appropriate management in order to avoid organic and metallic pollution, invasive species and the enhancement of coastal erosion, and is limited by the appropriate bottom depth, ship types and number. The present review inventories the environmental, health, safety, economical and forensic aspects of each practice.

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*Author for all correspondence

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LIST OF COAUTHORS:

Dr. Damien Devault, presenting author. Université des Antilles et de la Guyane, EA 929 AIHP-GEODE, groupe Biospheres, Campus de Schœlcher, Schœlcher Cedex, France. **Author to whom all correspondence should be addressed.**
E-mail: damien.devault@martinique.univ-ag.fr

Beilvert B. Ingemar EURL5, boulevard Vincent Gâche F-44262 Nantes cedex 2. Université de Nantes, BP 81307, Chemin de la Censive du Tertre, 44313 Nantes Cedex, France.

Pascaline H. Université des Antilles et de la Guyane, EA 929 AIHP-GEODE, groupe Biospheres, Campus de Schœlcher, Schœlcher Cedex, France.

OCEAN THERMAL ENERGY CONVERSION: OVERVIEW OF AN EMERGENT RENEWABLE ENERGY AND PRELIMINARY STUDY OF ENVIRONMENTAL IMPACT

Devault D.A.* (1), Giraud M. (2,3), Boye M. (3), Pascaline H. (1), Garçon V. (4), de la Broise D. (3).

(1) Université des Antilles et de la Guyane, EA 929 AIHP-GEODE, groupe Biospheres, Campus de Schœlcher, Schœlcher Cedex, France
(2) France Energies Marines, 15 rue Johannes Kepler, Technopôle Brest Iroise, 29200 BREST
(3) Lemar UMR 6539, IUEM Technopôle Brest-Iroise - rue Dumont d'Urville - 29280 Plouzané, FRANCE
(4) LEGOS/CNES 18, av. Edouard Belin, 31401 Toulouse cedex 9, France

Ocean Thermal Energy Conversion is a power source obtained by confrontation of cold and hot seawater sources in a heat pump. Regions where such technology could be implanted are restricted to hot and deep seas, especially Caribbean and Red ones –and all the Indo-Polynesian area. This green power source is useful in a green energy mix because of intermittence of the major other green power sources, i.e. solar and wind power ones.
However, because of the huge capital need for tropical area and because of technological gaps, OTEC power plants have never been built in an exploitation scope. Recent innovations and political awareness about power transition to green sources have allowed since few years to elaborate such power plant and, after a first engineering step in Reunion Island, Martinique was selected for harbor the first full-size OTEC power plant in the world for exploitation and demonstration.

Bases of OTEC technology, application field and limits will be summarized. The impact of environment on OTEC plant and the impact of OTEC plant on environment will be discussed.

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*Author for all correspondence

TYPE OF PRESENTATION: Oral

LIST OF COAUTHORS:

Dr. Damien Devault, presenting author. Université des Antilles et de la Guyane, EA 929 AIHP-GEODE, groupe Biospheres, Campus de Schœlcher, Schœlcher Cedex, France. **Author to whom all correspondence should be addressed.**
E-mail: damien.devault@martinique.univ-ag.fr

Giraud M. France Energies Marines, 15 rue Johannes Kepler, Technopôle Brest Iroise, 29200 BREST. Lemar UMR 6539, IUEM Technopôle Brest-Iroise - rue Dumont d'Urville - 29280 Plouzané, France.

Boye M. Lemar UMR 6539, IUEM Technopôle Brest-Iroise - rue Dumont d'Urville - 29280 Plouzané, France.

Pascaline H. Université des Antilles et de la Guyane, EA 929 AIHP-GEODE, groupe Biospheres, Campus de Schœlcher, Schœlcher Cedex, France.

Garçon V. LEGOS/CNES 18, av. Edouard Belin, 31401 Toulouse cedex 9, France

De la Broise D. Lemar UMR 6539, IUEM Technopôle Brest-Iroise - rue Dumont d'Urville - 29280 Plouzané, France.

STRATEGY FOR A SUSTAINABLE MANAGEMENT OF THE N-INPUT FROM ORGANIC WASTE IN AN ALMOND GROVE.

Gerardo López-López (1), Antoni Negre (1), Cristina Macci (2), José M. Rovira Alós (3), Ana M. Guerrero (4), Ana Ibáñez-Burgos (1), Grazia Masciandaro (2), Mª Carmen Lobo Bedmar (4), ***Isabel Sastre-Conde**. (3)**

(1) Consejería de Agricultura, Medio Ambiente y Territorio de Islas Baleares. Spain
(2) Institute of Ecosystem Studies (ISE), CNR, Pisa, Italy
(3) SEMILLA, Palma de Mallorca, Islas Baleares. Spain.
(4) IMIDRA, Madrid, Spain

The residual (R) and accumulated (A) effect of the application of different types of organic wastes as nitrogen source in an almond grove (Ferragnes variety) have been evaluated in a field experiment during two years. Sewage sludge (S), a mixture of manure+slurry+composted sewage sludge (MS) and waste from an organic farm (O) were applied at different doses 210 and 420 KgN/ha, according to current legislation. Residues were applied every year (A) and only the first year (R) in plots located in Majorca, Balearic Island (Spain). Every year, soil and plant were sampled with the object to evaluate the main soil characteristics and the almond nutrition and production. Biochemical-soil parameters such as potential metabolic index, soil enzyme activities (urease and dehydrogenase) and the water soluble carbon were evaluated in relation to the changes in soil chemical characteristics, as well as in almond nutrition and production. In the first year, all treatments seem to unbalance soil metabolism producing a drop of metabolic potential index, especially in soils where O and MS wastes were applied. Nevertheless, in the second year the metabolic potential index was re-established. The soil dehydrogenase activity showed a similar behavior. On the contrary, the soil urease activity did not change during the first year, except in soils where MS were applied at 420 kg N/ha. In this case, a significant decrease of the activity was observed. In the second year, urease activity increased in all the treatments assayed, especially at low dose applied. Even, in treatment with organic waste (O) the urease activity increased significantly in all soils. The almond crop production increased slightly in all treatments in the second year. Values were significant in those plots of soil where the treatment O at low dose was applied, in comparison to control soil. The water soluble soil carbon, as well as urease activity seems to have a major role in the changes promoted in production than water soluble carbon. This latter seem to be related with the content of macro and micro nutrients in almond leaves. In summary a low application of N from organic waste applied every year results more effective to restore the water soluble carbon necessary to improve the metabolic potential index in soil and consequently contribute to increase the almond crop production.

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*Author for all correspondence

TYPE OF PRESENTATION: Oral

LIST OF COAUTHORS:

Dra. Isabel Sastre Conde, presenting author.
SEMILLA,
C/Babieca, nº 2. 07198, Son Ferriol, Palma de Mallorca, (Balear Island). Spain

Author to whom all correspondence should be addressed.
E-mail: misastre@semilla-sau.es, isabelsastre2003@yahoo.es

Jose María Rovira Alós
SEMILLA
Same address as those of Dra. Isabel Sastre Conde

Ana Ibáñez Burgos, Consejería de Agricultura, Medio Ambiente y Territorio de Islas Baleares. C/ Eusebio Estrada, nº 145. Palma de Mallorca (Balear Islands)

Gerardo López López
Consejería de Agricultura, Medio Ambiente y Territorio
Same address as those of Ana Ibáñez

Antoni Negre
Consejería de Agricultura, Medio Ambiente y Territorio
Same address as those of Ana Ibáñez

Dra Carmen Lobo Bedmar,
Instituto Madrileño de Investigación y Desarrollo Rural Agrario y Alimentario. IMIDRA
Finca "El Encin" A-2, Km 38,2
28800 Alcalá de Henares (Madrid)

Ana María Guerrero
Instituto Madrileño de Investigación y Desarrollo Rural Agrario y Alimentario. IMIDRA
Same address as those Dra Carmen Lobo

Dra. Cristina Macci
Institute of Ecosystem Studies (ISE), CNR, Via Moruzzi, 1, 56124, Pisa, Italy

Dra. Grazia Masciandaro
Same address as those of Dra. Cristina Macci

IS MICROBIAL REMEDIATION OF SOILS IMPACTED BY THE POP CHLORDECONE (CLD, $C_{10}CL_{10}O$), FEASIBLE? WHAT CAN TELL US A THEORETICAL THERMODYNAMIC APPROACH, THE LITERATURE AND EXPERIMENTAL RESULTS WITH METHANOGENIC SLUDGE

Hervé Macarie* (1,2); Yoan Labrousse (1,2); Aurélie Amic (1,2); Alain Soler (3); Sébastien Bristeau (4); Christophe Mouvet (4); Isabel Sastre (5); Igor Novak (6); Alain Archelas (7); Jan Dolfing (8)

(1) IRD, IMBE, Campus Agro-environnemental Caraïbe (CAEC), Martinique, France;
(2) Aix Marseille Université, CNRS, IRD, Avignon Université, IMBE, UMR 7263, Marseille, France;
(3) CIRAD, UPR Systèmes de culture bananiers, plantains, ananas, CAEC, Martinique, France;
(4) Brgm, Orléans, France;
(5) Semilla, Palma de Mallorca, Islas Baleares, Spain;
(6) Charles Sturt University, Orange, NSW, Australia;
(7) Aix-Marseille Université, CNRS, UMR ISM2, Marseille, France;
(8) School of Civil Engineering & Geosciences, Newcastle University, Newcastle-upon-Tyne, UK.

CLD is an organochlorine insecticide that sorbs strongly to soils, is poorly soluble in water, and highly persistent in the environment. It accumulates in food chains, has potent chronic and acute toxicity, and is a proven carcinogen. It is now worldwide banned under The Stockholm Convention on Persistent Organic Pollutants (POPs). It is estimated that 1/6 (300 tonnes) of the CLD ever produced has been used in the French West Indies (FWI) islands against the banana weevil. This has resulted in a long-term pollution of the banana field soils, causing a diffuse contamination of water and food resources (crops, livestock, fishes, crustacean) that is presently responsible of an unprecedented sanitary crisis. Measures such as restriction to fishing and root vegetable cultures have allowed cutting consumers contamination but a final solution would consist to destroy the stock of CLD still presents in soils. Microbial remediation could be such a solution if applicable *in situ* using land farming procedures. Until recently, however, due to its cage structure and above-mentioned characteristics, CLD was considered as non-biodegradable, a feeling reinforced by the apparent absence of natural attenuation in FWI soils. In this talk thanks to the use of a theoretical approach, we will demonstrate that there is no thermodynamic reasons why CLD would not be amenable to full mineralization in presence of the main environmental electron acceptors (O_2, Fe^{3+}, NO_3^-, SO_4^{2-}, CO_2) or to partial transformations (e.g. dechlorination, reduction or oxidation of the ketone group). All these reactions moreover would liberate sufficient energy for microbial growth. We will show that such finding is in agreement with the few papers available in old literature suggesting that anaerobic and particularly methanogenic conditions would be favourable to achieve important chemical transformations of CLD. According to this information and the availability of free abundant sources of methanogens for a bioaugmentation process, a lab scale methanogenic reactor was set up in order to explore further the behaviour of CLD in methanogenic environments. The results obtained after 81 days of operation of this system will be presented.

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*Author for all correspondence

TYPE OF PRESENTATION: Oral

LIST OF COAUTHORS:

Hervé Macarie, PhD, Research Officer, presenting author and **author to whom all correspondence should be addressed**
IRD – IMBE – Campus Agro-environnemental Caraïbe, Quartier Petit Morne, BP 214, 97285 Le Lamentin, (Martinique) France.
Tel: +596 596 42 30 30, Fax. +596 596 42 31 00
E-mail: herve.macarie@ird.fr

Yoan Labrousse, B. Sc, Assistant Engineer
Same address as Hervé Macarie
E-mail: yoan.labrousse@ird.fr

Aurélie Amic, M. Sc.
E-mail: aurelie.amic@hotmail.fr

Alain Soler, PhD, Research Officer
CIRAD, Campus Agro-environnemental Caraïbe, Quartier Petit Morne, BP 214, 97285 Le Lamentin, (Martinique) France.
Tel: +596 596 42 30 92, Fax. +596 596 42 31 00
E-mail: alain.soler@cirad.fr

Sébastien Bristeau, M. Sc., Engineer
Brgm, Avenue C. Guillemin, F- 45060 Orléans Cédex 2, France
Tel: +33 2 38 64 35 40, Fax. +33 2 38 64 39 25
E-mail: s.bristeau@brgm.fr

Christophe Mouvet, PhD, Research Director
Brgm, Avenue C. Guillemin, F- 45060 Orléans Cédex 2, France
Tel: +33 38 64 39 08, Fax: +33 2 38 64 39 63
E-mail: c.mouvet@brgm.fr

Isabel Sastre, PhD, Research Officer
SEMILLA, C/ Esperanto, nº 8 07198. Palma de Mallorca, (Islas Baleares) Spain
Tel: +34 971 179 596 ext 69 750
E-mail: isabelsastre2003@yahoo.es, misastre@semilla-sau.es

Igor Novak, PhD, Full Professor
Orange, NSW 2800, Australia
Tel / Fax. +61 2 6365 7815
E-mail: inovak@csu.edu.au

Alain Archelas, PhD, Research Director
Aix Marseille Université, CNRS, UMR ISM2-UMR7313 Biosciences, case 332, Faculté des Sciences et Techniques de St Jérôme, Avenue Escadrille Normandie Niemen, F-13397 Marseille Cédex 20, France
Tel: +33 4 91 28 89 66, Fax. +33 4 91 28 84 40
E-mail: a.archelas@univ-amu.fr

Jan Dolfing, PhD, Research Officer
School of Civil Engineering & Geosciences, Newcastle University, Newcastle-upon-Tyne, NE1 7RU, United Kingdom
Tel: +44 191 208 8352 Fax. +44 191 208 6502
E-mail: jan.dolfing@newcastle.ac.uk