



The Sixth International
Symposium on Environmental
Biotechnology and Engineering



- And -

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Y CIENCIAS AMBIENTALES



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Rios-Vazquez, N.J.; Ulloa-Mercado, R.G.; Sánchez-Duarte, R.G.; Correa-
Murrieta, M.A.; Gortáres-Moroyoqui, P.

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Preface

The Sixth International Symposium on Environmental Biotechnology and Engineering (6ISEBE) was carried out in Instituto Tecnológico de Sonora (ITSON) at Ciudad Obregón Sonora México. This Symposium was focused on discussions and debates by recognized researchers and scientific leaders at several environmental topics such as water and soils remediation processes, biotechnology, nanotechnology and chemistry. Plenary lectures, short courses, roundtables (symposia), and research works (oral and poster) presented were around the following main topics: 1) Renewable and Alternative Energies and Biorefineries, 2) Sustainability and Environmental System Analysis, 3) Soil, Sediment, Groundwater and Aquifer Remediation, 4) Green Materials and Biomaterials, 5) Solid and Hazardous Waste Management and Treatment, 6) Microbial Ecology and Molecular Biology Applications to Environmental Problems, 7) Wastewater Treatment, 8) Risk Assessment and Environmental Impact, 9) Environmental Biotechnology, and 10) Climate Change.

Around 400 delegates attended the 6ISEBE; around 300 research works, ten plenary lectures, eleven symposia with 33 lectures were presented. Delegates attending came from different Mexican (30) and international research Institutions and Universities. Works received and delegates came from nineteen countries: Argentina, Brazil, Burkina Faso, Bolivia, Equador Canada, Chile, Coat Ivory, Colombia, England, France, Martinique, Morocco, Perú, Spain, Tunisia, Uganda, United States of America, and Mexico.

Thank you very much to come to ITSON.

Pablo Gortáres Moroyoqui



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The Sixth International
Symposium on Environmental
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Chapter 1 Renewable and Alternative Energies and Biorefineries



Use of a microalgae consortium from a wastewater plant for the treatment of residual effluents and biodiesel production

J. Suastes Rivas^{*(1, 2)}, R. Hernández Altamirano^(1, 2), L. R. Tovar Gálvez⁽³⁾ and I. Chairez Oria⁽⁴⁾

⁽¹⁾ Instituto Politécnico Nacional, Centro Mexicano para la Producción más Limpia, Av. Acueducto s/n, Col. La Laguna Ticomán, Ciudad de México, 07340, México.

⁽²⁾ Instituto Politécnico Nacional, Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad de Biocombustibles (LaNDACBio), Ciudad de México, 07340, México

⁽³⁾ Instituto Politécnico Nacional, Centro Interdisciplinario de Investigaciones y Estudios sobre Medio Ambiente y Desarrollo (CIEMAD), 30 de junio de 1520 s/n, La Laguna Ticomán, 07340 Ciudad de México, México.

⁽⁴⁾ Instituto Politécnico Nacional, Departamento de Bioprocesos, Unidad Profesional Interdisciplinaria de Biotecnología, Professional, Av. Acueducto s/n, Col. La Laguna Ticomán, Ciudad de México, 07340, México.

*Corresponding author: jeskaksr@hotmail.com

The interest in biofuels production from microalgae is because of their content of metabolites in the cell structure such as carbohydrates, proteins and lipids. The recent research has been focused on the extraction of the triglycerides from the lipid fraction of the microalgae and carry the transesterification process to obtain the fatty acid methyl acids (FAME, biodiesel). In the present study, a consortium of microalgae was collected from a wastewater plant with the objective to characterize them and report the main metabolites such as carbohydrates, proteins and lipids used to produce biodiesel. The microalgae consortium was cultivated in Bold's Basal Medium and BG-11 medium and the optimal biomass productions were 0.40 g/L and 0.55 g/L, respectively. The highest content of metabolites was 30% of carbohydrates, 20% of proteins and 50% of lipids. They were characterized and was obtained their biochemical content and FAME composition. The lipid accumulation was 4 mg L⁻¹ with BBM medium and 6 mg L⁻¹ with BG-11, during all the kinetics. These were transesterified and the fatty acid methyl esters (FAME) were analyzed by GC-MS. FAME compositions were mainly C16-C18. In addition, were predicted several quality parameters of biodiesel and compared with the corresponding specifications in the American and European biodiesel standards such as Cetane Number (CN), Iodine Value (IV), the cold filter plugging point (CFPP), cloud point (CP), kinematic viscosity (KV) and density.

Keywords: Microalgae, metabolites, lipids, triacylglycerides wastewater.



Design of a low environmental impact prototype for the manufacture of brickets from lignocellulosic residues



J. C. Paredes Rojas ⁽¹⁾, B. Bravo Diaz ⁽¹⁾, S. M. García Solares ⁽¹⁾, A. Flores Vela ⁽¹⁾, F. J. Márquez Rocha ⁽²⁾

⁽¹⁾ Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad en Biocombustibles (LANDACBIO) del Centro Mexicano para la Producción más Limpia, Instituto Politécnico Nacional de México, Acueducto de Guadalupe s/n. La laguna Ticomán, CP. 07340, Ciudad de México, México. Tel: 5729-6000 Ext. 52621.

⁽²⁾ Centro Regional para la Producción más Limpia, Unidad Tabasco, Instituto Politécnico Nacional de México, Parque Industrial Tabasco Business Center Circuito Tabasco Poniente S/N Edificio FINTAB Carretera Reforma - Dos Bocas Km. 17+920 Ranchería Pechucalco 2da. Sección, Cunduacán, Tabasco, México.

*Corresponding author: paredesrojasjc@gmail.com

In Mexico, the wood industry generates tons of organic residues, which are not used and become a waste that causes enormous social, environmental and health problems in the nearby communities. From the waste generated during the wood production process, you will find the crust, sawdust and some cuts. This biomass is an energy vector that can be basic in our society, from the energy and environmental point of view, as well as for the socioeconomic development of rural areas. That is why, in recent years, the industry of biomass production, in particular the manufacture of pellets and brickets, has experienced a great boom on a global scale. In order to contribute to the reduction of the problems generated by the residues of the wood industry in our country, a prototype was designed for the manufacture of brickets made from biomass. A technological development was designed applying one of the eight ecodesign strategies, these are: Selection of low impact materials; Reduction in the use of materials; Techniques to optimize production; Optimization of the distribution system; Reduction of the impact during its use; Optimization of the useful life; Optimization of the end-of-life system, and; Development of a new concept. This prototype is an alternative sustainable development for rural microenterprises in our country that, despite being rich in a wide variety of 100% renewable biomass resources, Mexico does not figure in pellet imports made by the European Union or other market.

Keywords: alternative energy, biomass, eco-design, pelletizing machine.



Design of a Centralized Bioenergy Unit: Modeling strategy to optimize

bioenergy production and reduce methane emissions



J. A. Silva González ⁽¹⁾, I. O. Hernández de Lira ⁽¹⁾, L. E. Montañez Hernández ⁽¹⁾, A. Rodríguez Martínez ⁽²⁾, N. Balagurusamy ^{*(1)}

⁽¹⁾ Laboratorio de Biorremediación, Facultad de Ciencias Biológicas, Universidad Autónoma de Coahuila, Carretera Torreón-Matamoros km 7.5, Torreón Coahuila, México.

⁽²⁾ Centro de Investigación en Ingeniería y Ciencias Aplicadas, Universidad Autónoma de Morelos, Av. Universidad, N° 1001, Cuernavaca, Mexico.

*Corresponding author. bnagamani@uadec.edu.mx.



Anaerobic digestion (AD) of the agro-industrial wastes to produce biogas has been used widely as an alternative to fossil fuels and as a strategy to mitigate greenhouse gases (GHG) emissions. However, unlike other countries where AD has been successfully implemented, currently in Mexico the production of bioenergy from biogas does not compete with conventional energy sources such as oil, coal and natural gas. The poor deployment of biogas bioenergy is related with the problems in the design and operation of biogas plants. Previous studies reported the potential of bioenergy production from cow manure (410 GW/year) in Mexico and Comarca Lagunera (21 GW/year) and a consequent reduction in GHG emissions to the tune of 21 Gg per year in CO₂ equivalent¹. A centralized bioenergy unit was simulated using SuperPro Designer software v 8.5, focused on the design and optimization of the manure transport chain, installation of centralized biogas plant, operation costs of the process, biogas upgrading and organic fertilizer production and as well as economic analyses. The area known as La Popular at Comarca Lagunera was chosen as study area due to the existing number of animal farms (16), livestock population (64,000 cattle heads) in an area of 50 km². Two scenarios were analyzed and they consisted of one and three clusters of biodigesters, for which optimal locations were determined using mathematical modeling. The bioenergy unit was designed with capacity to process 1,600 t/day of animal manure to produce biogas and other value added products such as biomethane (increased methane percentage by way of removing carbon dioxide, hydrogen sulfide and water vapour) and organic fertilizer. Further, different strategies to reduce net production cost were used by way of including the use of produced bioenergy to minimize their fossil energy requirements and by heat recovery from other processes. Results indicated that biomethane production was the more profitable option over energy production by using non-purified methane or through biogas selling. Biomethane production was calculated to be around 58,756 m³ /day, which is equivalent to 36,175 l of diesel. Economic analysis showed a net production cost of \$0.7994 and \$0.8021 USD per kg of biomethane, a profit margin of 12.2% and 11.9% and a payback period of 12.7 and 13.4 years for the scenarios 1 and 2, respectively. This study demonstrated the techno-economical potential for the implementation of a bioenergy system.

Keywords: Bioenergy, Biogas, Economic analysis, Process simulation.

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A biorefinery approach using acid pretreatment, dark fermentation and anaerobic digestion from fruit and vegetable wastes and corn stover



S Rodríguez-Valderrama⁽¹⁾, C Escamilla-Alvarado^{*(1)}, P Rivas-García⁽¹⁾, M Alcalá-Rodríguez, J-P Magnin⁽²⁾

⁽¹⁾ *Universidad Autónoma de Nuevo León, Centro de Investigación en Biotecnología y Nanotecnología (CIByN), Facultad de Ciencias Químicas. Parque de Investigación e Innovación Tecnológica, Km. 10 autopista al Aeropuerto Internacional Mariano Escobedo Apodaca, México.*

⁽²⁾ *Grenoble Institute of Technology, Laboratory of Electrochemistry and Physico-Chemistry of materials and Interfaces, 1130 Rue de la Piscine, St Martin d'Hères, France.*

*Corresponding author: cea_escamilla@yahoo.com.mx.



Biomass is a renewable and abundant carbon source (e.g. agriculture crop residues or municipal organic wastes) that has been receiving special attention because of its potential to be transformed into biofuels, energy and biobased products. The integrated used of biomass is related to the biorefinery concept. According to the aforementioned, the aim of this investigation was to develop a process for biohydrogen and biomethane production from organic wastes combination by a biorefinery approach. Fruits and vegetables wastes (FVW) and corn stover (CS) were used as substrates. The biorefinery was constituted of the following processes: acid hydrolysis, dark fermentation and anaerobic digestion. The acid hydrolysis was carried out on a 5×2^1 general factorial. The factors were the combination of co-substrates (0:100, 25:75, 50:50, 75:25, 100:0) FVW:CS %_{dry base} and the acid catalyst (HCl and H₂SO₄ 0.5 % v/v). Reducing sugars, monomeric sugars and inhibitory compounds (5-hydroxymetilfurfural, furfural and total phenolic compounds) were quantified. After acid hydrolysis, liquid hydrolizates (LH) and solid hydrolizates (SH) were separated by centrifugation. The LH were used as carbon source for hydrogen production by dark fermentation in a 3^2 factorial design, evaluating the inoculum/substrate ratio (0.8 to 1.2 g SV_{inoculum} g⁻¹ SV_{substrate}) and initial concentration of reducing sugars (10 to 16 g L⁻¹), whereas the SH was fermented by anaerobic inoculum varying the inoculum/substrate ratio (2, 2.5, 3, 3.5 and 4 g SV_{inoculum} g⁻¹ SV_{substrate}). The best catalyst was HCl for the ratio 75:25 FVW:RM. For this combination, concentration of glucose, xylose and arabinose were (g L⁻¹): 10.02, 3.67 and 2.93 respectively. The maximum production of hydrogen was 8.08 mmol H₂ for initial concentration of 13 g L⁻¹ and inoculum/substrate ratio of 1.2. Butyric acid was the most abundant liquid metabolite. The maximum cumulated methane and the maximum methane yield were 13.82 mmol CH₄ and 16.24 mmol CH₄ g⁻¹ SV, respectively for the inoculum/substrate ratio of 2.5 and 4. The biorefinery demonstrated the possibility to use HL for hydrogen production and the HS for methane production.

Keywords: acid hydrolysis, biorefinery, organic wastes.

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Optimization of process conditions for the biosynthesis of ligninolytic

enzymes in solid state fermentation using agricultural residues



Sosa-Martínez, Jazel⁽¹⁾, Nagamani Balagurusamy⁽¹⁾, Montañez-Sáenz, Julio⁽²⁾, Morales-Oyervides, Lourdes^{*(2)}

⁽¹⁾ Laboratorio de Biorremediación, Facultad de Ciencias Biológicas, Universidad Autónoma de Coahuila, Libramiento Torreón-Matamoros, Torreón, Coahuila, 27000, MÉXICO

⁽²⁾ Departamento de Ingeniería Química, Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Boulevard Venustiano Carranza SN, Saltillo, Coahuila, 25280, MÉXICO

*Corresponding author: lourdesmorales@uadec.edu.mx



Many industries such as, paper, food, cosmetics, plastics and textile, used dyes in order to color their products. This type of contaminants in water is negative for the environment, as affects photosynthesis, marine life and represents a harm for human health. White rot fungi are capable of excrete a complex enzymatic degrading of lignin, the organic compound most resistant to degradation. These extracellular enzymes have been used in the biodegradation of synthetic dyes. The representative fungus is *Phanerochaete chrysosporium*, which is capable of producing two peroxidases, lignin peroxidase (LiP) and manganese peroxidase (MnP) and one phenol oxidase, Lacasse (Lac). These enzymes are considered the responsables for the decolorization of dyes¹. Bioprocesses depend on a high number of variables; therefore, the objective of the present study was to optimize the production of ligninolytic enzymes by *Phanerochaete chrysosporium* in solid culture using the Taguchi methodology.

Corncob was used as a support and nutrients source for the solid culture of *P. chrysosporium*. The evaluated factors and levels were: humidity (60%-70%-80%), inoculum concentration (7.5×10^5 - 1×10^6 - 2.5×10^6 spores mL⁻¹), packing density (0.14-0.16-0.2 g mL⁻¹) and time (6-8-10 days). According to the ANOVA, for LiP and Lac, humidity was the factor with the highest influence, while for MnP, it was the time. We observed that the humidity presents a similar effect for the three enzymes; activity decreased when humidity increased. The optimal conditions were different for each enzyme; therefore, a Desirability function was used to unify the three responses in a single target. At optimum conditions the activities for LiP, MnP and Lac were (U L⁻¹): 5.4, 4.2 and 3.4, respectively. These values represent a high increment in activity compared to the unoptimized process.

The optimal conditions obtained will serve as a basis for scaling-up the process for the degradation of dyes in contaminated waters.

Keywords: *Phanerochaete chrysosporium*, optimization, ligninolytic enzymes, colored effluents.

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Environmentally relevant concentrations of glibenclamide induce

oxidative stress in common carp (*Cyprinus carpio*)



Martínez-Viveros E.M.G.⁽¹⁾, Dublán-García O.*⁽¹⁾, Gómez-Oliván L.M.⁽¹⁾, Islas-Flores H.⁽¹⁾, Galar-Martínez M.⁽²⁾, Hernández-Navarro M.D.⁽¹⁾

⁽¹⁾ *Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México. Paseo Colón intersección Paseo Tolloca s/n. Col. Residencial Colón, 50120 Toluca, Estado de México, México.*

⁽²⁾ *Laboratorio de Toxicología Acuática, Departamento de Farmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu Esq. Cda. Miguel Stampa s/n, Delegación Gustavo A. Madero. C.P.07738. Ciudad de México. México.*

*Corresponding author: octavio_dublan@yahoo.com.mx

The hypoglycemic pharmaceutical glibenclamide (GLB) is widely used around the world. This medication is released into the environment by municipal, hospital and industrial wastewater discharges. Although there are reports of its environmental occurrence in the scientific literature, toxicity studies on aquatic species of commercial interest such as the common carp (*Cyprinus carpio*) are scarce. The present study aimed to evaluate the oxidative stress induced on *C. carpio* by environmentally relevant concentrations of GLB. Biomarkers of oxidative damage such as hydroperoxide content, lipid peroxidation and protein carbonyl content were evaluated as well as the activity of the antioxidant enzymes superoxide dismutase and catalase. The concentration of GLB was determined in water as well as in gill, liver, muscle, brain and blood of carp at 12, 24, 48, 72 and 96 h. The findings obtained in the study prove that GLB induces increases in biomarkers of oxidative damage and antioxidant enzyme activity in *C. carpio*, in the same way, it was observed that the evaluate organs bioconcentrated this hypoglycemic agent. These findings allow us to conclude that the presence of GLB in water bodies represents a risk for aquatic species.

Keywords: Oxidative damage, Antioxidant enzymes, glibenclamide, teleost.



Valorization of microalgal biomass cultivated in 200 L raceway using piggery wastewater as substrate



Fernández-Linares, L.*; González Farfán, K.; Flores Aguilar, E.; Gutiérrez Márquez, A.

Unidad Profesional Interdisciplinaria de Biología - Instituto Politécnico Nacional (UPIBI - IPN).
Av. Acueducto s/n Col. Barrio la Laguna Ticomán, 07340, Mexico City, MEXICO.

* Corresponding author e-mail: lfernand36@gmail.com



The culture and valorization of biomass of microalgae for energy purposes has proved to be a promising option, since it does not compete with the production of food crops or arable land. However, the production and processing of biomass represent a cost that is not competitive in the current energy market. Microalgal production involve enormous amounts of nitrogen (N) and phosphorus (P), which are fundamental for microalgal growth. Thus, there is a need to look for alternatives that compensate this situation, making this process more viable. The use of wastewater to obtain biomass, energy and high added-value products, within the concept of biorefinery, make it feasible. A microalgal consortium was grown in four 200 L Raceway in semicontinuous regime under greenhouse conditions, using piggery wastewater, rich in essential nutrients for the growth of microalgae, as substrate. The harvesting cycles were carried out removing half of the culture volume and replaced it with the same volume of fresh water and piggery wastewater (until get 80 mg L^{-1} of NH_4). The productivity of biomass, lipids, carbohydrates, proteins and total pigments was evaluated over five cycles of thirteen days each. N-NH_4 , N-NO_2 , N-NO_3 , PO_4^{3-} and chemical oxygen demand (COD) were determined in the culture; besides, pH, temperature, dissolved oxygen, N-NH_4 and N-NO_3 , were monitored with a Hydrolab DS5 probe. Five cycles of the Semi-continuous culture were completed (97 days). The productivities of the evaluated metabolites were increasing with the cycles, except for lipids, which presented a maximum productivity in the 4th cycle. The maximum obtained productivities of lipids, carbohydrates, proteins and pigments were 29.23, 15, 33.15 and $2 \text{ mg L}^{-1}\text{d}^{-1}$, respectively. The production of biomass also increased with the cycles, reaching in the fifth cycle 1.67 g L^{-1} . PO_4^{3-} and COD removals of 82.22 and 92.9 %, respectively, were achieved; the maximum Nitrogen removal was 62.01%; an increase of N-total in culture media from the second half of the cycle (in all cycles) was observed, indicating processes of nitrogen fixation. The presence of nitrifying bacteria (Nitrosomonas and Nitrobacter) in the culture was proven. The ability of the system to eliminate fecal coliforms was determined. The pH increased from 7.2 to 9 throughout each culture cycle; the average temperature of the culture medium was 20°C .

Keywords: microalgae, piggery wastewater, raceways, semicontinuous culture.



Utilization of non-conventional media for the growth of microalgae for

biodiesel production



Fernández-Linares L.*; González Ambriz L.M.; García Cordero, J.M.

Unidad Profesional Interdisciplinaria de Biología - Instituto Politécnico Nacional (UPIBI - IPN).
Av. Acueducto s/n Col. Barrio la Laguna Ticomán, 07340, Mexico City, MEXICO.

* Corresponding author e-mail: lfernand36@gmail.com



Today there are increasing concerns over global climate change and the energy crisis, caused in part by increasing global energy demand¹. Bioenergy is considered to have the highest potential to satisfy the energy needs. Microalgae have been described as one of the most promising feedstock for biofuel production². Microalgae have higher oil content, higher rate of photosynthesis, higher biomass productivities than plants, the ability to grow in wastewater, and does not compromise the production of food. However, algal biomass as a raw material to produce biofuels is still expensive. Microalgae production in non-conventional media as wastewaters represents one of the best measures toward the reduction of the requirement of commercial nutrients, environmental impact and production costs. The aim of this research is to determine the feasibility to growth four microalgal strains in non-conventional media, as a raw material to produce biofuels. *Chlorella vulgaris*, *Chlorella protothecoides*, *Scenedesmus sp* and *Chlorococcum humicola* was grown in filtered urban wastewater, piggery wastewater diluted until get 80 mg L⁻¹ of ammonium and Bold's Basal Medium (BBM) under controlled conditions in a 0.5 L photobioreactors. Three kinetics of each medium and with each strain were carried out in triplicate. The biomass, lipids, proteins, carbohydrates and total pigments were determined. *Scenedesmus sp* and *C. protothecoides*, presented the highest production of biomass in urban wastewater, 1.80 and 1.13 g L⁻¹, respectively. Likewise, the highest lipid productivities of the four strains were obtained in urban wastewater (37 to 82 mg L⁻¹d⁻¹). This may be because the urban wastewater contained a lower concentration of nitrogen, reaching the N limitation stage, which increased the cellular accumulation of lipids. The highest biomass production in piggery wastewater was obtained with *C. vulgaris*, reaching 1.43 g L⁻¹. *Scenedesmus sp* presented the highest productivity of both biomass and lipids in urban wastewater. The four strains are viable as raw material for obtaining biofuels. The four strains removed more than 98% of the ammonium and phosphate, and 88-92% of the COD in both urban and piggery wastewater.

Keywords: biofuels, microalgae, wastewater.



Semicontinuous culture of *Scenedesmus* sp in 200 and 2000 L RW under

greenhouse conditions using commercial liquid fertilizer



ISEBE

Fernández-Linares L.*, González Ambriz L.M.; García Cordero, J.M.; Guerreo Barajas C.

Unidad Profesional Interdisciplinaria de Biología - Instituto Politécnico Nacional (UPIBI - IPN).
Av. Acueducto s/n Col. Barrio la Laguna Ticomán, 07340, Mexico City, MEXICO.

* Corresponding author e-mail: lfernand36@gmail.com



For the generation of biofuels or high value-added products from microalgae such as lipids, proteins, carbohydrates and pigments, it is necessary to produce biomass at large scale¹. The traditional commercial algal production requires a large amount of freshwater, fertilizer and carbon dioxide, which accounts for approximately 30% of the total production costs². Cultivating microalgae with conventional media (formulated with chemical products) is not sustainable³. An alternative is the use of non-conventional substrates, such as agricultural fertilizers, biofertilizers, organic fertilizers derived from municipal wastewater, animal farming waste products, and wastewater from the food and beverage industry². The objective of the work was to determine the effect of the bayfolan fertilizer as medium in the productivities of the *Scenedesmus* grown in open systems under greenhouse conditions. *Scenedesmus* sp. was grown under semicontinuous regime in two media, fertilizer (Bayfolan® Forte, 1mL L⁻¹) and Bold's Basal Medium in 200 and 2000 L raceways (RW) under greenhouse conditions. The biomass, lipids, proteins, carbohydrates and total pigments were determined. During the semicontinuous culture regime, the productivities were increasing with the cycles; reaching lipid productivities of 18.06 ± 0.59 and 20.00 ± 6.29 mg L⁻¹ d⁻¹ and biomass productivities of 111.4 ± 9.30 and 65.7 ± 9.30 ± 4.35 mg L⁻¹ d⁻¹ in BBM and Bayfolan medium, respectively in 2000 RW. In 200 L RW the highest growth was obtained in bayfolan medium, and the productivity of lipids and biomass was significantly lower (less than 50%) to that obtained in the RW of 2000L. It was shown that the fertilizer has the potential to replace the conventional media in the culture of *Scenedesmus* sp. for the production of biodiesel. Besides, when the nitrogen source was replaced by ammonium bicarbonate the maximum lipid productivity was reached (25.2 ± 3.7 mg L⁻¹ d⁻¹). The fatty acids were mainly composed of C16:0, C18:3n3 and C18:0. Palmitic acid (C16:0) was the main FAME accumulated along with stearic acid (C18:0) and myristic acid (C14:0). The utilization of the fertilizer allowed a threefold increase of the productivities of total pigments compared to BBM.

Keywords: fertilizer, microalgae, raceways, semicontinuous culture.

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A. Ramírez-Estrada*^(1,2), V. Y. Mena-Cervantes^(1,2), R. Hernández-Altamirano^(1,2) M. Camargo-Martínez^(1,2)

⁽¹⁾ Centro Mexicano para la Producción más Limpia, Instituto Politécnico Nacional, Av. Acueducto s/n, Col. La Laguna Ticomán, Ciudad de México, 07340, México.

⁽²⁾ Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad de Biocombustibles (LaNDACBio), Instituto Politécnico Nacional, Ciudad de México, 07340, México.

*Corresponding author: alexram0021@gmail.com



Biomass can act as a store of chemical energy to provide heat, electricity and transportation fuels, or as a chemical feedstock for bio-based products. The tree main technology conversion routes for converting biomass to biofuel can be grouped into thermochemical, physicochemical and biochemical processes. The sources of biomass feedstock for biofuels production include agriculture crops, forestry sources, forestry and agricultural residues, organic waste from urban residues. However, the heterogeneous nature of biomass feedstocks is the primary factors affecting the conversion efficiency and ethanol yield. Unfortunately, conventional physicochemical analysis (ASTM and NREL methods) has many drawbacks for chemical composition assessment of biomass; they are time-consuming, labor-intensive and expensive. In contrast, Infrared (IR) spectroscopy and thermogravimetric analysis (TGA), as a fast analytical alternative, have been successfully used in the food and wood industries so that both are potentially feasible in the bioenergy industry. The aim of this work is to provide knowledge about biomass properties and the application of IR and thermogravimetric methods in the feedstocks characterization for biofuel production. The IR spectra for biomass samples (cotton fiber, wood, maguey, corn cob, wheat straw, sugarcane bagasse, orange peel, pineapple peel and banana peel) show bands at 3500-3200, 3200-2800 and 1070-900 cm^{-1} are assigned to -OH, -CH- and C-O-C stretching vibrations of different groups in cellulose. The bands at 1730-1600 cm^{-1} is assigned to C=O stretching vibrations of the carboxyl and acetyl groups in hemicellulose. The bands at 1600-1270 cm^{-1} are assigned to C=C, C-O and C-H stretching or blending vibrations of different groups in lignin. Thus, IR spectroscopy is a powerful tool for identification of biopolymers and selection of biomass feedstock. TGA provides a biomass weight-change profile as a function of the sample temperature. The pyrolysis of hemicellulose occurred at 220-315°C, cellulose at 305-375°C and lignin covered a whole temperature range (250-900°C). Using the weight loss associated with those regions identified, it was possible to obtain an approximation of the chemical composition of the raw materials under study.

Keywords: biomass feedstock, lignocellulosic biomass, polysaccharides content.



Potential of microwave treatment in environmental biorefinery



Bichot, A.⁽¹⁾, Delgenès, J.P.⁽¹⁾, Méchin, V.⁽²⁾, Radoiu, M.⁽³⁾, **García-Bernet, D.*⁽¹⁾**

⁽¹⁾ LBE, Univ Montpellier, INRA, 102 Avenue des Etangs, 11100 Narbonne, France

⁽²⁾ Institut Jean-Pierre Bourgin, UMR 1318 INRA/AgroParisTech, 78000 Versailles, France

⁽³⁾ Microwave Technologies Consulting, 69140 Rillieux la Pape, France

*Corresponding author: diana.garcia-bernet@inra.fr



Objectives fixed by World governments for succeeding energy transition have aroused interest on lignocellulosic biomass utilization for bioenergy and green chemistry applications. The valorization of agricultural residues is appealing due to their renewable character and the non-competition with food chain since non-edible parts of the plants are used¹. But due to their recalcitrance, deconstructive pretreatments are necessary to render possible biological conversions of these lignocellulosic residues. Microwave (MW) treatment has already proved its worth in many biotechnology fields and biomass pretreatment for biorefinery purposes is another possible application². This work presents the effects of MW pretreatment applied to two underexploited agri-food residues of economic interest and available in Europe in high quantities: grape pomace and corn stalks. The studied parameters were the following: power level, power density, irradiation time, temperature (controlled or not), liquid to solid ratio and solvent. Effects were evaluated by a complete biomass characterization before and after treatment, in particular phenolics release and by biological conversion efficiency (enzymatic hydrolysis and/or biochemical methane potential). Energy balances and energy consumption are presented. Observed most important operation parameters for improving treatment efficiency were: power density and solvent, but they had different effect on studied biomass: release of phenolic compounds was more influenced by MW power density in grape pomace than by solvent, contrary to corn stalks, in which the present phenolics (hydroxycinnamic acids) decrease significantly cell wall accessibility, due to their characteristics, needing a though physico-chemical pretreatment involving higher chemical consumption. For both substrates, MW treatment allowed to improve Biochemical methane potential.

Keywords: Environmental biorefinery, lignocellulosic biomass pretreatment.

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Functional convergence of two diazinon microbial communities to a toxic shock by chlorpyrifos.

Arias-Ruiz Alfredo ⁽¹⁾, Ambriz-Mexicano Isabel ⁽¹⁾, Galíndez-Mayer Juvencio ^{*(1)}, Ruiz-Ordaz Nora ⁽¹⁾, García-Mena Jaime ⁽²⁾

⁽¹⁾ Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Av. Wilfrido Massieu s/n, Ciudad de México.

⁽²⁾ Cinvestav, IPN, Departamento de Genética y Biología Molecular. Av. Instituto Politécnico Nacional # 2508, Zacatenco, Ciudad de México

*Corresponding author: juvencio gm@yahoo.com



The pesticides diazinon (DN) and chlorpyrifos (CP) are potential carcinogenic compounds, and harmful to the aquatic biota. The application of mixtures of pesticides to combat more than one pest is common in agricultural activity. To remove them from the ground or to prevent their arrival to water bodies it is convenient to use microbial communities that allow the efficient and rapid degradation of mixtures of these compounds. The effect of joint toxic molecules in microbial communities (CM) has been little studied, so in this work, the kinetic response to a CP toxic shock was evaluated in two distinct CMs. The first one, CMT, is a mixture of four communities; each one of them with the proved capacity to degrade the pesticides DN, CP, BN (benomyl) and BF (bifenthrin), and the other (CMX), was acclimatized during a long time exclusively on diazinon. The toxic shock consisted of the increasing and additive supply of CP to each community. Their kinetic behavior was evaluated in parallel in two identical aerobic biofilm reactors, running in the same conditions. Gas chromatography coupled to mass spectrometry was used to evaluate the degradation kinetics of the pesticides and the formation of metabolic products. The CMX and CMT communities had a similar kinetic response when they grew on DN in a continuous regime. The increasing addition of CP to both reactors did not significantly alter the removal efficiencies of DN and CP, nor the pattern of accumulation of the metabolites product of the decomposition of DN and CP. The kinetic results showed that in both cases, a functional convergence of both CMs was presented, independently of the origin and previous acclimatization processes.

Keywords: diazinon, chlorpyrifos, functional convergence, multifunctional microbial community.



Development of a crude glycerol valorization process obtained as a by product of the technology IPN-GBD-1000 of biodiesel production



Tiscareño Ferrer * ^(1,2), V. Y. Mena Cervantes ^(1,2), R. Hernández Altamirano ^(1,2)

⁽¹⁾ Instituto Mexicano para la producción más limpia, Av. Acueducto S/N, Gustavo A. Madero, Ticomán, Ciudad de México, 07340, México.

⁽²⁾ Instituto Politecnico Nacional, Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad de Biocombustibles (LaNDACBio), Ciudad de México, 07340, México

*Corresponding author: alejandralf65@hotmail.com



The IPN-GBD-1000 process for the production of biodiesel produces as sub-product crude glycerol with a percentage of glycerol in the ranges of 20-25% weight that requires of a purification to achieve commercial value. For this reason, the main objective of this work is obtain glycerol with a purity of over 90%, through a primary treatment via chemical neutralization. Two acids were evaluated for neutralization, sulfuric acid (H_2SO_4) and citric acid ($C_6H_8O_7$) of which glycerol was obtained with purity of 98% and 86% and a treatment efficiency of 90% and 32%, respectively. The spectroscopic characterization was carried out to determine the functional groups of the crude and purified glycerol, by means of infrared spectroscopy (FTIR). The thermogravimetric analysis (TGA) was used to quantify the components present in the crude glycerol and the purity of the glycerol obtained by the neutralization treatments. Finally, the Nuclear Magnetic Resonance (RMN) of proton 1H and carbon ^{13}C to verify the purity of the obtained glycerol. The environmental viability of the process was evaluated through the calculation of green metrics such as the Environmental Factor (EF), Water Factor (WF), Mass Intensity (MS) and Energy Intensity (EI), from which 0.03 kg waste / kg EF product was obtained, 1.1 kg reactive / kg product MS and 0.08 kg H_2O / kg product. The results obtained indicate that it is possible to increase the economic and environmental viability of the Biodiesel production process by coupling this purification process.

Keywords: Glycerol, Biodiesel, Purification, Green Chemistry.



Simultaneous radiant and thermal energy collection with a parabolic

cylindrical collector for biotechnological applications



Ruelas-Ramírez E.H⁽¹⁾, Velumani Subramanian⁽²⁾, Reyna-Velarde R^{*(1)}

⁽¹⁾ *Universidad Mexiquense del Bicentenario, Unidad de Estudios Superiores Tultitlán, Av. Ex-hacienda de Portales S/N, Col. Villa Esmeralda, Tultitlán Estado de México, México*

⁽²⁾ *Laboratorio de Eco-Nano Energías, Centro de Investigación y de Estudios Avanzados del IPN, Av. Av. Instituto Politécnico Nacional 2508, Gustavo A. Madero, San Pedro Zacatenco, Ciudad de México, México.*

*Corresponding author: r.reyna@umb.mx, fenixrv@yahoo.com.mx

Nowadays, microalgal biotechnology presents important advances. However, one of the main problems to solve are the low mass yields. Several studies show that light is directly linked to the yield in the cultures of these microorganisms. Microalgae cultures require light and temperature control. Several technologies, in the field of solar energy, have been developed and one of them are parabolic cylindrical collectors, which concentrate energy in a linear point ⁽¹⁾.

The aim of this work is to integrate a system of capture of light by means of optical fiber to a parabolic cylindrical thermosolar collector, with modifications that allow to work both systems simultaneously.

In our system, the optical fiber recovers the residual luminous radiation that is wasted in the solar thermal systems and its directed to the interior of an airlift, double-riser photobioreactor, with the purpose of increasing mass yields in a native photosynthetic culture, comparing it versus the same culture applying direct sunlight only.

Our results show that the system effectively contributes with significant quantity of light to the photobioreactor, so we can achieve superior mass yield, and this can open the possibility to use solar collection in photosynthetic cultures without any other energy investment to increase light input.

Keywords: Microalgae, Native culture, Photobioreactor, Solar collector.

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Analysis of pollutant emissions in a diesel engine using biodiesel as fuel



J. C. Paredes Rojas⁽¹⁾, F. J. Martínez Pineda⁽²⁾, R. Vázquez Medina⁽³⁾, R. A. Romero Merino⁽²⁾

⁽¹⁾ Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad en Biocombustibles (LANDACBIO) del Centro Mexicano para la Producción más Limpia, Instituto Politécnico Nacional de México, Acueducto de Guadalupe s/n. La laguna Ticomán, CP. 07340, Ciudad de México, México. Tel: 5729-6000 Ext. 52621. e-mail: paredesrojasjc@gmail.com

⁽²⁾ Escuela Superior de Ingeniería Mecánica y Eléctrica, Unidad Culhuacán, Instituto Politécnico Nacional, Avenida Santa Ana 951, San Francisco Culhuacán, Zona Urbana Ejidal San Andrés Tomatlán, 09080 Ciudad de México, México.

⁽³⁾ Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada del Instituto Politécnico Nacional (CICATA) Querétaro, Cerro Blanco No. 141 Col. Colinas del Cimatarío, C.P. 76090 Querétaro, Querétaro. MÉXICO.



Despite the greater efficiency of the diesel engine it is regarded by the public as being generally dirtier than petrol engines because the pollution it produces is visible and audible⁽¹⁾, so tests were carried out to verify the viability of the use of biodiesel as fuel (used in the form of a diesel-biodiesel mixture), and thus, reduce the polluting emissions caused by the vehicle fleet. Mixtures of different percentages of biodiesel were made taking as reference a volume of one liter of fuel for each test, the tests were carried out with fuel B10, B20, B30, B40 and B50. The measurements were taken 5 minutes after the engine was started, each measurement lasted 5 minutes and the engine was left running for 5 minutes without measurement between each test, 3 measurements were taken for each mixture, for a total of 30 minutes of engine operation. They were measured directly from the engine exhaust with a probe connected to a combustion efficiency and environmental emissions analyzer. On the other hand, operating values were obtained, as revolutions per minute of the engine with help of an electronic circuit to maintain similar operating conditions for all tests. With the combustion analyzer it was possible to obtain data on the percentages and parts per million of particles present during the burning of the fuel, with these information comparative graphs of pollutant emissions could be made, being able to observe the decrease of these particles in relation to the use of pure diesel. The results of the mixture B50 gave us a decrease of 12.5% of hydrocarbons (HC), a decrease of 3.99% of Carbon monoxide (CO), an important decrease of 50% of Sulfur dioxide (SO_2) and a significant increase of 114% of Nitrogen oxides (NOx).

Key words: biodiesel, diesel engine, polluting emissions.

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Study of extracellular enzymatic activity in native fungi isolates from

Yaqui Valley



S.R. Maldonado Bustamante ⁽¹⁾, I. Mondaca Fernández ^{*(1)}, E.A. Ciprián Uribe, R.A. Zúñiga Zamora, S. De los Santos Villalobos ⁽¹⁾, P. Gortares Moroyoqui ⁽¹⁾, J.J. Balderas Cortés ⁽¹⁾, M.M. Meza Montenegro ⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 5 de Febrero, 818, Ciudad Obregón, México

*Corresponding author: iram.mondaca@itson.edu.mx.



Lignocellulose is a matrix of three polymers: cellulose, hemicellulose, and lignin. There are different methods to give an added value to the lignocellulosic material, whether chemical, physical-chemical and biological. For biological methods, white rot fungi have been widely studied for delignification, and they are known for its ability to secrete ligninolytic enzymes and to degrade phenolic compounds the dyes present in textile effluents. The search of native strains has been considered as an alternative to ensure the survival of inoculum in a full-scale production if it comes from a similar substrate. This work aimed to determine the ability of *Pleurotus ostreatus* (P.o.) and TSM36 native fungi to secrete lignin-cellulolytic enzymes. The fungi were grown on malt extract agar (MEA) at 30 ° C. Three dry grams of wheat straw of 2 cm of length was put in a flask and were inoculated with five disc of 1 cm² of fungi on MEA with 1: 4 V/W of nutritive solution accord Thakur ⁽¹⁾. Enzyme extraction was performed every three days, which extract was centrifuged at 8500 r.p.m. for 15 minutes and the enzymatic activity of Manganese peroxidase (MnP) was determined, by monitoring the oxidation rate of 2,6 Dimethoxyphenol⁽¹⁾ for Xylanases (Xyl); Cellulases (Cel) activity were determined by the method of Miller⁽²⁾ for analysis of released reducing sugars. The production of MnP, Xyl, and Cel, by P.o and TSM36, was studied for nine days, where P.o. had the highest enzymatic activity of MnP 4.96 ± 0.19 IU / g of dry substrate at the third day, Xyl 10.96018 ± 3.77138 IU / g of dry substrate at sixth day and Cel of 11.73410 ± 0.92718IU / g of dry substrate at ninth day, it has been reported that P.o. had the maximum Xyl expression at eight day with 10 UI/g of dry substrate, and a concentration of MnP of 562.60 UI/gr dry substrate at the eighth day⁽¹⁾ although in this study the maximum was presented at the third day. TSM36 presented the highest enzymatic activity of Cel 14.70110 ± 2.23493 IU / g of dry substrate at the third day, for Xyl TSM36 had 38.03126 ± 2.42465 IU/g of dry substrate at the third day, MnP 0.00364±0.00104 at sixth day. It is concluded that the strains have the potential for their use in biopulping of lignocellulosic biomass.

Keywords: cellulose, Manganese peroxidase (MnP), xylanase, wheat straw

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Phenomenological model for the production of ethanol from a photosynthetic microbial consortium



D.J. Hernández-Melchor^{*(1)}, I.J. Rodríguez-Meléndez⁽²⁾, J. Alarcón-Bonilla⁽²⁾, J.E.S. Martínez-Calderón⁽²⁾, J.C. Rosas-Aveland⁽²⁾, E. Ríos-Leal⁽⁴⁾, P.A. López-Pérez⁽³⁾

⁽¹⁾ Colegio de Postgraduados, Carretera México-Texcoco Km. 36.5, Estado de México, México

⁽²⁾ Universidad Tecnológica de Tecámac, Carretera Federal México - Pachuca Km 37.5, Estado de México, México.

⁽³⁾ Escuela Superior de Apan-UAEH Carretera Apan-Calpulalpan Km.8, Hidalgo, México

⁽⁴⁾ Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Av. Instituto Politécnico Nacional 2508, Ciudad de México, México

*Corresponding author: dulcejazz@hotmail.com



For many decades, fossil fuels have been the main energy source and also the main engine of the world economy, however, due to the current problems, it is necessary to search for sustainable and environmental friendly alternatives. That is why the production of biofuels from microalgae is an option in this problem¹. Likewise, a novel phenomenological model was used to describe the kinetics of ethanol concentration. Growth kinetic was performed in 500 mL Erlenmeyer flasks using the BG-11 mineral medium supplemented with 10% rice water. For 10 days was evaluated the biomass production, nitrogen consumption, the amount of reducer sugars and the production of ethanol. At day 8 the maximum concentration of ethanol (4 g/L) was obtained. The quantity of ethanol obtained in this study is similar to the reported by Harun et al. (2011), who use the microalgae biomass as carbon source to the fermentation with *S. cerevisiae* obtaining 7 g/L of ethanol. The process proposed for these authors resulted more complex and expensive than the one we suggested in the present work. The kinetic model was able to reproduce the experimental data based on the correlation coefficient; finally, the parameters were optimized by a non-linear algorithm and a parametric sensitivity analysis which allowed establishing the confidence intervals to improve the quality of the prediction of the model.

Keywords: bioethanol, kinetic model, microbial consortium

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Obtaining photoheterotrophic microbial consortiums for the production of biohydrogen



D.A. Campos-Vivanco^{(1) (2)}, V.Y. Mena-Cervantes⁽¹⁾, L.R Tovar-Gálvez, B. Reyes-Romero^{(1) (2)}, J.A. Cruz-Pelaez^{(1) (2)}, S.M. García-Solares,^{(1)*}

⁽¹⁾ Centro Mexicano para la Producción más Limpia IPN, Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad de los Biocombustibles. Av. Acueducto S/N, Gustavo A. Madero, Ticomán, 07340 Ciudad de México, CDMX. Tel. 57296000, ext 52621.

⁽²⁾ Unidad Profesional Interdisciplinaria de Biología (UPIBI), Av. Acueducto, 550, Ciudad de México, México.

⁽³⁾ Centro Interdisciplinario de Investigaciones y Estudios sobre Medio Ambiente y Desarrollo IPN 30 de Junio de 1520 s/n, La Laguna Ticoman, 07340 Ciudad de México, CDMX

*Corresponding author: smgarcias_25@outlook.es;

At present, the increase of greenhouse gases in the atmosphere due to the burning of fossil fuels, as well as the reduction of oil reserves has warned countries worldwide to consider a greater generation of clean or renewable energies and in this way counteract the environmental, economic and social impacts that can occur in a future cause of the excessive use of fossil fuels. One of the alternatives that was sought was the production of biofuels to contain products extracted naturally, which are inexhaustible in the environment. Biohydrogen, although not as well-known as bioethanol and biodiesel, makes it a viable fuel for the generation of premium energy since its main product is water. In this work, we intend to implement the leachate extracted from the sanitary landfill westbound as an inoculum for the generation of Biohydrogen using acetic acid and ethanol as a substrate. The production of hydrogen was evaluated in dark fermentation and light fermentation determining parameters such as pH, temperature and cell concentration. A variation of the cellular concentration presented when the pH decreased and temperature was elevated. The pH variation is attributed mainly to the acid phase in which the ethanol is converted to acetic acid. The consortium microbial grew favorably in the imposed conditions.

Keywords: biofuel, biohydrogen, consortium microbial, fuel fossil



Evaluation of acid and enzymatic pretreatment in *Opuntia ficus-indica* for the production of assimilable sugars and bioproducts



Abimael Iván Avila-Lara ⁽¹⁾, Santiago Rodríguez-Valderrama ⁽¹⁾, José Antonio Pérez-Pimienta ^{*(2)}, Rubén Morones-Martínez ⁽¹⁾, Julián Cano-Gómez ⁽¹⁾, Elvira Ríos-Leal ⁽³⁾, Héctor M. Poggi-Varaldo ⁽³⁾, Carlos Escamilla-Alvarado ⁽¹⁾

⁽¹⁾ Universidad Autónoma de Nuevo León, Universidad Autónoma de Nuevo León, Centro de Investigación en Biotecnología y Nanotecnología (CIByN), Facultad de Ciencias Químicas. Nuevo León, 66629, Mexico.

⁽²⁾ Universidad Autónoma de Nayarit, Basic Sciences and Engineering, 63155, Tepic Nayarit,

⁽³⁾ Centro de Investigación y de Estudios Avanzados del Cinvestav, Department of Biotechnology and Bioengineering, 07360 México City.

*Corresponding author: cea_escamilla@yahoo.com.mx

Opuntia ficus-indica can become a source of carbohydrates for the generation of bioproducts such as biofuels and green chemicals. Generally, pretreatment processes are required to reduce the recalcitrance of the crystalline structures of biomass to make it suitable for bioprocesses¹. One of the most employed pretreatments is diluted acid as a method to release pentoses and attack celluloses; recently the removal of calcium oxalate by concentrated acid is considered to avoid the constraints to the saccharification stage fostered by this compound^{2,3}, lastly, enzymatic saccharification is an effective option to produce sugars from cellulose or hemicellulose. In this study, the potential of *Opuntia* for the generation of fermentable sugars was evaluated through a series of experimental designs in search of an optimal region. At first, in a 2³ design the solids loading, reaction time and acid concentration factors were evaluated. Later, a 2² central composite design (CCD) was evaluated to search for an optimization point for the production of sugars. This point was not found yet, and instead a steepest ascent method was formulated. Regarding the pretreatment of calcium oxalate removal, it consisted of introducing the biomass in an acid solution at 80 °C for 30 min; the removal of calcium oxalate was analyzed by the change in the X-ray diffraction patterns. Next, the hydrolyzed biomass without calcium oxalate was treated using commercial enzymes Novozymes and Cellic HTec2. From the 2³ factorial, a maximum point at 12% solid load and 3% acid concentration was determined; the reaction time was found to be not significant. In the CCD no optimization region was found, because sugar release was shown to increase as solids load and an acid concentration increased. From the steepest ascent method, sugar release reached up to 54 g/L. Currently, the enzymatic treatments is underway of execution.

Keywords: Hydrolysis, *Opuntia*, oxalate, sugars

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Organic Solid Waste Anaerobic Digestion for Methane Production

B. G. Arellano⁽¹⁾, R. Campuzano^{*(1)}, G. Trejo-Aguilar⁽¹⁾, A. Ferreira-Rolón⁽¹⁾, F. Ramírez-Vives⁽¹⁾ and O. Monroy⁽¹⁾

⁽¹⁾ Biotechnology Department, Universidad Autónoma Metropolitana, Av. San Rafael Atlixco 186, Col. Vicentina, C.P. 09340 Delegación Iztapalapa CdMx, México.

*Corresponding author: rcampuzano.a@gmail.com



The anaerobic digestion (AD) is a proven technology for biogas production from organic solid wastes (OSW) as alternative energy¹ but in emerging countries has not been implemented in a commercial scale. In this order, prototypes in pilot scale are necessary to know the operational factors that can affect the process. Two stage AD (2S-AD) allows to improve the hydrolysis process and to obtain value-added byproducts as volatile fatty acids (VFA) in the first stage, as precursors for rapid methane production in a second stage. The objective of this work is to evaluate the operation of a 2S-AD pilot plant in order to quantify de methane recuperation from real and variable organic solid wastes. During 2017, 2S-AD pilot plant was operated with OSW from the university cafeteria and a nearby restaurant. First stage is carried out in hydrolysis leaching bed reactors (AHLBr) with a capacity of 1 T/d, during 2017 the average load was 100 kg/d, processing 18 ton of OSW with an average organic loading rate of 5 kgVS/m³·d and 19 m³ of leachate generation (62±17 gCOD/L). The volatile solids and COD reduction were 58 and 52%, respectively. Leachate was mixed with the wastewater generated in the University and fed to an upflow anaerobic sludge blanket (UASB) reactor for biogas production in the second stage. From the leachate only, 690 m³ of biogas was produced, which is cleaned with a membrane system and 295 kg of methane have been recuperated. Nowadays the process capacity was increased to 280 kg/d looking forward increase it to 1 ton/d in 2019 and different adaptations are being done for the process automation and optimization. This pilot system will eventually use more organic wastes generated elsewhere.

Keywords: Hydrolysis, methane, organic solid waste, Two stage anaerobic digestion.

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Comparison of the lignin degrading activity of three recombinant

laccases from *Pycnoporus sanguineus*



A. Cerdan-Cabrera ⁽¹⁾, E. Alarcon* ⁽¹⁾, A. Camas ⁽²⁾, F. Ziarelli ⁽³⁾, L. Sanchez-Velasquez ⁽¹⁾, M.L. Osorio ⁽¹⁾.

⁽¹⁾ Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana, Av. de las Culturas Veracruzananas No. 101, Campus para la Cultura, las Artes y el Deporte, Col.

⁽²⁾ Emiliano Zapata, C.P. 91090, Xalapa, Veracruz, México Centro de investigación en estudios avanzados del IPN, Unidad Irapuato. Km 9.6 libramiento

⁽³⁾ Norte Carr. Irapuato – Leon, CP 36821. Irapuato, Gto. México Fédération des Sciences Chimiques de Marseille FR CNRS 1739, Spectropole, av. Escadrille Normandie Niémen, case 511, F-13013 Marseille, France.

*Corresponding author: enalarcon@uv.mx

White rot fungi, like *Pycnoporus sanguineus*, have the ability to secrete a combination of lignocellulose degrading enzymes among which are laccases. Laccases are strong oxidizer enzymes; this property allows multiple applications in biotechnological industry. Our interest focuses on pretreatment sugar cane bagasse (or other lignocellulosic substrates) for bioethanol production. Our *P. sanguineus* strain has already been used for lignin degradation through solid state fermentation but we think that expressing three of its laccase genes in recombinant yeast cultures will allow us to overproduce the enzymes and optimize the lignin degradation of our substrate. Some works have already been done to study recombinant laccases yet none has compared lignin degradation of three isoforms from *P. sanguineus* in the same culture conditions. In order to do such comparison, we isolated, cloned and overexpressed separately the three laccase genes with the highest activity reported in the Genbank for *P. sanguineus* in a *Pichia pastoris* strain. First, a specific pair of primers was designed to amplify the sequence of each gene. Total RNA was extracted from the mycelium of our *P. sanguineus* strain and a RT-PCR was performed using the designed primers to obtain the cDNA of each of the three laccase genes. Afterwards, cDNAs will be cloned separately in an *E. coli* strain for preservation and sequencing and then they will be cloned and overexpressed in *Pichia pastoris* cell cultures. The recombinant laccases will be biochemically characterized and their lignin degrading activity over sugar cane bagasse will be measured and compared through ¹³C Solid State Nuclear Magnetic Resonance.

Keywords: laccase, pretreatment, *Pycnoporus sanguineus*, recombinant.



Simultaneous fermentation and saccharification of sugar cane-bagasse

for ethanol production by *S. cerevisiae* RP2-BGL



Castro-Eddy Luis ⁽¹⁾, Ramirez-Reyes Itzel ⁽¹⁾, Poggi-Varaldo Hector ⁽²⁾, Cristiani-Urbina Eliseo ⁽¹⁾, Ponce-Noyola Teresa ⁽¹⁾

⁽¹⁾ Departamento de Biotecnología y Bioingeniería – CINVESTAV Unidad Zacatenco, Mexico City, Mexico

⁽²⁾ Departamento de Ingeniería Bioquímica – ENCB, IPN Unidad Zacatenco, Mexico City, Mexico

*Corresponding author: tponce@cinvestav.mx



The production of bioethanol from lignocellulosic residues, in a Simultaneous Saccharification and Fermentation Process (SSF) requires cellulolytic enzymes and an efficient ethanologenic strain. In this way in the present work we evaluate in a SSF, the production of ethanol from sugarcane bagasse (SCB) using *Cellulomonas flavigena* PR-22 enzymes [1] and *Saccharomyces cerevisiae* RP2-BGL[2] that expresses an active β -glucosidase. *C. flavigena* PR-22 enzymes and *S. cerevisiae* RP2-BGL were inoculated in a bench bioreactor (0.5 L) using 2 and 6% of SCB. The ethanol at the end of the fermentation was 2.4 and 3.3 gL⁻¹ respectively. In previous studies our research group carried out a sequential processes ethanol production (SESF), achieving 1.5 gL⁻¹ of ethanol from 2.2% of SCB. The SSF represented a 60% increase of the final concentration. The implementation of SSF processes can reduce the time and production costs of 2G bioethanol, since the sugars are consumed as soon as they are released, diminishing the feedback inhibition of the cellulases. These results also show that *S. cerevisiae* RP2-BGL is an efficient microorganism for using in SSF for the production of bioethanol.

Keywords: Bioethanol, lignocellulosic residues, Saccharification.

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Pretreatment Process.



C. E. Molina-Guerrero^(1,2), G. de la Rosa⁽²⁾, H. Castillo-Michel⁽³⁾, A. Sánchez⁽⁴⁾, C. García-Castañeda⁽²⁾, A. Hernández-Rayas⁽²⁾, I. Valdez-Vazquez⁽⁵⁾, S. Suarez-Vázquez⁽⁶⁾

⁽¹⁾ Autonomous University of Chihuahua, New Campus University, Circuito 1, Chihuahua, Mexico

⁽²⁾ Autonomous University of Guanajuato, Loma del Bosque, 103, León, Mexico

⁽³⁾ European Synchrotron Radiation Facility, ID21, BP220-F-38000, Grenoble, France.

⁽⁴⁾ Cinvestav-Unidad Guadalajara Advance Engineering Unit, Av. del Bosque, 1145, Zapopan, Mexico.

⁽⁵⁾ UNAM-Juriquilla Engineering Institute, Blvd. Juriquilla, 3001, Juriquilla, Mexico.

⁽⁶⁾ Autonomous University of Nuevo León, Av. Universidad, S/N, San Nicolás de los Garza, Mexico.

*Corresponding author: cemolina@uach.mx

Pretreatment of Lignocellulosic Biomass (LB) comprises a key stage in biorefinery schemes for glucose production and its subsequent transformation into bioethanol or bio-based, high-value-added products. Nevertheless, the changes occurring during the depolymerization of lignocellulosic biomasses are not yet fully understood. Synchrotron micro-Fourier Transform InfraRed (μ -FTIR), Raman Spectroscopy (RS), X-Ray Diffraction (XRD), and X-Ray Fluorescence (XRF) were used for better characterization of Wheat Straw fibers during a continuous pretreatment process: Conditioning (C), Extrusion (E), Steam Explosion (SE), and Enzymatic Hydrolysis (EH). μ -FTIR revealed functional groups as phenylpropanoid polymers, ethers, and aliphatic alcohol. RS revealed acetoacetate, methyl and phenol groups after SE. The crystallinity index (CrI) was: 54.5%, 27.1%, 31.6%, and 26.7% for C, E, SE and EH respectively. The silica content was: 2.5%, 2.7%, 1.9% and 5.6% for C, E, SE and EH respectively. To our knowledge, this is the first time that a synchrotron source has been used to analyze a WS after a continuous conversion process.

Keywords: Synchrotron μ -FTIR, Wheat straw, Steam explosion, Silica content.



Design and Construction of a Fresnel-type Linear Solar Concentrator



J. Álvarez-Sánchez, J. Bernal-Tolano, **C. A. Duarte-Ruiz***, G. E. Dévora-Isiordia, J. Saldivar-Cabrales

Instituto Tecnológico de Sonora, 5 de Febrero, 818 sur, Cd. Obregón, México.

*Corresponding author: andres.duarte@itson.edu.mx



The need to understand and go against climate change effect, as well as the future demand for clean water, involves the study and development of new technologies through renewable energies (1). The solar concentrator is a type of solar collector that, through mirrors or lens, concentrates a big quantity of light in a small area (2). For local conditions, an optical, geometric and thermal design was carried out, as well as the construction of a Fresnel-type linear solar concentrator (FLSC). The FLSC technology represents an option that had not yet been studied in the Yaqui Valley; with possible applications in brackish water desalination, crystallization and power generation. The results show that operating variables for local conditions that allow reaching the highest collection efficiencies. The use of solar energy through FLSC is a viable option for the Yaqui Valley.

Keywords: Drinking water, Renewable energy, Solar concentration, Yaqui Valley.

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Inhibition of hydrogen production by sodium polyacrylate from dark

fermentation of a diaper-like substrate



Perla X. Sotelo-Navarro⁽¹⁾, Héctor M. Poggi-Valardo^{*(2)}, Sylvie J. Turpin-Marion⁽¹⁾

⁽¹⁾ Department of Energy, Sustainable Technologies Laboratory, UAM-A, México, D. F. Av. San Pablo #180 Col. Reynosa Tamaulipas, Ciudad de México, D. F. C. P. 02200.

⁽²⁾ Environmental Biotechnology and Renewable Energies Group GBAER-EBRE, Department of Biotechnology and Bioengineering, Centro de Investigación y Estudios Avanzados del IPN, P.O.Box 14-740, Ciudad de México D.F., 07000, México.

*Corresponding author: e-mail. r4cepe@yahoo.com



In Mexico, waste diapers (WD) constitute an environmental problem. Little is known of WD reclaiming by producing biohydrogen. Furthermore, less is known about the effect on bioenergy process by sodium polyacrylate (SAP), a polymer that is added to diapers for absorbing/retaining moisture (urine of babies). This research evaluated the inhibitory or toxic effects of SAP on hydrogenogenic dark fermentation of a model substrate that resembles waste diapers. Three types of substrate bond paper and filter paper (P, substrate control), only sodium polyacrylate (SAP control or hydrogel, HG) and paper-hydrogel (P-HG) were tested in hydrogenogenic batch bioreactors at 37 °C. The bioreactors were operated using the anaerobic hydrogenogenic fermentation with intermittent venting and headspace flushing (SSAHF-IV) process. The reactors were loaded with the substrate at ca. 25 % of total solids and 10% w/w inoculum. It was found that the SAP hydrogel did not produce hydrogen. Units loaded with P-HG showed H₂ productions ca. 25% lower than that of bioreactors loaded with only paper. Our results suggest that diaper manufacturers could consider SAP replacement in diapers considering the optimization of H₂ yields during the treatment of waste diapers. For sustainability sake, SAP could be replaced by special starches such as those used by the food industry, that are known to absorb important amounts of water and are still degradable by dark fermentation. In the meantime, for dealing with SAP in diapers, the inoculum used for hydrogenogenic fermentation could be acclimated to acrylate degradation by using increasing amounts of acrylic acid or low molecular weight acrylates spiked in the feed to of semi-continuous bioreactors fed with a cellulosic waste.

Keywords: sodium polyacrylate, dark fermentation, diaper-like, hydrogen.



Evaluation of the culture of *Dunaliella tertiolecta* in f/2 Guillard and

effluent of shrimp with variable temperature in biomass production



Hernández-Ferreira J.J.⁽¹⁾, Ortega-Clemente L.A.^{*(1)}, Valadez-Rocha V.⁽¹⁾, Pérez-Legaspi I.A.⁽¹⁾, León-Rangel E.⁽¹⁾,

⁽¹⁾ Tecnológico Nacional de México. Instituto Tecnológico de Boca del Río. División de Estudios de Posgrado e Investigación. Km. 12 Carr. Veracruz-Córdoba, Boca del Río, Veracruz. 94290. México.

*Corresponding author: alfclemen2002@yahoo.com.mx



In recent years, important advances have been made in the use of microalgae for various purposes such as human health, cosmetology, purification of wastewater, prevention of aquatic contamination, pharmaceutical industry, aquaculture, production of pigments and antibiotics, biofuels, and among others. Microalgae have a high content of antioxidant compounds (carotenoids, phycobilins and fatty acids) that are used in the biotechnology industry, using different temperatures to obtain a higher growth (1). The objective of the present work was to evaluate the effect of temperature on cell density, production and productivity of *Dunaliella tertiolecta* biomass cultivated in f/2 Guillard medium and shrimp effluent. The culture systems in both media were 1.2 L flasks with a culture volume of 1L at 24, 28, 32 and 36 ° C and at a luminous intensity of 20,000 K. The results show that the best growth was observed between 28 ° C and 24 ° C. At 24 ° C, the highest cell density was observed in both f/2 Guillard medium and shrimp effluent, in stationary phase (4.5×10^6 and 3.05×10^6 cel ml⁻¹, respectively) on the sixth day in both treatments. The highest production observed in both media was at 24 ° C in stationary phase with 722 and 312 mg L⁻¹ in f /2 Guillard medium and shrimp effluent, respectively. However, the highest productivity in both media was at 24 ° C and 28 ° C (144 and 77 mg L⁻¹ d⁻¹) in the f/2 Guillard medium and shrimp effluent in stationary and exponential phase, respectively. It can be concluded with the observation that the optimum temperatures for the growth of *D. tertiolecta* are between 24 ° C and 28 ° C in both treatments. On the other hand, although the growth in f/2 Guillard medium is better in all the treatments, the price of this medium is high which opens a window of opportunity to the effluent of shrimp due to its low cost.

Keywords: *Dunaliella tertiolecta*, f/2 Guillard, shrimp effluent, variable temperature

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Comparison of the production of bioH₂ between a microbial consortium

photoheterotrophic and *Rhodopseudomonas palustris*



S.M. García-Solares ⁽¹⁾; H.M. Poggi-Varaldo ^{(2)*}

⁽¹⁾ Centro Mexicano para la Producción más Limpia IPN, Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad de los Biocombustibles. Av. Acueducto S/N, Gustavo A. Madero, Ticomán, 07340 Ciudad de México, CDMX. Tel. 57296000, ext 52621.

⁽²⁾ Centro de Investigación y de Estudios Avanzados del IPN, Av. Instituto Politécnico Nacional 2508, Gustavo A. Madero, San Pedro Zacatenco, 07360 Ciudad de México, CDMX. Tel. 57473800, ext 4321, 4324

*Corresponding author H.M. Poggi-Varaldo



An alternative to the problems caused by the production and use of fossil fuels is the biological production of hydrogen (H₂) which is considered a clean fuel. The non-sulphur purple bacteria (NSPB) are photosynthetic microorganisms that use organic, simple and economical substrates for the anoxic production of H₂. The objective of the project was to evaluate the effect of the type of culture, type of medium and lighting regimes on photoheterotrophic hydrogen production (P_{H₂}) by selected NSPB. Mixed cultures of NSPB were obtained from a Winogradsky column (WC) that was incubated at room temperature and an intensity of light of 2500 to 3000 luxes. In the second part, a factorial design of 3 factors was implemented: two levels of substrate (medium 27 and Pfennig), two biocatalysts (the consortium of the WC and NSPB pure strain *Rhodopseudomonas palustris* (*Rp*), and two lighting regimes (continuous and cycles of 10 and 14 hours, light intensity was 2500 lux.) The process was carried out at room temperature and batch mode; the main response variable was P_{H₂}. Medium 27 was leached from an anaerobic digester and spiked with a mixture of volatile fatty acids and alcohols. In general, *Rp* bioH₂ production was much higher than that of the consortium, contrary to expectations. Higher P_{H₂} was obtained with Medium 27 than with Pfennig one. Finally, the lighting mode did not result in P_{H₂} differences. Results were encouraging regarding the use of Medium 27 that would allow the coupling of bioH₂ from dark fermentation to production of bioH₂ from photoheterotrophic fermentation that would lead to increased yields of bioH₂ from organic wastes. Yet, more research is needed to develop a NSPB-consortium with bioH₂ yields as high as those of pure strains of NSPB.

Key words: non-sulphur purple bacteria (NSPB), photoheterotrophic hydrogen.



Effect of water depth and plant density in rooting of the energy crop

Arundo donax L. for propagation by stems



Cano-Ruiz J, Amorós M.C, Sáez-Marugán D, Montalvo S.G, Lobo M.C, Mauri P.V,
Ruiz-Fernández, J
IMIDRA. Finca "El Encín" A-2 Km 38,2, 28800 Alcalá de Henares (Madrid), Spain
Corresponding author*: judith.cano@madrid.org



The promising energy crop Giant reed (*Arundo donax*) is almost sterile in terms of seed production. The main used method for crop establishment is the rhizome propagation, which is unpractical and expensive. Other methodology, the reproduction of culture in vitro, supposes an extra cost for farmers. The rooting of the stems is the cheapest way to produce own plants, being necessary to do it in the best way to ensure the survival of the plant during the first year of development. The objective of this work is to determine if density or depth are determinant factors in rooting and sprouting of *Arundo donax* for crop establishment. Stem cuttings were collected from secondary buds, from a thirteen-year-old crop. Three water depths and three plant densities were evaluated. Depths were: P1 (6 cm of water); P2 (12 cm of water); P3 (18 cm of water). Densities were: D1 (1 cm between stems); D2 (2 cm between stems); D3 (stems grouped in bunches). The treatments included the combination of both factors. Ten secondary stems with two branches were cut at 40 cm length and included in each tray. Three trays by treatment were evaluated. The experiment was carried out for 4 months in greenhouse conditions. After this period, different parameters were evaluated, percentage of rooting and sprouting of stems; root number; root length; sprout number; sprout length and weight of roots and sprouts. No significant differences were observed between groups in sprouting or rooting percentage; neither in root length, dry weight root, sprout length or dry weight of sprout. Differences between groups in root number and sprout number were observed either with depth and density, as well as with the interaction of both factors. The best treatment was P3D2, (18 cm of water and 2 cm of separation between stems). This is the deepest treatment where plants were more separated. In all evaluated experiments the higher number of roots or sprouts were obtained in the density D2. In this sense, a higher space between plants favors a better rooting and sprouting for the implantation of *Arundo donax* as energy crop.

Keywords: energy crops, alternative energy, implantation, biomass

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Biogas production and characterization from different mixtures of agro-industrial wastes

I. Silva-Ortiz⁽¹⁾, A. Pérez-Hernández⁽²⁾, I. Pérez-Reyes⁽¹⁾, S. B. Pérez-Vega⁽¹⁾, C.E. Molina-Guerrero⁽¹⁾, I. Salmerón^{*(1)}

⁽¹⁾ *Universidad Autónoma de Chihuahua, Facultad de Ciencias Químicas, Circuito Universitario s/n, Campus II, Chihuahua, México.*

⁽²⁾ *Centro de Investigación en Materiales Avanzados, Parque Industrial, Chihuahua, México.*

*Corresponding author: isalmeron@uach.mx.



The production of biogas from the anaerobic digestion of agro-industrial wastes is a sustainable alternative for the production of energy without generating a high environmental impact; additionally and added value product like biofertilizer is obtained. The objective of this study was to evaluate the production of biogas generated by various mixtures of agro-industrial wastes abundant in the state of Chihuahua such as cow manure, cheese whey, apple pomace and corn stover. The dry biomass of these feedstocks was pulverized in a ground mill. Mixtures were adjusted to a carbon-nitrogen ratio (C:N) of 20:1 using a fix concentration of cow manure. Anaerobic fermentations were carried out at 30 °C for a period of 10 days in 200 mL biodigestores with a value of 10% w/v of biomass and inoculum size of 5% w/v. The inoculum was from a local dairy farm biodigestor. A water displacement method was used for the measurement of the gas volume produced. It was observed that with only cow manure the rate of biogas production was very slow until the tenth day when this value increased, however when combined with cheese whey, corn stover and apple pomace the biogas production was significantly higher obtaining volumes of 755 mL in 4 days. Nevertheless, this fast microbial growth and gas production generates an acidification of the media that inactivates the microbiota in the biodigestor lowering the production of biogas. In future work it is expected to optimize the mixtures of agro-industrial wastes varying the ratio of the formulations and controlling the pH of the biodigesto.

Key words: bioenergy, biogas, agro-industrial wastes, methane.



Obtaining Renewable Diesel from castor oil

Adid Olaff Santoyo López ^{*(1,2)}, R. Hernández Altamirano ^(1,2), F.S. Mederos Nieto ^(1,2)

⁽¹⁾ Centro Mexicano para la producción más limpia, Av. Acueducto S/N, Gustavo A. Madero, Ticomán, Ciudad de México, 07340, México.

⁽²⁾ Instituto Politécnico Nacional, Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad de Biocombustibles (LaNDACBio), Ciudad de México, 07340, México

*Corresponding author: adidolaff@gmail.com



With the intensification of the search for a sufficient to oil-based energy, the development of alternative energy, the development of alternative energy sources becomes increasingly relevant. Fuels derived from biomass or biological sources have received a lot of attention. Faced with the depletion of fossil fuels and the search for diversification of the energy matrix for México, there is a need to take advantage of natural resources in a sustainable manner, generation Jobs among society, promoting the economy in rural areas and generating more fuel. Friendly to the environment that are incorporated into the carbon cycle. The objective this work is to obtain renewable diesel from the castor from the state of Morelos, based on obtaining methyl esters from the reactive extraction by homogeneous catalysis of the castor bean seed and in the hydrotreatment of the methyl esters in a Batch reactor characterizing the products. The extractive reaction was carried out by conditioning the seed and adding a solvent with catalyst so that the castor oil extraction was carried out and in the same stage obtaining the castor methyl esters with the aim of reducing a step in this process and agreeing to the saving of resources and energy, the product obtained from the extractive reaction was characterized by the Fourier transform infrared (FTIR) and thermogravimetric analysis (TGA). The hydrotreating reaction was carried out at certain pressure conditions and different temperature levels in a Batch reactor in order to give properties very similar to fossil diesel (number of cetans) so that no type of adaptation is necessary in the engines of the vehicles to which is added renewable diesel.

Keywords: Renewable diesel, Transesterification, Hydrotreating, Methyl esters of castor oil.



Aquaponics system for *Salicornia bigelovii* cultivation

Escoboza-Barceló D. O.^{(1)*}, Gortáres-Moroyoqui P.⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 5 de Febrero, 818, Ciudad Obregón, México.

*Corresponding author: dorlando.eb@gmail.com.



The Sonora state (Northwest of Mexico) is part of the most arid zone in the country. Despite that condition, Sonora is among the top food producers nationally, predominantly producing conventional crops as wheat and maize, while saline aquaculture has too an outstanding role in state's economy. These activities result in harmful environmental effects. The recent paradigm of saline agriculture implies the production of high salt and drought tolerant crops in marginal lands to produce cattle, human feed or biofuels, linking aquaculture systems with ecosystem services resulting in a reduction of the impact of human activities. *Salicornia bigelovii* is a halophyte plant that has a high tolerance to the salinity, so it can be sowed in saline soils, and irrigated with seawater during their annual phenologic cycle. Since recent decades, *S. bigelovii* has been object of environmental interest because of seed has a high oil content, which can be a source of feedstock for biofuels like renewable jet fuel (RJF). Our main objective is to set an aquaponic system in order to produce *S. bigelovii* seedlings. *Salicornia bigelovii* seeds from Baco-chibampo bay (Sonora, Mexico) were collected from dead plants. The seeds were sowed in germination trays with expanded perlite as substrate, irrigated with seawater by intermittent subirrigation. Once reached enough maturity for a strong and defined radicular system, the youth plants will be transplanted to individual pots to finish their vegetative development, and then will be irrigated with aquaculture wastewater until reach a full-grown and seed productive plant. Under our conditions, we have 53% of germination after 12 days of sowing. The seedlings were transplanted to a tray filled with substrate and irrigated with aquaculture wastewater. After 121 days after sowing, the last one seedling dries and die. At 109 days of the experiment, we weren't able to find the optimal laboratory conditions that would lead to a further vegetative growth in seedlings. Then, we need to explore other laboratory conditions (light, temperature, humidity, water) that help the development of the seedling to its final phenological stage.

Keywords: Aquaponic system, Biofuels, *Salicornia bigelovii*.



Effect of *Nannochloropsis oculata*, added in a commercial diet, on the growth, survival and immune system of *Litopenaeus vannamei*



García-Medel D.I. (1), Luna-González A.(2), **Ortega-Clemente L.A.**(1) *, Álvarez-Ruiz P(2).
(¹) *Tecnológico Nacional de México. Instituto Tecnológico de Boca del Río. División de Estudios de Posgrado e Investigación. Km. 12 Carr. Veracruz-Córdoba, Boca del Río, Veracruz. 94290. México.*
(²) *Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, Unidad Sinaloa, Sinaloa, México.*
*Corresponding author: alfclemen2002@yahoo.com.mx



In aquaculture, the continuous use of antibiotics for the control of pathogens has resulted in the generation of strains resistant to antimicrobials. Due to this, it is necessary to find safer alternatives such as the use of immunostimulants. The present work evaluated the effect of the microalga *Nannochloropsis oculata*, added to a commercial diet, on the growth, survival and immune system of the white shrimp *Litopenaeus vannamei* challenged with *Vibrio parahaemolyticus*. For this, a bioassay (36 days) was designed in triplicate where different concentrations of the microalga (0, 2.5, 5.0 and 7.5 g kg⁻¹) were tested and added to a commercial feed, at day 30 of culture was inoculated with *V. parahaemolyticus* (LC50 = 180,000 CFU mL⁻¹). After 30 days of bioassay, shrimp fed at a dose of 5.0 and 7.5 g kg⁻¹ of *N. oculata* showed a significant difference ($P < 0.05$) in the specific growth rate (6.35 and 6.34%, respectively) in relation to the control (5.64%). Regarding the productivity index, only the dose of 5.0 g kg⁻¹ with 105.7 was different ($P < 0.05$) to the control (69.3). There were no significant differences between the treatments with respect to the amount of *Vibriosis* present in the shrimps. Likewise, there were no significant differences in the survival of the shrimps challenged with *V. parahaemolyticus* between the four treatments. Despite this, treatment with 2.5 g kg⁻¹ presented the highest survival value with 25.8%, unlike the 12.1% control. Although there were no significant differences between the four treatments in relation to final weight, gain in weight, feed conversion factor, treatment with 5.0 g kg⁻¹ presented the most optimal values of all treatments. The results obtained indicate that *N. oculata* improves the growth of *L. vannamei* and may be a good candidate to be used as an immunostimulant, so it is recommended to carry out new studies evaluating different doses and frequencies.

Keywords: Immune system, *Litopenaeus vannamei*, *Nannochloropsis oculata*, *Vibrio parahaemolyticus*.



Microalgal production with potential for obtaining omega-3 fatty acids and phycoremediation of aquaculture wastewater

López-Carrillo Z. ⁽¹⁾, Robledo-Narváez P.N. ^{*(1)}, Ortega-Clemente L.A. ⁽²⁾, Vázquez-Larios A.L. ⁽¹⁾, Gutiérrez-Rivera B. ⁽¹⁾

⁽¹⁾ Tecnológico Nacional de México, Instituto Tecnológico de Tierra Blanca, Maestría en Ciencias de los Alimentos y Biotecnología, Prol. Av. Veracruz, S/N, Tierra Blanca, Veracruz, México

⁽²⁾ Tecnológico Nacional de México, Instituto Tecnológico de Boca del Río, División de Estudios de Posgrado e Investigación, Km. 12 Carr. Veracruz Córdoba, P.O. Box 94290, Boca del Río, Veracruz, Mexico

*Corresponding author: paurobnar@gmail.com; pau_robnar@yahoo.com.mx.

Microalgae have the capacity to eliminate nitrogen and phosphorus from the environment. They are capable of accumulating appreciable amounts of lipids [1], among their fatty acids are the ω -3 and ω -6. The present work of investigation evaluated the algal production of *Chlorella vulgaris* and an isolated microalga in the Superior Technological Institute of Tierra Blanca named AVL cultivated in effluent of tilapia (ET) and in Bold's Basal Medium (BBM) as control, with light-darkness cycles, continuous lighting (CS) and intermittent lighting (IS), in phases of exponential (EX) and stationary (ST) growth. The biomass production, lipid content, ω -3 fatty acids and compound removal efficiency were evaluated. We used an initial factorial experimental design 2^4 and a 2^2 to evaluate the phytoremediation. The photoreactors were acrylic tubular with an operation volume of 2.0 L, inoculated at an initial cell concentration of 1.0×10^6 cells mL^{-1} at 21 ± 1 °C. The results indicate that the highest cell density was obtained with *C. vulgaris* in BBM with CS (28.5×10^6 cells mL^{-1}). In the analysis of main effects on the production of biomass and its lipid content, it was observed that biomass production was higher in *C. vulgaris* in BBM in CS and ST phase (1.31 ± 0.03 g L⁻¹); however, the highest lipid content was presented by AVL in ET in the EX phase in SI ($81 \pm 7\%$). The highest content of ω -3 fatty acids found was in the cultures with ET. The microalgae AVL was the one that presented the highest production of this type of fatty acids, the best treatment was 7.04% of ω -3 in ST phase, which represents 6.11% of ω -3 in dry weight of the biomass. The average removal efficiencies (%) of ammonium, nitrites, nitrates and phosphate did not vary between treatments. The lowest observed removal was for AVL phosphate in IS (75%), in ammonium the lowest was in AVL with CS (80%), in AVL nitrate with IS (88%) and in nitrites the behaviors were very similar in both microalgae with 100% and 96%, respectively. The values show that AVL is an alternative for the production of lipids with ω -3 fatty acids with the potential to treat aquaculture wastewater.

Keywords: *Chlorella vulgaris*, fish farm effluent, phycoremediation, omega-3 fatty acids

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and *Nannochloris oculata* in effluents of Tilapia farming for the production of fatty acids with potential in the production of biofuels. African Journal of Biotechnology. 14 (20) 1710-1717.



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Chapter 2 Sustainability and Environmental System Analysis



Local soil salinization caused by irrigation of horticultural and fruit crops

in Majorca

Tortella LL.⁽¹⁾, Vadell J.⁽²⁾, Lobo M.C.⁽³⁾, Llompart A.⁽¹⁾, Bonet J.⁽¹⁾, Rovira J.M.⁽¹⁾, and **Sastre-Conde I.**^{(1)*}

⁽¹⁾ SEMILLA-INAGEA, Babieca, 2. 07918, Son Ferriol, Palma de Mallorca (Balearic Island). SPAIN

⁽²⁾ UIB. Ctra. Valldemossa, km 7.5, 07122 Palma, (Balearic Island). SPAIN.

⁽³⁾ IMIDRA. Ctra. Barcelona, km 38,200. 28805. Alcalá de Henares. Madrid. SPAIN

*Corresponding author: isabelsastre2003@yahoo.es; misastre@semilla-caib.es



Localized fertilization by drip irrigation causes an impact on the soil that manifests in the short or long term in problems of soil degradation, or crop susceptibility. Random plots with irrigated horticultural and fruit crops were chosen in three different geographical areas of Majorca. The main objective of this work was to study the impact that irrigation causes on the fertility and soil quality of the selected horticultural areas corresponding to Son Ferriol (SF), Felanitx (F) and Manacor (M), in order to diagnose the state of sustainability of agricultural soils. The analysis of the soil fertility was done at three levels (1) globally for all the samples independently of their geographical origin, (2) by geographical origin, (3) by comparing the ridges under crops and the furrows for each plots. The physicochemical characteristics of the soil samples were determined in accordance with the official methods of analysis from Spanish Ministry of Agriculture. In addition, glucose-induced respiration has been analyzed as a quality biological index. Likewise, a set of waters corresponding to the irrigation water of different plots in the different sampling areas have been analyzed. In general, all the studied soils presented moderately basic pH, with high values of interchangeable cations, such as calcium and magnesium, and even potassium, which causes that in areas such as SF the relationships between them, for example, Ca / Mg, are unbalanced (excess or deficiency). The irrigation waters analyzed can be classified as low-quality waters in SF, and as acceptable quality for F and M. It should be taken into account that in the three cases there is the risk of soil salinization, which is accentuated in the SF zone, where there is a real danger of salinization. These facts point out that the soil EC should be constantly monitored, because it is likely that it could increase to levels that endanger the structure of the soil making them not functional for agricultural activity. Equally, both SF and M presented high levels of nitrates, which can be an advantage for agriculture, but are responsible for a deterioration of the aquifer quality. Excessive salt contents have been detected in SF that endanger the state of soil sustainability. On the other hand, the low biological activity observed in SF shows that induced respiration is a good indicator of the health of all the physicochemical and biological parameters of the soil layer. The statistical analysis of the soils sampled on the ridges under cultivation and the furrows denotes high values of electrical conductivity and sodium content in the ridges compared to the furrows, which shows that there is an accumulation of salts due to fertigation. Finally, it can be concluded that this study reveals that not all waters are suitable for irrigation. It is necessary to study the frequency and type of fertigation which can be done according to water quality and soil characteristics

Keywords: furrows, irrigation, ridges, soil



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The water and carbon footprint as partners for a sustainable irrigated agriculture

Tortella LL. ⁽¹⁾, Rovira J.M. ⁽¹⁾, Llompарт A⁽¹⁾., Bonet J. ⁽¹⁾, Garau M.C. ⁽²⁾, Lobo M.C. ⁽³⁾, Vadell J. ⁽⁴⁾, and **Sastre-Conde I.** ⁽¹⁾

⁽¹⁾ SEMILLA-INAGEA, Babieca, 2. 07918, Son Ferriol, Palma de Mallorca (Balearic Island). SPAIN

⁽²⁾ IRFAP-INAGEA, Eusebio Estrada, 145,07009. Palma de Mallorca (Balearic Island). SPAIN

⁽³⁾ IMIDRA. Ctra. Barcelona, km 38,200. 28805. Alcalá de Henares. Madrid. SPAIN

⁽⁴⁾ UIB. Ctra. Valldemossa, km 7.5, 07122 Palma, (Balearic Island). SPAIN.

*Corresponding author: isabelsastre2003@yahoo.es; misastre@semilla-caib.es



In the Mediterranean area, agricultural sustainable management practices are key to meet the needs arising from the climate change and, especially, water scarcity. Limitation of water in agriculture is more crucial in islands due to the shortage of surface water. Groundwater is then commonly the sole water available for irrigation but this source is frequently under great pressure due to a growing touristic activity, often causing a deterioration of its quality. On the island of Mallorca the fertility of around 150 soils of three areas under irrigation crops (fruits and horticultural), were investigated for analyzing how irrigation affects the soil agroecosystem. The soil physicochemical properties and their glucose-induced respiration were studied. Likewise, soil under dryland farming conditions and non-cultivated soils with a natural Mediterranean vegetation were also sampled, as a reference to the soil evolution process due to the irrigation practices. Double Na values, and 10 times higher values of electrical conductivity (EC), as well as a reduction in the carbon content (C), and the C/N ratio were found in the irrigated soils. The soil EC differed fundamentally in irrigated soils of the three areas. The soil induced respiration (SIR) seemed to change fundamentally with those soil parameters that represent the soil structure, therefore its stability and that marking the soil microhabitats characteristics, with positives relationships with the clays and soil organic matter (SOM) content, and negative relationships with the water surface area (WSA). Thus, soil parameters like EC, SOM, WSA, and SIR, should be considered in an environmental footprint global model due to the fertigation that likewise it reflects the need on evaluating jointly the water footprints and carbon.

Keywords: irrigation, quality, respiration, soil.



Novel design of biopesticide-fertilizer mixture using capsaicin and anaerobic digestion products



M. Ortíz León⁽¹⁾, J. A. Mendoza Pérez⁽¹⁾, M. M. Monroy Medienta⁽¹⁾, T. A. Fregoso Aguilar⁽¹⁾, O. Gómez-Guzmán⁽²⁾, G. Calva Calva⁽²⁾, N. A. Rivera Casado*⁽¹⁾

⁽¹⁾ Escuela Nacional de Ciencias Biológicas-IPN, Wilfrido Massieu s/n, Unidad Adolfo López Mateos, México City, México

⁽²⁾ CINVESTAV-IPN, Instituto Politécnico Nacional 2508, San Pedro Zacatenco, México City, México

*Corresponding author: noemia.rivera@gmail.com

Agrochemicals are helping in increasing agricultural productivity since Green Revolution, however it has caused adverse effects on soil health, water quality, greenhouse gases emissions, insect resistance, genetic variation in plants and toxic residues food and feed¹. Relied on natural fertilizers could reduce the use of chemical fertilizers that has achieved about 97% as common practices on agricultural production in Mexico. Swine manure has applied as soil amendment for crop production because it contains essential plant nutrients, however too many pigs carry *E.coli*, salmonella, parasitic worms and a host of other organisms in their manure. Anaerobic digestion of animal manure and slurries offers several benefits either improving their fertilizer qualities, reducing odors and pathogens or as a practical method to solve the disposal problem of about 1.04 x10⁵ Ton of this /day². The present study suggest a mixture coupling of anaerobic co-digestion products using a mixture of swine manure and landfill leachate combined with capsaicin extract in order to enhance tomato (*Solanum lycopersicum*) plant growth. The digester had fed at 32% with landfill-manure mix and it was operating by 64 days. The results showed that anaerobic digestion product was keeping pH values between 7-8 and fewer carbon:nitrogen ration. Furthermore, habanero chilli methanolic extract had 4.96 ± 1.29 mg_{(capsaicin + dihydrocapsaicin)/g} fresh biomass according to Salgado-Garciglia and Ochoa-Alejo (1990) extraction method³ and HPLC UV/VIS analysis. Chilli oleoresin with a yield of 41.3% (µg_{capsaicinoids/g} FB), was used to analyze this effect as bioinsecticide using *Drosophila melanogaster* as model organism. It reached efficiencies above 95% in larval stage showing that mortality rate was directly proportional with capsaicinoids amount. These results suggest an ecofriendly alternative to livestock waste and landfill leachate management in order to improve crop yield, combined with the used of insecticides made from natural products.

Keywords: capsaicin, *Drosophila melanogaster*, *Solanum lycopersicum*, swine-manure.

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DHA by *Aurantiochytrium limacinum* SR21



R. D. Múnera Soto* ⁽¹⁾, J. M. Delgado Naranjo ⁽¹⁾, M. Peñuela Vásquez ⁽¹⁾

⁽¹⁾ Grupo de Bioprocesos, Universidad de Antioquia, Calle 67 Número 53-108, Medellín, Colombia

* Corresponding author: ruben.munera@udea.edu.co.

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) – Docosahexaenoic Acid (DHA) among them– are of great importance in a healthy diet¹. Its traditional source is fish-capture of deep ocean species such as Salmon and Tuna, the increase in the demand of DHA has put a lot of pressure in these marine environments causing the depletion of many species. Thus, studying new ways to produce DHA will release the pressure in these environments. Accordingly, marine microorganism DHA arises as an alternative², but its high production costs makes this technology difficult to implement as prices compared to fish-capture DHA will be significantly higher. With this in mind, decreasing the costs of the carbon source, which accounts for 40 to 50% of the total process cost, is the most efficient way to achieve a cheaper fermentation process³. Barley-Spent Grain (BSG) from beer brewing processes is an alternative carbon source to glucose due to its abundance and availability. Nevertheless, BSG needs to be hydrolyzed for releasing sugars and make them readily available for the microorganism to metabolize them. Biotechnological processes have become a firsthand solution in the production of specialty compounds, *Aurantiochytrium limacinum* SR 21 has stood out for its high growth rates and high lipid content when grown in a 25:1 C:N ratio. This study evaluated the behavior of *A. limacinum* in two different culture media: glucose and hydrolyzed BSG; in both cases yeast extract was the nitrogen source. After the fermentation process, glucose yielded 36%/DCW and BSG 20%/DCW in total fatty acids. The cultures were performed at 23°C, 160rpm during 6 days in 250mL flasks. Finding new sources of n-3 PUFAS with cheaper production processes will decrease the pressure on the marine species that are being depleted while at the same time more people will have access to this essential nutrient.

Keywords: DHA, *A. limacinum* SR21, alternative carbon source.

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eutrophication effect in the lower basin.



Mendivil-García, K.¹, Amabilis-Sosa, L.E.^{2*}, Rodríguez-Mata, A.², Rangel-Peraza, J.G.¹, Cedillo-Herrera, C.I.G.¹

⁽¹⁾ Instituto Tecnológico Nacional de México-Instituto Tecnológico de Culiacán, Av. Juan de Dios Batiz, No. 310, Culiacán, México.

⁽²⁾ CONACYT-Instituto Tecnológico de Culiacán, Av. Juan de Dios Batiz, No. 310, Culiacán, México

*Corresponding author: leoamabilis@yahoo.com.mx



The main objective in this research is to study the spatial and temporal distribution of water quality along the Culiacan river basin for the analysis of the eutrophication process observed in the lower basin. The study describes the monitoring of 23 sampling sites. In each of them, the total nitrogen (TN), total phosphorus (TP) and dissolved oxygen (DO) concentrations were quantified with bimonthly samples from 2013 to 2018. Through an analysis of time series and mass balances of the nutrients, the sampling sites that significantly influence the eutrophication process, as well as its periodicity, were determined. The results indicated that three sampling points of the lower basin presented a critical state in terms of the measurement of dissolved oxygen, with maximum values of 1.0 mg / L, which can be attributed to the establishment of a meat product plant registered in the area in which they slaughter 3,500 cattle/day. On the other hand, 16 sampling sites were identified in the middle and lower basin, which provide a high content of nutrients, but are susceptible to seasonality, since the contribution of TN and TP fluctuates between 10 mg/L and 45 mg/L, and 2 mg/L minimum and 6 mg/L maximum respectively depending on the rainy season or low season. Likewise, it was found that the relationship between nitrogen and dissolved oxygen is inversely proportional, since in areas where there was a critical amount of dissolved oxygen, a high amount of nitrogen was registered up to 45 mg/L. On the other hand, in the lower basin of the Culiacan River, the lowest TP values were registered and it is just the zone in which the excessive algal growth that characterizes the eutrophic bodies of water is observed. The low concentrations of TN and TP in the eutrophic section of the Culiacan River, are related to the proliferation and high rate of assimilation of nutrients that the species *Eichhornia crassipes* presents, with biomass density of up to 2.5 kg/m². Considering the environmental services of the Culiacan River, including potable water supply, the results of the present investigation suggest mitigation measures to preserve the quality and quantity of the resource, for example, through the implementation of sewage treatment plants in some basin sites or incursion in drip irrigation techniques that significantly reduce the generation of agroindustrial wastewater.

Keywords: eutrophication, land use, nutrients, water quality.



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Determination of the degree of eutrophication in the Coastal Lagoon of

Coyuca de Benítez, Guerrero, period 2016 - 2017



Raúl Arcos Ramos^{(1)*}, Eduardo Méndez Ramírez⁽²⁾

⁽¹⁾ *Facultad de Estudios Superiores Zaragoza UNAM Batalla 5 de Mayo S/ N. Col. Ejército de Oriente C.P. 09300 CDMX.*

⁽²⁾ *Facultad de Estudios Superiores Zaragoza UNAM Batalla 5 de Mayo S/ N. Col. Ejército de Oriente C.P. 09300 CDMX.*

*Corresponding author: biolar13@gmail.com.



The lagoon of Coyuca de Benítez is part of the coastal lagoons of the central part of the State of Guerrero. This is of vital importance because it generates jobs for fishermen and tourism service. This system has increased its eutrophication process, as a consequence of a nutritional increase, due to anthropogenic activities, it is necessary to maintain a permanent monitoring of the changes presented by the macronutrients as well as the physical and chemical parameters in the system. This work presents nutritional evaluations in two different periods, relating to the physical and chemical parameters of water at two different levels of depth in five monitoring points established by virtue of human activities. A Van Dorn bottle was used for the samples, for the chemical and physical determinations the HANNA multi-parameter model HI9829 was used and for the determination of the nutrients, the HANNA multifotometer model HI83200. With the obtained results, bar graphs were made to determine the behavior in the physical and chemical parameters as well as in the NH_3 , NO_3 , NO_2 and PO_4 , taking into account the comparison of these with those established by the NOMs and in the CE-CCA 001/89), in addition, univariate statistical tests were carried out, as well as multivariate statistical tests using the statistical package Stat Graphic Centurion XVI.II. The analysis of the results determined that the parameters have a non-normal distribution, (dissolved oxygen, BOD_5), with a homogeneous behavior, however, other parameters there are significant variations, especially in the nutrients where the points of monitoring. The bar and the river present concentrations slightly higher than the other points of monitoring. In addition to this, the chemical parameters show that there is a slight increase in the concentrations of organic matter. In conclusion, it is established that anthropogenic activities are essential for the system, mesotrophic characteristics, with a slight tendency to feed, as well as nutrients, since these variations can significantly alter the nitrogen cycle in the column of water, causing the chain and the food pyramid to be affected permanently. What is necessary for the intervention of conservation, mitigation and improvement programs, as well as a constant and convenient control for the physical, chemical and nutrient parameters of the system due to its economic and ecological importance.

Keywords: Eutrophication, Macronutrients, Mesotrophic



Evaluation of biodigester slurry application as an organic fertilizer on

different crops of Comarca Lagunera and its effect on soil enzyme activities



D. A. Fraire García⁽¹⁾, C. E. López Ortiz⁽²⁾, I. O. Hernández De Lira⁽¹⁾, N. Balagurusamy^{*(1)}

⁽¹⁾ *Laboratorio de Biorremediación, Facultad de Ciencias Biológicas, Universidad Autónoma de Coahuila, carretera Torreón-Matamoros km 7.5, Torreón, México*

⁽²⁾ *Department of Biology, West Virginia State University, Institute, USA*

*Corresponding author: bnagamani@uadec.edu.mx



Inorganic fertilizers (IF) are being used to increase the crop yields. This year the global consumption of IF rose to 180 million tons and Mexico uses more than 6.8 million tons per year¹ and Comarca Lagunera is one of the large consumers within Mexico. The excessive application of IF provoke various environmental problems such as soils acidification, eutrophication and emission of greenhouse gases. An alternative to mitigate or minimize this environmental problem is use of organic fertilizers. Biodigester slurry (BS) can be considered as a high quality organic fertilizer due to its N, P, K content and organic matter, which increase the nutrient availability and microbial activities in soil. This study is focused on the effects of the BS as organic fertilizer application at different rates on the growth and yield of regional crops such as tomato, alfalfa, maize and pepper and as well as its effect on different soil enzyme activities, such as urease, alkaline phosphatase, dehydrogenase, arylsulfatase and β -glucosidase. The soil used was collected from Comarca Lagunera. Pots containing 5 kg of soil with five different fertilizations such as, T1, control with no fertilization, T2, recommended dose (RD) of Inorganic fertilizer (IF), T3, RD of BS (25t/ha), T4, a mix of 50% RD BS + 50% RD of IF, and T5, 100% BS application. Pot study was carried out for four months, until their harvest time in a greenhouse and triplicates were maintained for each treatment and crops. Soils samples were collected and analyzed during the germination, flowering, fruit and harvest stage for all enzymes tested in this study. Results showed that T4 treatment showed a significant increase in the total wet biomass yield of tomato and maize (208% in tomato and 169% in maize) over T2. In case of enzymes, a significant increase ($p < 0.05$) of urease, phosphatase and arylsulfatase activities of the soil applied with BS was observed. Further, dehydrogenase and β -glucosidase were found to increase in BS treatments. It can be concluded that application of bioslurry as organic fertilizer at optimal rates and mixed proportions increase the soil fertility, microbial activities, aid in increasing the growth and yield of plants and decrease the IF consumption by 50 per cent.

Keywords: biodigester slurry, crop productivity, organic fertilizer, soil enzymes

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Effect of organo-mineral amendments in the zaï system on the productivity and soil organic matter evolution in Sudano-Sahelian zone of Burkina Faso.

Edmond HIEN^(1,2) * ; Der SOME⁽¹⁾ ; Dominique MASSE⁽³⁾ ; Jean Jacques DREVON⁽³⁾ ;

⁽¹⁾ Université Ouaga I Pr Joseph KI-ZERBO, BP 7021 Ouagadougou 03, Burkina Faso;

⁽²⁾ IRD, UMR Eco&Sols, BP 182 Ouagadougou 01, Burkina Faso;

⁽³⁾ IRD, UMR Eco&Sols, 2 Place Viala, F34060, Montpellier, France.

*Corresponding author: edmond.hien@ird.fr ; Tel : +226 70 26 36 36



The study was carried out in the sudano-sahelian zone of Burkina Faso. The aim was to assess the impact of the rock phosphate used in combination with organic manure in ecological zaï system on the productivity and organic matter evolution of degraded soils cultivated in cowpea and sorghum. A Fisher Factorial Blocs with three replicates was used as experimental design on a plinthosol. The treatments consisted by digging zaï holes and applying or not compost and manure alone or mixed with rock phosphate. The addition of phosphate to compost and manure has led to the increase of grain yields of cowpea and sorghum compared with compost and manure alone. However, this practice has tended to reduce the soil carbon content in the two crops. The use of organo-mineral amendments based on rock phosphate increase the productivity of degraded soils. It seems, however, limiting the reconstitution of the MOS. The phosphate intake associated with organic manure seems essential to restore the productivity of degraded soils despite its seemingly adverse effect on C storage in soil.

Keywords: phosphate, manure, yields, carbon.



Typology and socio-economic logic of cropping systems in Sub-Saharan agro- ecosystems of Burkina Faso

Edmond HIEN ^(1,2) *, Koulibi Fidèle ZONGO ⁽¹⁾ , Jean-Jacques DREVON ⁽³⁾ , Didier BLAVET⁽³⁾,
Dominique MASSE ⁽³⁾ , Cathy CLERMONT-DAUPHIN⁽³⁾

⁽¹⁾ Université Ouaga I Pr Joseph KI-ZERBO, BP 7021 Ouagadougou 03, Burkina Faso;

⁽²⁾ IRD, UMR Eco&Sols, BP 182 Ouagadougou 01, Burkina Faso;

⁽³⁾ IRD, UMR Eco&Sols, 2 Place Viala, F34060, Montpellier, France.

*Corresponding author: edmond.hien@ird.fr ; Tel : +226 70 26 36 36



In Sub-Saharan zone of Burkina Faso, legumes and cereals mixed cultivation systems occupy most of the cultivated area. However, yields are low and do not cover the food needs of the households. In order to improve the productivity, it is necessary to understand the socio-economic logic driving of these intercropping systems. 60 family farms were surveyed in the northern region of Burkina Faso. Multiple correspondences analysis (MCA) showed 4 types of cropping systems characterized on the one hand, by organic matter amendment and weeding frequency and on the other hand, by the presence of permanent structures for the conservation of water and soil. Organic matter use and frequent weeding are associated with farms with high family labor, livestock, especially small ruminants, and cultivated area, while 70% of farmers have an off-farm activity. The age of household head and the number of their wives are good indicators of farmers' income. Most of farmers' monetary recipes vary widely from 25 to 300 KFCFA. These aspects must be taken into consideration for reasoning suitable technical recommendations for the farmer's in this region.

Keywords: Mixed cultivation, typology, logic.



Enhanced oil recovery by hydrophobins from *Lecanicillium lecanii*

Zaizy Rocha-Pino,⁽¹⁾ Jesús I. Ramos-López,⁽¹⁾ Miquel Gimeno,⁽²⁾ Fernando Barragán-Aroche,⁽³⁾
Cecilia Durán-Valencia,⁽³⁾ Simón López-Ramírez,^{*(3)} Keiko Shirai^{*(1)}

⁽¹⁾ Universidad Autónoma Metropolitana-Iztapalapa, Departamento de biotecnología, Av. San Rafael Atlixco No. 186, C.P. 09340 Mexico City, Mexico.

⁽²⁾ Universidad Nacional Autónoma de México (UNAM), Depto. Alimentos y Biotecnología, Facultad de Química (FQ), Ciudad Universitaria (CU), C.P. 04510, Mexico City, Mexico.

⁽³⁾ UNAM, Depto. Ingeniería Química y Unidad de Servicios para la industria petrolera (USIP), Facultad de Química, Ciudad Universitaria, C.P. 04510, Mexico City, Mexico.

Corresponding author: ^{(3)}slr@unam.mx ^{*(1)}smk@xanum.uam.mx



The oil production is carried out by primary and secondary methods which pressurizing the reservoir by the natural gas into the wall or by the gas or water injection, respectively, thus oil displacement ahead of the reservoir occurs. These recovery methods can reach up to 60% of the reservoir's original oil in place (OOIP), though, the viscosity, gravitational and capillary forces within the rocky mantle of the wells challenges the production of profitable amounts. A tertiary method for oil extraction is the enhanced oil recovery (EOR), an alternative to level up the oil production, this is focused on reducing the forces within the rocks to release the oil trapped. EOR employs biological agents such as microorganisms and enzymes, as well as biological surfactants such as the rhamnolipids from *Pseudomonas* species, however, it is related to virulence factors in the human host.¹ In this regard, hydrophobins (HFBs) are proteins with surfactant properties that offer the advantage to be biodegradable molecules from no human pathogen microorganism and to be chemical and thermal resistance that can be useful for EOR. *Lecanicillium lecanii* is an entomopathogenic fungus used as a commercial biopesticide which is able to produce class I and class II HFBs in solid-state culture (SSF).² The aim of this work was test the capability of HFBs to form emulsions and to recover oil from core plugs for its application in EOR. Class I and class II HFBs were obtained from *L. lecanii* in SSF using polyurethane foam as support and colloidal chitin as carbon source. Interfacial tension (IFT), Emulsion index after 24 h (E_{24}) at 25 and 100°C and oil recovery factor from original oil in place (RF_{OOIP}) were determined using solutions of HFBs class I and class II with critical micellar concentration (CMC) and oil from a mature oil reservoir in Poza Rica, Veracruz, Mexico. A process of protein flooding using Indiana limestone core plugs under depleted oil well in-water conditions at 26°C and 1500 psi were tested for RF_{OOIP} .³ The reduction of IFT of oil in solutions of HFBs was higher with class I HFBs (CI) than with class II HFBs (CII) which did not show thermal resistance with the test of E_{24} at 100°C. However, CI HFBs with CMC of 242.2 μ g/mL reduced IFT of oil from 29.2 \pm 0.1 to 9.0 \pm 0.03 mN/m, E_{24} of 100% at 25°C and E_{24} of 95% at 100°C and RF_{OOIP} of 14.2%. Class I HFBs from *L. lecanii* have been associated to the adhesion of the fungus to hydrophobic surfaces while the class II HFBs were related to hydrophilic surfaces as a protection against the drying of mycelium, their structural and functional differences influence on their biotechnological application.^{3,4} In this work, class I HFBs from *L. lecanii* were able to interact and recover oil from core plugs comparable to the rhamnolipid. The authors acknowledge to DGAPA by the postdoctoral fellowship to ZRP and financial support by CONACYT project No. 237292. We also thank to engineers Callejas, Galicia, Alonso for oil well simulation experiments.

Keywords: Biosurfactant, Enhanced Oil Recovery, Emulsion index, Hydrophobins.

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Production of hydrophobins from *Lecanicillium lecanii*: proteins with biosurfactant properties

Andrea Ramírez de la O,⁽¹⁾ José Luis Vázquez Sánchez,⁽¹⁾ **Zaizy Rocha-Pino,**⁽¹⁾ Gabriel Viguera,⁽²⁾ Keiko Shirai*⁽¹⁾

⁽¹⁾ *Universidad Autónoma Metropolitana-Iztapalapa, Biotechnology Department, Av. San Rafael Atlixco, No. 186, C.P. 09340 Mexico City, Mexico*

⁽²⁾ *Universidad Autónoma Metropolitana-Cuajimalpa, Processes and Technology Department, Mexico City, Mexico*

*Corresponding author: [*smk@xanum.uam.mx](mailto:smk@xanum.uam.mx)

Nowadays the interest in biosurfactants is due to their application in food, pharmaceutical, chemistry industries as well in bioremediation applications because they are no toxic, biodegradable and biocompatible molecules, additionally, their production avoids contamination generated by chemical surfactants. Different microorganisms are able to produce molecules with surfactant activity.¹ This is the case of *Lecanicillium lecanii*, an entomopathogenic fungus able to produce hydrophobins (HFBs), amphipathic proteins characterized by containing a hydrophobic patch on their exposed surface that contributes to their surface activity. HFBs are classified in two groups; class I HFBs which are soluble with formic acid and resistance to 100 °C and class II HFBs that are soluble in SDS 2% solutions but they showed thermal sensibility at 60 °C.² The HFBs from *L. lecanii* have shown the capability to be applied successfully in enhanced oil recovery (EOR).³ They are able to reduce the hydrophobicity of interfaces, surface, and interfacial tension, and to form stable emulsions with oil. However, extrinsic factors like the kind of culture and carbon source modify their production and surface properties.^{2,3} For this reason, the objective of this work was to determine the effect of aeration on the production and surface activity of HFBs from *L. lecanii* in solid state fermentation (SSF) in order to develop a method for their production at large scale and facilitate their application in EOR. *L. lecanii* was grown in SSF using colloidal chitin as carbon source and polyurethane foam (PUF) as inert solid support during 6 days at 25 °C with a constant airflow of 3, 1.5 and 0.8 mL/min/g of moist material. The internal temperature in the reactor and class I HFB production was determined at 3 height level of a column reactor of 8 L of work volume. Results showed that the airflow has an influence on the internal temperature in the reactor that was 2 – 4 °C higher than the external temperature due to the metabolism of *L. lecanii*. Although PUF porosity facilitates the heat transfer into the reactor, the low aeration hinders the heat transference and reduced the yield of class I HFBs from 10% to 2.2 % for 1.5 to 0.8 mL/min/g air flow, respectively. This can be attributed to the oxygen requirements of *L. lecanii* which is an aerobic fungus as well to the biological function of the HFBs as protection to avoid the drying of hyphae and to mediate their adhesion to surfaces. Thus, the airflow influence on heat transfer into the reactor and the production of class I HFBs from *L. lecanii* as a biological response to its development. SSF with a proper airflow could be a successful method for large-scale production of class I HFBs. The authors acknowledge CONACyT project No. 237292 by the financial support.

Keywords: Hydrophobins, *Lecanicillium lecanii*, solid state fermentation, surface activity.

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Chapter 3 Green Materials and Biomaterials



Study of the relationship between the concentration of Pb, As and Hg with the pH in soils of mine tailings for phytostabilization in an area of Morelos-Zacatecas

Luis Enrique Silva Aparicio⁽¹⁾, Consuelo Letechipía de León⁽²⁾, Rebeca Basurto Gaytán⁽¹⁾, Héctor René Vega-Carrillo⁽²⁾, Víctor Martín Hernández Dávila,⁽²⁹⁾ Ana Isabel Veyna Gómez.⁽³⁾

⁽¹⁾ Unidad Académica de Ciencias de la Tierra, Universidad Autónoma de Zacatecas. Calzada Universidad 108, Progreso, 98058 Zacatecas, Zac., México.

⁽²⁾ Unidad Académica de Estudios Nucleares, Universidad Autónoma de Zacatecas. Ciprés 10, Peñuela, 98060 Zacatecas, Zac., México.

⁽³⁾ Unidad Académica de Ingeniería, Universidad Autónoma de Zacatecas. Ramón López Velarde 801, Zacatecas Centro, 98000 Zacatecas, Zac., México.

*Corresponding author: luisasilvaa18@gmail.com

The soil contamination by heavy metals represent threat and an environmental concern for its toxic effect, bioavailability and biomagnification¹. Likewise, the mining tailings are characterized by having high concentrations of heavy metals, scarce presence of nutrients, lack of organic matter, and for being difficult to recover². However, one of the technological alternatives to stabilize these places is the phytostabilization process, where it is use a vegetation cover for the immobilization of the heavy metals, which can be presented in the soil, therefore it turns out to be transcendent to evaluate physicochemical parameters of these metals, to analyze their bioavailability and importance as a natural resource in the performance of the homeostasis functions in the ecosystems³. Consequently, the objective of the study was to determine the characteristics of the soil with mining tailings located to the northwest of the capital of Zacatecas and evaluate its content of Pb, As and Hg. According to the NMX-AA-132-SCFI-2006, it was obtained 25 samples compounded by the tresbolillo method, to which was made an evaluation of the physicochemical parameters (texture, pH, electrical conductivity, and organic matter). The concentration of Pb, As and Hg was determined by the technic of AAS-Flame and HG. As a result, it was obtained an average value for Ph of 4.46, EC 3.305 dS/m, the percentage of the organic matter was 1.104%, whereas the more predominant texture was silty-loam. Respecting to the average concentration in the area was, for Pb 720.12±0.0005 mg/kg, As 42.61±0.0021 mg/kg and Hg 5.97±0.0003 mg/kg. According to the NOM-147-SEMARNAT/SSA1-2004, it is concluded that for the study area, the pH of the soil is strongly acid, whereby it could facilitate the solubility of the potentially toxics elements, making them more bioavailable for the plants, in addition, the conductivity exhibit a soil moderately saline, representing a possibly risk for the mobility of the metals. Likewise, the concentration of Pb and As could be an environmental risk in the area by its possible biomagnification, thus this data could be use to establish a strategy of phytostabilization in the study area.

Keywords: heavy metals, mining tailings, pH, soil.

¹ Gil-Loaiza, J., White, S. A., Root, R. A., Solís-Dominguez, F. A., Hammond, C. M., Chorover, J., & Maier, R. M. (2016). Phytostabilization of mine tailings using compost-assisted direct planting: Translating greenhouse results to the field. *Science of The Total Environment*, 565, 451-461. doi: <http://dx.doi.org/10.1016/j.scitotenv.2016.04.168>

² Ciarkowska, K. H.-F., Ewa; Gambuś, F.; Muszyńska, E.; Czech, T. (2017). Phytostabilization of Zn-Pb ore flotation tailings with *Dianthus carthusianorum* and *Biscutella laevigata* after amending with mineral fertilizers or sewage sludge. *Journal of Environmental Management*, 189, 75-83. doi: <http://dx.doi.org/10.1016/j.jenvman.2016.12.028>

³ Zhao, L., Li, T., Zhang, X., Chen, G., Zheng, Z., & Yu, H. (2016). Pb Uptake and Phytostabilization Potential of the Mining Ecotype of *Athyrium wardii* (Hook.) Grown in Pb-Contaminated Soil. *CLEAN – Soil, Air, Water*, 44(9), 1184-1190. doi: 10.1002/clen.201400870



**Enzymatic response in *e. Hortensis* and *I. Terrestris* exposed to 3-, 4-,
and 5-ring hydrocarbons amended with biosurfactant**



S. Popoola, D. Purchase*, H. Jones, L. Pantoja-Munoz
*Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, The
Burroughs London NW4 4BT United Kingdom.*

*Corresponding author: D.purchase@mdx.ac.uk



Phase I and II detoxification and antioxidant enzymes are responsible for the transformation, detoxification and excretion of xenobiotics such as PAHs. These enzymes serve as indicators for oxidative stress ⁽¹⁾. Rhamnolipid is a biosurfactant (surface active agent produced by microorganisms) that makes PAHs bioavailable ⁽¹⁾. In this study we examined the combined effect of 180mg/Kg phenanthrene (PH), fluoranthene (FL) and benzo(a)pyrene (BAP) on the production of indicator enzymes in *E. hortensis* and *L. terrestris* and the influence of 100mg/L rhamnolipid amendment. We investigated the enzymatic activities of CYP1A1's ethoxyresorufin-O-deethylase (EROD), methoxyresorufin-O-deethylase (MROD) and glutathione-S-transferase (GST) activity in a time response relationship over a 28-day period. Ultra-performance liquid chromatography (UPLC) analysis showed that both EROD and MROD activities were induced between days 0 and 2 of exposure, the level of increase in enzymatic activities were similar in both genera (between 1.2 and 1.7 fold increase in EROD and between 2 and 2.5 fold increase in MROD activity). Enzymatic activities decreased by day 7, with complete mortality of *L. terrestris* by day 28. In contrast, an increase in GST activity was observed from day 2 in both genera. The addition of rhamnolipid alone did not appear to affect the levels of indicator enzymes, suggesting that it is non-toxic. However, the presence of the biosurfactant significantly enhanced vermiremediation ($p = <0.05$) and reduced the time for the hydrocarbon to become undetectable in the spiked soil sample from day 0 to 28. Our results suggested the use of rhamnolipid in conjunction with vermiremediation can offer a cost-effective and environmental friendly option to tackle lands contaminated by recalcitrant hydrocarbons.

Keywords: biosurfactant, enzyme activity, soil contamination, vermiremediation.

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Tolerance to Cd and As in barley plants enhanced by sulphur



Gil-Díaz M⁽¹⁾, Pérez-Ríquez C.A⁽¹⁾, Sastre-Conde I⁽²⁾, Mancho C⁽¹⁾, Alonso J⁽¹⁾, González A⁽¹⁾, Lobo M.C.⁽¹⁾

⁽¹⁾ IMIDRA. Finca "El Encín" A-2, km 38,2. 28800 Alcalá de Henares (Madrid). Spain.

⁽²⁾ SEMILLA-INAGEA, Babioca, 2. 07918, Son Ferriol, Palma de Mallorca (Balearic Island). Spain

*Corresponding author: mar.gil.diaz@madrid.org

Metal(loid) contamination is a widespread problem in European soils being necessary to found sustainable remediation strategies to recover these sites. Phytoremediation is a friendly technology which uses the plant capability to absorb or immobilize pollutants in soil minimizing the risk of transfer to the trophic chain. Some authors have observed that the addition of sulphur to metal(loid) polluted soil can improve the plant phytoremediation capacity¹. This study evaluates the effect of sulphur application to a polluted soil to enhance barley tolerance to As and/or Cd. A greenhouse experiment was carried out using 4-L pots filled with a clay loam soil spiked with 60 mg/kg As or 500 mg/kg Cd. After 15 days, sulphur (CaSO₄·2H₂O) was applied at a dose of 3 Mg/ha and two barley plants previously seeded, were transplanted in each pot, using eight pot per treatment. At the end of the growing cycle, plant biomass, content in nutrient and metal(loid)s in plant as well as soil characteristics were evaluated. Different physiological and biochemical parameters were analyzed in order to evaluate the impact of the treatments on plant.

Barley plants showed a different behaviour in relation to the pollutants, showing higher tolerance to Cd than to As. This latter induced a significant increase in the malonaldehyde content (MDA) at 15 days of growth. The sulphur application decreased the oxidative stress in the plants growth in As-polluted soils showing a significant decrease in MDA values. Barley plants grown in soils treated with sulphate showed higher sulphur uptake. In both polluted soils, the sulphur induced an increase in the metal(loid) content in root, which could favor phytostabilization processes. Further studies should be necessary to evaluate the effect of sulphur at different conditions on the plant tolerance to metal(loid)s, and, consequently on the phytostabilization efficiency.

Keywords: *Hordeum vulgare*, metal(loid)s, phytoremediation, soil.

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Polyacrylic-polyether-polyurethane coating biodegradation by the enriched microbial community BP8



I. Gaytán-Enríquez⁽¹⁾, M. J. Cruz-Gómez⁽²⁾, H. Loza-Tavera^{*(1)}

⁽¹⁾ Departamento de Bioquímica, ⁽²⁾ Departamento de Ingeniería Química, Facultad de Química, Universidad Nacional Autónoma de México. Ave. Universidad 3000, Col. UNAM, Ciudad de México, 04510, MÉXICO.

*Corresponding author: hlozat@unam.mx



Polyurethanes (PU) are plastic polymers that have allowed the development of essential devices for modern life. However, its excessive use, high durability, and the lack of an effective recycling process have generated a major environmental problem worldwide. Since environmental microorganisms are constantly evolving, bacteria able to attack plastics have been naturally selected. This work investigates the capability of the enriched microbial community BP8 to degrade the polyether-polyurethane-acrylic (PE-PU-A) coating PolyLack[®]. The coating also contains additives such as N-methyl-2-pyrrolidone, 2-butoxyethanol, isopropanol, dipropylene glycol butyl ether, and dipropylene glycol monomethyl ether. BP8 grew diauxically along 20 days culture and consumed 50% of the carbon in a mineral medium with PolyLack[®] (0.3%) as the only carbon source, indicating that more than one coating component is degraded by the activity of the community. By FTIR and thermal analysis, we found that the bacterial growth was supported by chemical modifications in the ester, ether, urethane, and aromatic groups. Culture supernatants were analyzed by gas chromatography (GC) showing that BP8 metabolized all the coating additives during the first five days of culture. Therefore, during the following 15 days of culture, the community has to grow by degrading the PE-PU-A copolymers. GC analysis at different culture times revealed 42 degradation products, such as organic acids, alcohols, aldehydes, esters, ethers, alkanes, amines, amides, oxides, and aromatic rings. Some of these compounds are complex molecules bound to simple organic acids such as acetic acid, propionic acid or butyric acid, which may allow their incorporation to microbial metabolism. Because the PolyLack[®] is a complex mixture of copolymers and additives, bacterial accessibility to the chemical groups to be degraded might be limited. However, modifications in cell hydrophobicity, emulsification index and particle size of the BP8 culture suggest that interactions between the microbial cells and the components of the coating may favor the biodegradation process. Our results demonstrate the biodegradative activity of the BP8 community, not only on the PE-PU-A component, but also on the xenobiotic compounds containing the highly recalcitrant ether bond present in the coating.

Keywords: plastics, polyurethane, ether bond, biodegradation.

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Removal of diesel by soil washing technologies using a non-ionic

surfactant



Carolina Guatemala Hernández¹, Josefina Barrera-Cortés², Ma. Teresa Ponce Noyola³, Mauricio Carbajal Tinoco⁴

^{1,2,3}Dept. Biotechnology and Bioengineering, CINVESTAV – IPN. Av. IPN 2508. Col. San Pedro Zacatenco. Ciudad de México, C.P. 07360

⁴Depto de Física, CINVESTAV – IPN.

*Corresponding author: jbarrera@cinvestav.mx



A large number of soils highly polluted with recalcitrant hydrocarbons and the limitation of the current bioremediation methods are still the drawback for an efficient recuperation of these under safe conditions. In this regard, soil washing by degradable surfactants is an alternative option knowing the capacity of surfactants to desorb oily organic compounds. The aim of this study was the establishment of the washing conditions of a soil polluted with diesel, using a nonionic surfactant. A soil polluted with diesel was used. This was collected near to a polluted railway station zone. The soil was dried at room temperature and sieved to a mesh size 10 for its physicochemical and biological characterization. Washing of the polluted soil was performed with surfactant solutions in a 1:5 ratio (5g of soil per 25 mL of the surfactant solution). This was carried out at 28 ± 1 °C and 150 rpm for 72 hours. The factors tested were the Tween 80 surfactant concentration (1, 2, 5 and 10%) and the treatment time. Residual diesel concentration was determined every 24 h. The soil was of a sandy loam texture with a low concentration of organic matter (3.68%) and conductivity ($0.016 \text{ dS} \cdot \text{m}^{-1}$). The level of alkalinity was moderate (7.63) and the total petroleum hydrocarbons (TPH) of $11,600 \pm 1058.38$ ppm. The high TPH content could explain the low microbial count of $1.1 \cdot 10^5$ determined as UFC per gram of dried soil. Within the range of the surfactant concentration tested for washing the polluted soil under study, TPH removal increased proportionally with the surfactant concentration. 5080.8 ± 422.2 ppm (43.8 ± 3.64 %) was the maximal concentration of TPH removed after 72 h of contact with a surfactant pollution at 10%. Despite the high percentage of hydrocarbons removed, it is assumed that a higher concentration of these could be removed if the washing process is extended or is carried out by stages. Soil washing through the use of surfactants as a desorbing agent was found to be a viable and effective technology for the rapid recovery of soils highly polluted with recalcitrant hydrocarbons.

Keywords: diesel, hydrocarbons, soil washing, tween 80.



Nickel and Zinc Salphen compounds.



A.A. Lopez-Pacheco⁽¹⁾, M.V. Escárcega-Bobadilla⁽²⁾, L. Mondragón-Camarillo⁽²⁾, A.C. Hayano-Kanashiro⁽¹⁾, A. Varela-Romero⁽¹⁾, R. Vílchez-Vargas⁽⁴⁾ and K. Calderón^{*(1)}

⁽¹⁾ *Department of Scientific and Technological Research (DICTUS), University of Sonora. Edificio 7G, Blvd. Luis Donaldo Colosio s/n. Col. Centro, C.P. 83000 Hermosillo, Sonora, Mexico.*

⁽²⁾ *Department of Organic Chemistry, School of Chemistry, UNAM. Mexico.*

⁽³⁾ *UBIBPRO, Facultad de Estudios Superiores Iztacala, UNAM. Mexico*

⁽⁴⁾ *Department of Gastroenterology, Hepatology and Infectiology. University Hospital Magdeburg. Germany*

*Corresponding author: kadiya.calderon@unison.mx

Soil provides fundamental ecosystem services such as nitrogen cycle, which is highly driven by the composition of the soil microbiota, and in turn, it is affected by the presence of heavy metals and metalloids. High concentrations of Nickel and Zinc are introduced in soils by conventional agricultural practices, contaminated wastewater and mining industry activities. Therefore, metal ion ligands like Metal-Salphen compounds (M-Salphen) have been implemented to increase the bioavailability of these elements so that, cells can metabolize them quickly¹. In order to evaluate the effects of this contamination, a microcosm system study was performed with agricultural soil from the coast of Hermosillo, México, disturbed with M-Salphen complex of Nickel (Ni²⁺) and Zinc (Zn²⁺) to evaluate their impact on the soil microbiota and its effect on their functioning along time based on the nitrogen cycle. The obtained results from quantification analyses of inorganic nitrogen mobilization during long-time combined treatment, revealed a significant effect of the interaction of time and metal applied ($p < 0.05$), regardless the form or dosage applied. This result could suggest the promotion of the soil nitrification process under metal contamination.

Keywords: microbiota, M-Salphen, nitrogen cycle, soil.

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Assessing long-term impact of zero-valent iron (nZVI) nanoparticles on soil microbial community function

P. García-Gonzalo^{1*}, M.M. Gil-Díaz¹, E. Arroyo¹, E. Rodríguez-Valdés², J.R. Gallego², M.C. Lobo¹

⁽¹⁾IMIDRA, Finca "El Encín" A-2, Km 38,2 28800 Alcalá de Henares (Madrid), Spain.

⁽²⁾Indurot, Universidad de Oviedo, Mieres (Oviedo) Spain.

*pilar.garcia.gonzalo@madrid.org



In the last few years, nanoscale zero-valent iron (nZVI) has gained widespread use to remediate soil and ground-water contamination by metal(loid)s immobilization. Despite its growing use, information regarding the impacts of engineered nanoparticles on soil microbial communities is currently limited. The aim of the present study was to determine the effect of nZVI on soil microbial communities, enzymatic activities and microbial biomass in a long-term experiment for 14 months, at field conditions, in a brownfield contaminated with arsenic (As) and mercury Hg. The study site is a former mine "El Terronal" (Asturias, Spain). Mining activity were carried out between the 1960s and the early 1970s in order to obtain mercury (Hg) and arsenic (As) compounds. According to Hg and As concentration, two zones (A, B) were selected for the study. Two plots of 5m² were treated with nZVI (Nanofer 25S, NANO IRON, Czech Republic) at a dose of 2,5%. Composite soil samples were collected at 1, 2, 8 and 14 months at 0-10cm depth. The availability of As and Hg was evaluated according to the TCLP test (USEPA 1311). Functional diversity of microbial communities was determined using Biolog Ecoplates. Microbial biomass was assessed by substrate-induced respiration (SIR). Soil samples were analyzed for individual enzymes including, β -glucosidase, β -galactosidase, acid and alkaline phosphatase, urease and arylsulphatase. After 14 months of nanoparticle application a reduction on the availability of As and Hg was observed in both zones. Available As was reduced 76% (A) and 71% (B) and available Hg was reduced 86% (A) and 82% (B). Comparison of Shannon diversity index along different collecting periods, showed that microbial communities were sensitive to nanoremediation treatment, as a significant decrease of functional diversity was observed in both plots. However at the end of the sampling period a slight recovery was noticed in A and B zones. The same pattern was found for microbial biomass and enzymatic activities except arylsulfatase. Among enzymes, β -glucosidase showed the highest microbial activity reduction 82% (A) and 87% (B) and acid phosphatase were recovered near the same activity level than control in B zone. An enhanced arylsulfatase activity was found along the sampling period as values increased 163% (A) and 56% (B), suggesting that nanoremediation treatment causes significant changes in the available pool of sulfur in these contaminated soils.

Keywords: field experiment, microbial activity, microbial communities, nanoremediation



Searching for proteins with capacity to break the urethane group in

Alicyclophilus sp. BQ1, a bacterium capable to degrade polyurethane



J. Fuentes-Jaime, M. Vargas-Suárez and H. Loza-Tavera*

Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México.
Ave. Universidad 3000, Col. UNAM, Ciudad de México, 04510, MÉXICO.

*Corresponding author: hlozat@unam.mx



Polyurethane (PU) is a synthetic polymer with a high versatility highlighting its use as foam, elastomer, adhesive, paint, coating and elastic fiber. Its production is based on the high reactivity of the isocyanate double bond that binds either ester or ether polyols, generating the urethane groups, by a condensation reaction. PU physicochemical properties and chemical structure give it such a design to resist degradation under environmental conditions, that its overall decomposition is difficult and very slow. Several microorganisms capable of PU attack have been isolated, and most of the activities correlated with the attack have been identified as esterases, lipases and proteases. However, enzymes with capacity to attack the urethane group, the most recalcitrant of the polyester-PU structure have not been reported. In our laboratory, we work with the bacterial strain *Alicyclophilus* sp. BQ1 able to grow in a minimal medium supplemented with Impraniil[®]DLN, a polyester-PU varnish, as the only carbon source. We characterized BQ1 growth in Impraniil[®]DLN by quantifying the amount of cytosolic and membranal protein, and observed a log phase from 12 to 28 h. The clearing of cultures containing Impraniil[®]DLN, measured spectrophotometrically, is indicative of its biodegradation. Initially the medium is milky and progressively becomes transparent after 48 hours of incubation reaching 86% of degradation. Moreover, by FTIR spectroscopy we demonstrated that BQ1 is able to hydrolyze urethane groups, which suggest that an amidase activity must be involved in PU biodegradation. Searching for amidase activities, spectrophotometric and zymographic techniques were implemented by using urethane as substrate. A protein of ~150 kDa, capable of cleaving the urethane was detected. Biochemical characterization showed that the use of McIlvaine buffer pH 8 improves the urethanase activity; at 10% glycerol it tolerates up to 0.6 M NaCl and the activity is significantly reduced in the presence of benzamidine. Purification by ion exchange and molecular exclusion chromatography, and identification by LC- TOF/MS suggest that the urethanase is either the carbamoyl phosphate synthase (EC 6.3.5.5) or an amidohydrolase (EC 3.5.1.32). Experiments are undertaking to define the real nature of this novel activity and to characterize its activity.

Keywords: biodegradation, polyurethane, urethanase, *Alicyclophilus*.

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Characterization and whole genome sequencing and analysis of a

hydrocarbon-degrading negativicute isolated of sediment from the Gulf of México



L Escobar⁽¹⁾, P Lara⁽¹⁾, K Juárez^{*(1)}.

⁽¹⁾ Instituto de Biotecnología, UNAM. Av. Universidad 2001, Chamilpa, C.P. 62210, Cuernavaca, Morelos, México.

*Corresponding author: katy@ibt.unam.mx.



Hydrocarbons are commonly present in marine environments, both from natural and anthropogenic sources. These compounds are highly toxic for diverse ecosystems and considered priority contaminants. Marine hydrocarbon pollution is naturally attenuated by bacterial degradation both aerobic and anaerobically. While aerobic degradation has been extensively studied, knowledge of anaerobic degradation is limited and it has been demonstrated only in a small number of microorganisms. The main objective of this work is extend the knowledge of anaerobic degradation of hydrocarbon as well as the isolation and characterization of bacteria with notable capabilities related with these metabolisms. We isolated from marine sediment of Gulf of Mexico, a strain of *Pelosinus* sp. This microorganism is able to growth in presence of toluene, benzene and benzoate. Moreover, we found that this strain is able to grow with fumarate in absence of another electron donor or acceptor, and it is aerotolerant anaerobe. We performed the whole genome sequencing and its analysis revealed that it possessed genes probably involved in hydrocarbon degradation, e.g. UbiX/UbiD, benzoate CoA ligase and benzyl CoA reductase reported to benzene degradation pathway, as well as a 4-hydroxyphenylacetate decarboxylase previously associated with oxygen-dependent aromatic hydrocarbon degradation. In other hand, we also found putative transcriptional regulators related to hydrocarbon utilization like CatR and IclR. Other notable features are under current characterization, but it is important to mention that *Pelosinus* genus never has been isolated from marine environments, neither reported to degrade hydrocarbons.

Keywords: Anaerobic hydrocarbon degradation, Gulf of Mexico, *Pelosinus* sp.



Microbial diversity and contamination in Reconquista River sediments:

detection of bacteria with potential biotechnological applications.



Natalia Porzionato⁽¹⁾, Agustina Ziliani⁽¹⁾, Celeste Grimolizzi, Ana E. Tufo⁽¹⁾, Susana Vázquez⁽²⁾,
Angela Cabezas Da Rosa⁽³⁾, Gustavo Curutchet^{(1)*}

⁽¹⁾ Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, Bs. As., Argentina.

⁽²⁾ NANOBIOTEC UBA-CONICET, Cátedra de Biotecnología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, CABA, Argentina

⁽³⁾ Department of Environmental Sciences, Technological University (UTEC), Francisco Antonio Maciel s/n, CP: 97000, Durazno, Uruguay.

*Corresponding author: gcurut@gmail.com



Sediments from contaminated water courses accumulate large amounts of organic matter and toxic persistent pollutants like heavy metals and pesticides. The stress caused by severe contamination leads to the selection of microorganisms which, in addition to being involved in *in situ* self-purification processes, have a clear biotechnological potential. In this work, we studied the microbial communities from fluvial sediments in areas with different degree and type of contamination at the Reconquista River basin, one of the most polluted watercourses in the metropolitan area of Buenos Aires, Argentina. Our aim was not only to get insight on the composition of bacterial communities in these habitats but also to recover in culture bacteria capable of growing at the expense of the pollutants. The sediment samples were analyzed to determine their physicochemical parameters and respirometry experiments were performed. Bacterial community structure in sediment samples was analyzed through 16S rRNA gene amplicon sequencing (V4 variable region, Illumina MiSeq platform, and data were processed using Mothur and R packages). Cultures were performed to enrich in sulphate and iron reducers and sulfur and iron oxidizers using selective media, and isolates were obtained in solid media. Taxonomic identification of isolates was achieved by partial sequencing of the 16S rRNA gene after DNA extraction using the Wizard® Genomic DNA Purification Kit (Promega) and PCR amplification using the universal primers 27F/1492R for bacteria and ITS5/ITS4 for yeasts. Growth kinetics and metabolites production were studied for the enrichment cultures and isolates obtained. Bacterial communities from areas with different contamination status differ in their structure (and therefore, in their functional potential and performance). Aerobic and anaerobic isolates, affiliated to *Acidithiobacillus*, *Clostridium*, *Desulfovibrio*, and *Shewanella* among others, were obtained. These microorganisms possess a great potential for applications in environmental biotechnological processes.

Keywords: Bacterial communities. Persistent pollutants. Reconquista River.



Evaluation of soil physical and chemical properties of degraded lands of

Hermosillo Coast, Sonora Mexico



H. Celaya Michel^{*(1)}, M. A. Barrera Silva⁽¹⁾, R. F. Osuna Chávez⁽¹⁾, y J. Sosa Castañeda⁽¹⁾.

⁽¹⁾ *Departamento de Agricultura y Ganadería, Universidad de Sonora. Km 18.5 Carretera a Bahía Kino, Hermosillo Sonora, México 83000.*

*Corresponding author: hernan.celaya@unison.mx



Land degradation and desertification is an environmental problem that worries and puts at risk the services demanded by the population and which in turn continue to increase with the increase in population. The arid and semi-arid zones are the most vulnerable to degradation, due to the natural limitations in resources they present. The physical, chemical and biological properties of the soil can be an indicator of degradation and desertification risk. This work was carried out in a degraded area of the coast of Hermosillo, Sonora, Mexico. Soil plots were studied under tree, with grass cover, with bare soil and with bare soil added with pruning branches of native trees. Contents of soil organic matter (SOM), bulk density (BD), soil surface temperature (T) and volumetric moisture after rain events were determined. The measurements were of the first 10 centimeters of soil, except for the temperature that was superficial. The results indicate that the soil with vegetative cover of trees and grasses presented higher SOM content and lower DA ($p > 0.05$) than the other treatments; however, the most outstanding result was the higher moisture content of soil under tree, grass and bare soil with branches, than bare soil ($p > 0.05$). The literature provides evidence that plant cover and covering with mulch favors conditions for the development of healthy soils with less risk of degradation, our data coincide and also add soil cover with pruning branches as a possible alternative to recover degraded soils. These results could contribute to future remediation strategies of eroded soils.

Keywords: arid zones; desertification; erosion; hydrology.



U(VI) removal by nanoscale zero-valent iron (nZVI) supported on raw and

modified montmorillonite



- Valiente, R.⁽¹⁾, Marco-Brown, J.L.*⁽¹⁾, Ramos, C.P.⁽²⁾, Torres Sánchez, R.M.⁽³⁾, Candal, R.⁽¹⁾
- ⁽¹⁾ Instituto de Investigación e Ingeniería Ambiental (3iA), Escuela de Ciencia y Tecnología, UNSAM, CONICET, Av. 25 de Mayo y Francia, San Martín, Buenos Aires, Argentina.
- ⁽²⁾ Departamento de Física de la Materia Condensada, GlyA-CAC-CNEA, CONICET. Av. Gral. Paz 1499 (1650), San Martín, Buenos Aires, Argentina.
- ⁽³⁾ Centro de Tecnología de recursos Minerales y Cerámica, Gonnet, Buenos Aires, Argentina.
- *Corresponding author: joseluis.marcobrown@unsam.edu.ar



Water contamination by uranium is a problem of particular concern for people living near mining areas or nuclear fuel production plants. Immobilization of soluble U(VI) to suitable sorbents and reduction to less soluble U(IV) are ways to remove or diminish U(VI) mobility. In this work, the removal efficiency of U(VI) from water by a combination of adsorption and reduction/precipitation processes using nanoscale zero valent iron (nZVI) and nZVI supported on raw and Fe/Al pillared montmorillonite (MMT) was evaluated. It is expected that nZVI immobilization on support materials enhance the stability of nZVI compared with non-supported nZVI. Fe/Al pillared clays were prepared following a previously reported method¹, with Fe/(Fe+Al) molar ratio of 0.33 (FeMMT0.33) or 0.5 (FeMMT0.5). nZVI were synthesized via Fe(III) reduction with NaBH₄ and obtained as it or supported on raw MMT (nZVIMMT) or FeMMT0.33 (nZVIFeMMT0.33). Additionally, the Fe(III) present in FeMMT0.5 sample was reduced with NaBH₄ to Fe(0) (ZVIFeMMT0.5). Samples were characterized by XRD and Mössbauer spectroscopy and by N₂ adsorption-desorption isothermal analysis. Removal kinetics of U(VI) by the different materials were studied in anoxic batch systems at pH 4 and 6; U(VI) concentration, pH and oxidation-reduction potential (ORP) were measured during kinetics. Fe(0) presence was determined by Mössbauer in materials containing Fe(0) (ZVI materials). U(VI) removal was higher at pH 6 than at pH 4. U(VI) removal from solutions with initial U(VI) concentration: [U(VI)]₀ = 50 mgL⁻¹ and pH 6 varied according to the material. Total U(VI) removal was achieved by nZVIMMT and ZVIFeMMT0.5 in about 10 and 25 min respectively. After 60 minutes, a complete removal of U(VI) was not achieved neither using nZVI, nor nZVIFeMMT0.33. The occurrence of reduction process of U(VI) was proven when ZVI materials were used by the analysis of ORP vs time plots. Raw MMT, FeMMT0.33, FeMMT0.5 removed less than 30% of U(VI) after 60 min by adsorption process. In conclusion, U(VI) removal using of nZVI supported on raw or pillared MMT proved to be more efficient than using non-supported nZVI. Supported nZVI are projected into the future as a suitable material for the development of U(VI) removal technologies.

Keywords: adsorption, kinetics, reduction/precipitation, uranium removal.



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Metal removal capacity by microalgae and effect on growth



R.J. Aguilar-Ruiz⁽¹⁾, M.R. Martínez-Macías^{*(1)}, D.I. Sánchez-Machado⁽¹⁾, J. López-Cervantes⁽¹⁾, G. E. Dévora-Isiordia⁽¹⁾.

⁽¹⁾ Instituto Tecnológico de Sonora, Av. 5 de febrero, 818 sur, Cd. Obregón, Sonora, México.

*Corresponding author: maria.martinez@itson.edu.mx



Mineral and metal demand in the world has been increasing due to population growth, as a result, the mining industry has become of great importance in the world economy. This industry generates in extraction processes, high amounts of acid water, which are toxic to the environment and human¹. Acid mine water is characterized by having a low pH and high heavy metals concentrations, that cannot be degraded biologically. In northwest Mexico, the poor control of acid water discharges causes problems to the ecosystems and aquifers. Acid water has become an attention focus and their remediation are of great importance at present. The microalgae cultures are best technologies to the metal removal because there have mechanisms extracellular and intracellular to face up to the environmental toxicity by the presence of metals. Their ability to grow in the presence of heavy metals proves their practical application at bioremediation of the acid water. In this sense, the goal of this work was to evaluate the removal capacity of Cu and Fe metals, present in mine acid water by the microalga *Nannochloropsis oculata* and their effect on cell density, specific growth rate and removal percent. *N. oculata* culture was carried at different concentrations in tubular photobioreactors with saline water, a light intensity of $166 \mu\text{E m}^{-2} \text{s}^{-1}$ by 24 hours and controlled temperature of 25-26 °C; the nutritive medium used was the Algal medium (4mM of N₂) added every 72 hours. The results show at a culture medium concentration of $4.64 \text{ mg Cu L}^{-1}$, the cell density (cells mL^{-1}) decreased to 25% compared to the control cultures. The specific growth rate (d^{-1}) did have a significant difference between the treatments with metals and the control culture ($p < 0.05$). The highest biomass productivity ($0.229 \pm 0.001 \text{ g L}^{-1} \text{ d}^{-1}$) was obtained in cultures with concentrations of $1.74 \text{ mg Cu L}^{-1}$. The highest removal of copper and iron was recorded at 94.576% and 99.832% respectively in culture with 1.74 and $1.16 \text{ mg Cu L}^{-1}$. Additionally, in cultures concentration with 1.74 and $4.64 \text{ mg Cu L}^{-1}$, the cellular dry weights were of 6.37 and $5.51 \text{ pg cells}^{-1}$ respectively. In conclusion, the microalga *N. oculata* has a high capacity to remove Cu and Fe at different concentrations of metals. There are favorable results.

Keywords: Effect- Metal-Microalgae-Removal.

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Fe(II)/Fe(III)-oxides coupling



Jean-Pascal ANDRAUD^{*(1)}, Thierry WOIGNIER⁽¹⁾, Stéven CRIQUET⁽¹⁾, Benjamin OURSEL⁽¹⁾, Stéphane GREFF⁽¹⁾.

⁽¹⁾ Aix Marseille Univ, Univ Avignon, CNRS, IRD, IMBE, Marseille, France.

⁽²⁾ IRD, IMBE, Campus Agro-environnemental Caraïbe, Le Lamentin, Martinique, France.

*Corresponding author: jean-pascal.andraud@imbe.fr



Today, chlordecone (CLD), an organochlorine pesticide spread on the soil to control the populations of the banana weevil, is responsible for an unprecedented health, environmental, social and political crisis in the French West Indies (FWI). Despite the health management measures implemented by the authorities to prevent CLD from reaching the consumer's plate, the population continues to be exposed. A definitive solution to the problem would be to destroy the CLD stock in the soil. To this end, the ISCR (In Situ Chemical Reduction) remediation process, which consists of adding zero valent iron to the soil, has shown the capacity to significantly reduce the concentration of CLD in the main types of FWI soils by reductive dechlorination. However, the cost of this technique, linked to Fe⁰ imports, limits its applicability 1,2. FWI soils are very rich in iron, which accounts for 7 to 11% of their dry weigh 2. As these soils are oxidic, iron is found mainly in the form of Fe(III)-oxides, and more precisely in the form of goethite, hematite and ferrihydrite whose proportions vary according to the type of soil 1. The potential (E⁰) of the redox couples formed by Fe(III)-oxides/Fe(II) and Fe(II)/Fe⁰ (E⁰ ≈ -470 mV) are similar. This indicates that the Fe(II) resulting from the Fe(III)-oxides microbial reduction could be an electron donor as effective as Fe⁰ for attacking CLD. In order to verify this theory, which could led to a new bioremediation process, tests of abiotic dechlorination of CLD were performed in aqueous medium in presence of FeSO₄ and the Fe(III)-oxides mentioned above. Mesocosms were prepared in an anaerobic glove box ([O₂] atm (1,2), Hervé MACARIE ≤ 1 ppm) in serological bottles later closed with Teflon-lined septa. These mesocosms consisted of 62.5 µg CLD in 25 mL ultrapure water, with 7.20 mg FeSO₄ heptahydrate and an amount of Fe(III)-oxides defined according to the specific surface area of each Fe(III)-oxide. Appropriate controls without Fe(III)-oxides and/or FeSO₄ were also prepared. The CLD stock solution was made in degassed acetonitrile under argon. Incubation took place under stirring, at room temperature and in the dark. Four replicates of each modality were randomly selected and sacrificed after 4 hours, 2, 7, 14, 21 and 30 days of reaction. Three were used for the quantification of chlorides, CLD and possible dechlorination products. The fourth bottle was used for pH and redox measurements. These last two measurements were performed maintaining the anoxia after bottle opening. Chloride concentration was measured by ion chromatography and dechlorination intermediates followed by LC-MS. The results of these experiments are in course of acquisition and will be presented during the conference.

Keywords: chlordecone, dechlorination, iron oxides, iron-reduction.



An electrogenic microbial community from the polluted Reconquista

River as revealed by molecular and kinetic analysis



Prados MB⁽²⁾, Vázquez, S⁽³⁾, Lescano M⁽³⁾, Porzionato, N⁽¹⁾, Ziliani, A⁽¹⁾, Fernandez Albanesi L⁽⁴⁾, Pasquevich D⁽²⁾, Curutchet G^{*(1)}.

⁽¹⁾ *3iA, Escuela de Ciencia y Tecnología, Univ. Nac. De San Martín, Buenos Aires, Argentina.*

⁽²⁾ *Instituto de Energía y Desarrollo Sustentable, Centro Atómico Bariloche (CAB), Comisión Nacional de Energía Atómica (CNEA), Av. Bustillo 9500, S. C. de Bariloche, Argentina*

⁽³⁾ *NANOBIOTEC UBA-CONICET, Cátedra de Biotecnología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, CABA, Argentina*

⁽⁴⁾ *Gerencia de Investigación Aplicada, Centro Atómico Bariloche, Comisión Nacional de Energía Atómica.*

*Corresponding author: gcurut@gmail.com

With the aim of developing a bioelectrochemical system (BES) for the immobilization of heavy metals from effluents, we selected an electrogenic consortium of microorganisms from sediments of the highly polluted Reconquista river, Buenos Aires, Argentina (presented at the 5ISEBE symposium). This consortium (RE480) developed a dense and thick biofilm over graphite rod electrodes potentiostatized at 480 mV vs Ag/AgCl, which served as the sole electron acceptor in the BES. In this work, we present the characterization of such consortium, with the purpose of establishing the operation parameters of the BES in which these microorganisms will be used. RE480 was grown on DSMZ 579 medium with acetate as electron donor and fumarate as electron acceptor. The ability of RE480 to grow at different pH (4, 5, 7, 9 and 10) and temperatures (15, 20, 30, 40 and 45°C), and the combination of them, was tested under anaerobiosis. Additionally, we tested glucose and pyruvate as electron donors, employing the same medium, at pH 7 and 30°C. For every culture condition, we measured cell growth by turbidimetry, gas composition by respirometry and infrared spectroscopy (FTIR), cell phenotype by differential staining and scanning electron microscopy. The phylogeny of the RE480 was studied by 16S sequencing. Additionally, to isolate the components of RE480, the microorganisms were grown in selective media under anaerobic (Postgate, DSMZ 579) or aerobic (PCA, TSI) conditions. The optimum growth temperature of the microbial consortium was 30°C, although growth was observed between 20-40°C, at a pH range between 6-9. No gases other than CO₂ were detected, suggesting the complete oxidation of the provided substrates. These parameters should be considered for the operation of the BES in addition to the possibility to use complex organic compounds, which could generate a higher current yield. At least six genera were identified in consortium RE480 dominated by *Clostridium* and *Aeromonas*, highlighting the presence of *Shewanella*, one of the best characterized electrogenic microorganisms so far. These microorganisms are facultative anaerobes and have a great potential for application in BES. Interestingly, our results show that the electrogenic species found in the biofilm grow in community with other non-microorganisms, suggesting that this type of mixed biofilm structures could be more robust than those formed by a single species.



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Keywords: Bioelectrochemical systems, heavy metals, wastewater.



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Physicochemical changes and fatty acid profile during bioremediation of palm oil polluted soil using amendments

J.A. Rojas Alejo ⁽¹⁾, M. Barajas Aceves ⁽²⁾, G. G. Medina Mendoza ⁽²⁾, E. Ríos Leal ⁽²⁾, O. Gómez-Guzmán ⁽²⁾, G. Calva Calva ⁽²⁾, N. A. Rivera Casado* ⁽¹⁾

⁽¹⁾ *Escuela Nacional de Ciencias Biológicas-IPN, Wilfrido Massieu s/n, Unidad Adolfo López Mateos, México City, México*

⁽²⁾ *CINVESTAV-IPN, Instituto Politécnico Nacional 2508, San Pedro Zacatenco, México City, México*

*Corresponding author: noemia.rivera@gmail.com



As a result of high international demand of palm biofuel, the annual production of oil Palm in Mexico have shown great increase during last years, reaching up to 462 thousand tons. The grinding process related to the oil extraction from the oil Palm plants has produced severe problems of environmental pollution, mainly in Chiapas and Campeche sites. Several technologies have been developed and applied to treat the polluted wastewater from the palm oil mills, commonly named palm oil mill effluent (POME). However, actual challenge is to develop effective and simple methods for treatment of the oil palm waste included in soil (POMS). The objective of this study was to propose several mixtures of amendments and oil palm polluted soil in order to decrease palmitic and oleic fatty acid levels in soil matrix and recovery soil fertility. POMS was from an Oil Palm Industry established near to Rio Candelaria at Campeche, México. Soil was mixed with three organic amendments (Bokashi, Vermicompost and Green mountain) adjusted at 10%, 30% and 50%. The moisture of the amended soil was adjusted at 50% and every system was monitoring and evaluating during 168 days. The results suggested that any of the organic amendments enhanced the pH and electric conductivity increments related with ion fluxes, nitrification rate, chemical oxidation and the soil bacterial and fungal metabolic activities. The Gas chromatography analysis, showed that after four weeks, the palmitic acid (C16:0) and oleic acid (C18:1) amount dropped considerably in the amended mixtures adjusted at 30% and 10%. These results contribute to the bioremediation of novel organic pollutants, and encourage the use of eco-friendly technologies.

Keywords: oil palm milling, vermicompost, bokashi, Green mountain



Biodegradation of hydrocarbon-contaminated sediments: Using microbial bioelectrochemical systems B. 1

Cruz sotelo ^(1,2), E. Alvizo ⁽²⁾, L. Vega-Alvarado ⁽³⁾, A. Álvarez Gallegos ⁽¹⁾, K. Juárez Lopéz*⁽²⁾

⁽¹⁾Centro de Ingeniería y Ciencias Aplicadas, Av. Universidad 2001, Cuernavaca, México.

⁽²⁾Instituto de Biotecnología -UNAM, Av. Universidad 2001, Cuernavaca, México

⁽³⁾Instituto de Ciencias Aplicadas y Tecnología -UNAM, Ciudad Universitaria, México

*Corresponding author: katy@ibt.unam.mx, b_ere28@hotmail.com



Microbial bioelectrochemical systems (MBS) have gained a lot of attention as a mode of converting organic matter into electricity, and potentially enhancing biodegradation of recalcitrant pollutants [1]. In these devices the organic matter is oxidized by microorganisms, producing electrons, which are transferred directly or indirectly to a final electron acceptor, which is reduced [2]. The typical design of a microbial fuel cell consists of the anode chamber (where the anode is located), the proton exchange membrane (PEM) and the cathode chamber (where the cathode is located). The anode functions as the final electron acceptor. There are several factors involved in the energetic performance of a microbial fuel cell, for example, the complexity of the substrate, the inoculum, the type and material of the electrodes, the oxygen reduction reaction, etc. Exploring complex organic matter such as hydrocarbons to produce clean energy through microbial fuel cells has been of great importance recently, but it requires research work that allows to know how they work, the identity of microorganisms involved in the process and the optimal engineering conditions for maximum electrochemical performance. This work aims to evaluate the energy potential of sediments contaminated with hydrocarbons using microbial fuel cells, for this purpose, a set of these devices were designed and built, which contained sediment naturally contaminated with hydrocarbon, as substrate, and natural inoculum. The electrochemical characterization of these fuel cells was documented, the voltage according to the external load (Ω), the current density (I) was calculated from OHM's law to obtain the polarization curves and power curves. The energetic potential was explored according to factors of device design, multiple anodes and anodic material. We conclude that we can obtain energy from petroleum contaminated sediment with and this increased when using small anodes instead of large, we obtain 6.71 mW/m² in the best design. In addition, we performed microbial community analyses in order to identify the electrochemically active microorganism and/ or oil degraders. We identify bacteria and archaea groups reported as degraders, electrochemically active and others with syntrophic relationships.

Keywords: Microbial Fuel cells, hydrocarbon-contaminated sediments

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Last news about the microbial biodegradability of the organochlorine

insecticide chlordecone and the possibility to set up soil bioremediation



H. Macarie*, J.-P. Andraud, Y. Labrousse

⁽¹⁾IRD, Aix Marseille Univ, Avignon Université, CNRS, IMBE, Marseille, France

*Corresponding author: herve.macarie@ird.fr



Chlordecone (CLD), of raw formula C₁₀Cl₁₀O, was used from 1972 to 1993 in the French West Indies (FWI) to control the populations of the banana weevil. Nowadays, 25 years after the ban on its use, CLD is still present in the soils where it was spread and from where it causes a diffuse pollution of the other environmental matrices (surface & underground water, coastal areas) and of the associated food resources (crops, livestock, fish, crustacea, etc.). A recent study showed that CLD is detected in the blood of 92% of the FWI population with health consequences whose extent just begin to be appreciated: increased risk of prostate cancer and premature birth as well as negative effects on the development of children cognitive and fine motor skills. Today, the sanitary and societal crises linked to CLD are globally contained thanks to the management measures taken by the authorities to avoid the dietary exposure of the populations (for example by fishing bans) and to recreate an economic activity where it has been lost. Nevertheless, a definitive solution to the problem would be to eliminate the source of CLD, which means to destroy the stock of CLD still present in soils. One of the least costly ways of destruction that seems well suited to the case of FWI is microbial degradation, since it involves techniques that could be incorporated without much difficulty into normal agricultural practices and therefore be applied in situ. Until recently, however, due to its chemical characteristics (cage structure, high steric hindrance, low solubility and high hydrophobicity), CLD was considered to be non-biodegradable. During the presentation by using a thermodynamic approach, we will demonstrate that there are no energetic reasons why the chemical structure of CLD should not be accessible to a microbial attack whatever the environmental conditions considered (aerobic, anaerobic). From a thermodynamic point of view, CLD is in fact very similar to other organochlorine compounds whose biodegradability is established for over 2 decades. Based on the old literature generated after the 1975 CLD crisis in the USA, but also on more recent results and on the knowledge acquired on other organochlorines, we will then show that CLD degradation should be favored initially by anaerobic conditions, but that its ultimate mineralization will probably not be achievable without an aerobic final step. This will lead us to propose hypotheses to explain why, despite the detection of traces of a CLD dechlorination intermediate in soils, a significant degradation of this molecule has not been observed under natural FWI conditions yet, and what could be done by man to try to reverse the situation.

Keywords: soil remediation, thermodynamics, French West Indies, banana plantations



Biodegradation of potassium cyanide by two bacterial isolates from contaminated soil

Y. Maiga*⁽¹⁾, S. Young⁽²⁾, K.D. Orner⁽³⁾, M. Peterson⁽³⁾, J.R. Mihelcic⁽³⁾, V.J. Harwood⁽²⁾, A.S. Ouattara⁽¹⁾

⁽¹⁾ Laboratory of Microbiology and Microbial Biotechnology, University Ouaga 1 Pr Joseph Ki-Zerbo, 03 BP 7021 Ouagadougou 03, Burkina Faso

⁽²⁾ Department of Integrative Biology, University of South Florida, Tampa, FL 33620, USA

⁽³⁾ Department of Civil and Environmental Engineering, University of South Florida, Tampa, FL 33620, USA

*Corresponding author: yvoussa.maiga@gmail.com



The West African region including Burkina Faso is facing an unprecedented increase in industrial and traditional gold mining with the release of toxic chemicals such as cyanide (used to extract gold from the ore) into the environment (ONU, 2009; Carayol, 2010). Due to the adverse effects of cyanide on humans and the environment, it is necessary to develop remediation techniques for contaminated soils, of which bioremediation is considered a promising method. Therefore, soil samples were collected from a cyanide contaminated industrial site in order to isolate cyanide resistant strains to perform its biodegradation. Two bacterial strains (S11 and S13) were isolated and tested for their cyanide biodegradation potential at two different initial pHs (7 and 9) using potassium cyanide. Then, the effect of the initial concentration of cyanide on their biodegradation capabilities was tested at the most optimal pH. The biodegradation medium was prepared using M9 medium (without a nitrogen source) supplemented with potassium cyanide (as a nitrogen source). The bacterial growth was estimated using a nanodrop spectrophotometer while the cyanide removal was estimated by measuring the ammonia released. For both bacterial strains, the biodegradation was more efficient at pH 9 than pH 7. This finding is important for the development of cyanide bioremediation techniques because pH < 9 promotes cyanide volatilization (HCN being highly toxic). The generation of ammonia (i.e. degradation of potassium cyanide) increased when the initial concentration of potassium cyanide was increased from 1 mM to 5 mM for both strains. The results also showed that strain S13 was more efficient in cyanide biodegradation than strain S11 regardless of pH and the tested concentration of potassium cyanide. Results could be used for the development of cyanide bioremediation in contaminated environments.

Keywords: bioremediation, hazardous waste, mining, pH, soil bacteria.

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Production of glycolipid Biosurfactants by bacterial strains isolated from marine environments



Curiel-Maciel N.F.⁽¹⁾ Martínez-Morales F.⁽¹⁾, Rosas-Galván N.S., Bertrand B., Morales-Guzmán D., Trejo-Hernández M.R.*⁽¹⁾

Av. Universidad No. 1001, Col. Chamilpa, Cuernavaca, Morelos, CP. 62209.

*Corresponding author: mtrejo@uaem.mx.

Glycolipids are biosurfactants (Bs) that contain one or more carbohydrate molecules in combination with acidic aliphatic hydroxyl chains. They reduce surface and interfacial tension, form stable emulsions, and are produced by microorganisms¹. Bs are biodegradable, non-toxic and present activity under extreme conditions of temperature, pH and salinity². They have great potential for application in different industries (cosmetic, food, agricultural, petroleum etc.)³. The objective of this study was to improve the production of glycolipidic Bs from marine environments through experimental design. *Enterobacter* sp. C11McH isolated from the Gulf of Mexico was the strain used in this work. A Box-Behnken experimental design was employed to improve culture conditions, considering the following factors: carbon source and concentration, NaCl and temperature. A Taguchi design was previously used to select the most adequate factors. The response variables were reduction of surface tension, ST (mN/m) and emulsion index, EI₂₄ (%). The results obtained showed that the interaction between sucrose and temperature at low levels had a significant effect on Bs production resulting in an EI₂₄ of 47.5 % after 216 h. Additionally, NaCl also favored Bs activity (emulsion) at lower concentrations. In terms of ST, the best results were obtained with high levels of sucrose and low levels of NaCl with a reduction from 63.0 nN/m to 40.0 mN/m after 144 h approximately. Additionally, low temperatures tended to favored the reduction in surface tension.

Keywords: Bio surfactant, Box-Behnken design, Glycolipids, Marine environment

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bioremediation: Review



B. Camacho-Perez⁽¹⁾, E. Andrade-Collantes⁽²⁾, S. Montaña^{*(2)}

⁽¹⁾ Centro de Investigaciones Biológicas del Noroeste, S.C. , Instituto Politécnico Nacional N° 195, La Paz, México.

⁽²⁾ Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Ciudad Universitaria s/n, CP 80010, Culiacán, Sinaloa, México.

*Corresponding author: mmontano@uas.edu.mx



Agrochemicals are a fundamental part of the technological package of modern agriculture. They have been widely used in order to obtain higher yields by protecting crops from different diseases, weeds or insects. The indiscriminate use of agrochemicals is not only restricted to the agriculture use, there are administrated for different purposes like control pest in animals, as well as commercial, residential and industrial application even on public health campaign. However, the inappropriately and indiscriminately uses, concern about the persistence on the environment, bioaccumulation and the consequently adverse health effects for human¹ and others forms of life². In the recent years the proposal of bioremediation against pesticides has increase and the bioinformatics applications in this fields has became a fundamental helpful^{3,4}. Review methodology consisted a systematic informative searching of original research papers peer-reviewed literature and screened for data compilation, related to the impact of pesticides on the Northwest of Mexico and analyze principal advance into *in silico* bioremediation. This review encompasses the following topics: a) detection of agrochemicals in different complex matrices, b) adverse effects on environment by the excessive use the agrochemical, c) occupational health risk evaluation related to pesticide exposure and, d) *In silico* bioremediation using enzymes and molecular dynamics simulation as well as docking studies.

Keywords: agrochemicals, environmental, health, and *In silico*

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Identification of native plant species from an area with mining history in Morelos-Zacatecas and possible potential for phytostabilization

Rebeca Basurto Gaytan⁽¹⁾, Consuelo Letechipía de León⁽²⁾, Luis Enrique Silva Aparicio⁽¹⁾, Ana Isabel Veyna Gómez⁽³⁾, Héctor René Vega-Carrillo⁽²⁾, Miguel Ángel Salas Lúevano⁽⁴⁾.

⁽¹⁾ Unidad Académica de Ciencias de la Tierra, Universidad Autónoma de Zacatecas. Calzada Universidad 108, Progreso, 98058 Zacatecas, Zac., México.

⁽²⁾ Unidad Académica de Estudios Nucleares, Universidad Autónoma de Zacatecas. Ciprés 10, Peñuela, 98060 Zacatecas, Zac., México.

⁽³⁾ Unidad Académica de Ingeniería, Universidad Autónoma de Zacatecas. Ramón López Velarde 801, Zacatecas Centro, 98000 Zacatecas, Zac., México.

⁽⁴⁾ Unidad Académica de Agronomía, Universidad Autónoma de Zacatecas. Carretera Zacatecas-Guadalajara Km. 15.5 Zacatecas, Zac., México

*Corresponding author: rbc.snrrie@gmail.com

For years, the mining tailings have been placed in the environment without an adequate previous treatment, generating a potentially toxic risk to the environment. Nowadays, there are different methods for their treatment and the natural remedies with plants become an attractive and effective proposal. Therefore, the recently investigations focus on the native vegetation of different contaminated areas. So, by selecting tolerant plants a self-sustaining cover can be develop in a low cost and with the capacity to reduce the toxicity of heavy metals, as well as their biodistribution and biomagnification, achieving this way the restoration of the soil. Reason for which the target of the research was to identify the native botanical species from the surrounding area Zacatecas-Morelos and evaluate the possible potential for the phytostabilization. Thus, it was performed a study of the floristic composition of the area, covering approximately 57,700 m², also it was analyzed the concentrations of As and Pb in three plant tissues (root, stem, leaf) for 21 samples, by the technique of AAS-Flame and HG. It was identified 10 native botanic species, grouped in 8 families, where the more predominant were *Lupinus campestris* followed by *Buddleja scordioides* and *Pennisetum villosum*. It was calculated the factor of bioconcentration (BCF) and taking it as a reference any specie was consider as hyper-accumulator of As and Pb. However, *Buddleja scordioides* can be identified as accumulator of As, the rest are excluded of these elements. According with the translocation factor (TF) *B. scordioides*, *L. campestris*, *S. molle* and *N. glauca*, they show the capacity to phytoextracts As and only the first two have the ability to phytoextracts Pb. The rest of the species have the potential to be used in the phytostabilization. This data generates relevant information to be consider in the approach of a reforestation strategy of the study area.

Keywords: heavy metals, mining tailings, native vegetation, phytostabilization.

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Characterization and whole genome sequencing and analysis of a hydrocarbon-degrading negativicute isolated of sediment from the Gulf of México.

L Escobar⁽¹⁾, P Lara⁽¹⁾, K Juárez^{*(1)}.

⁽¹⁾ Instituto de Biotecnología, UNAM. Av. Universidad 2001, Chamilpa, C.P. 62210, Cuernavaca, Morelos, México.

*Corresponding author: katy@ibt.unam.mx



Hydrocarbons are commonly present in marine environments, both from natural and anthropogenic sources. These compounds are highly toxic for diverse ecosystems and considered priority contaminants. Marine hydrocarbon pollution is naturally attenuated by bacterial degradation both aerobic and anaerobically. While aerobic degradation has been extensively studied, knowledge of anaerobic degradation is limited and it has been demonstrated only in a small number of microorganisms. The main objective of this work is extend the knowledge of anaerobic degradation of hydrocarbon as well as the isolation and characterization of bacteria with notable capabilities related with these metabolisms. We isolated from marine sediment of Gulf of Mexico, a strain of *Pelosinus* sp. This microorganism is able to growth in presence of toluene, benzene and benzoate. Moreover, we found that this strain is able to grow with fumarate in absence of another electron donor or acceptor, and it is aerotolerant anaerobe. We performed the whole genome sequencing and its analysis revealed that it possessed genes probably involved in hydrocarbon degradation, e.g. UbiX/UbiD, benzoate CoA ligase and benzyl CoA reductase reported to benzene degradation pathway, as well as a 4-hydroxyphenylacetate decarboxylase previously associated with oxygen-dependent aromatic hydrocarbon degradation. In other hand, we also found putative transcriptional regulators related to hydrocarbon utilization like CatR and IciR. Other notable features are under current characterization, but it is important to mention that *Pelosinus* genus never has been isolated from marine environments, neither reported to degrade hydrocarbons.

Keywords: Anaerobic hydrocarbon degradation, Gulf of Mexico, *Pelosinus* sp.



Effect of pollution on the physicochemical, structural and surface

characteristics of the sediments of the Reconquista River, Argentina



Ana E. Tufo⁽¹⁾, Natalia Porzionato⁽¹⁾, Agustina Ziliani⁽¹⁾, Celeste Grimolizzi⁽¹⁾, Gustavo Curutchet^{*(1)}

⁽¹⁾ Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, Bs. As, Argentina.

⁽²⁾ CONICET. Consejo Nacional de Investigaciones Científicas y Técnicas

*Corresponding author: gcurut@gmail.com



The Reconquista river basin (located in the province of Buenos Aires) represents one of the most polluted areas of Argentina. In previous ISEBE conferences, we presented a bioremediation technique for the treatment of sediments with high contents of heavy metals¹ and the study of how the physicochemical and structural parameters of the treated sediments were modified during the process². In line with these investigations, in this work we have analyze the influence of the contamination on some physicochemical, structural and superficial characteristics of the sediments. To fulfill this aim, two sites from the river basin were sampled: Km 2 of Autopista Camino del Buen Ayre (CBA) (a highly contaminated site) and Dique Ing. Roggero (DR) (a non-contaminated location). The dissolved oxygen (DO), the oxidation reduction potential (ORP), the pH and other parameters of the water were determined in situ. Cores of 30 cm deep of sediment were extracted with a specially designed device. The pH, ORP, organic matter (MO) and volatile acid sulfides (AVS) were determined every 5 cm depth at the extracted cores. A sequential extraction of metals was carried out at samples and implemented the BCR method. The sediments were characterized by means of XRD, BET, porosity and particle size techniques. We found that the pH is slightly more alkaline for the contaminated site and remains constant along the depth at both sites (9.5-9 for CBA and 8-8.5 for DR). The CBA samples shown typical parameters of strong contamination and pollutants accumulation: ORP values of approximately -300 mV, OM values from 13 to 6 % with respect to the depth, and concentration of AVS from 3500 to 2500 mg/kg. On the contrary, the sediments of the DR shown values around +200 mV, the OM was less than 5% and AVS concentration was negligible. Regarding the accumulation of metals in the sediments, it should be remarked that in contaminated places the metal concentration at surface is generally higher than at 30 cm depth, suggesting continuous input from water column. For example, for Zn, we found at CBA 280 mg/kg surface, decreasing to ~150 mg/kg at 30 cm depth. DR shown superficial concentration of 7 mg/Kg. The increase of Zn concentration en CBA site due to contamination is dramatic. XRD and surface area analysis shown changes in the whole sediment and in it's clay fraction that could have strong influence on the fate of pollutants in the sediments. The whole set of results will allow us to understand how the degree of contamination, the accumulation of metals and the rest of examined parameters affect the intrinsic characteristics of the sediments and it's role in regulating pollutants processes.

Keywords: Reconquita river, Pollution, Sediments, XRD, Texture, Heavy Metals.

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Production of glycolipid Biosurfactants by bacterial strains isolated from marine environments



Curiel-Maciel N.F.⁽¹⁾ Martínez-Morales F.⁽¹⁾, Rosas-Galván N.S., Bertrand B., Morales-Guzmán D., Trejo-Hernández M.R.*⁽¹⁾

Av. Universidad No. 1001, Col. Chamilpa, Cuernavaca, Morelos, CP. 62209.

* Corresponding Author mtrejo@uaem.mx.

Glycolipids are biosurfactants (Bs) that contain one or more carbohydrate molecules in combination with acidic aliphatic hydroxyl chains. They reduce surface and interfacial tension, form stable emulsions, and are produced by microorganisms¹. Bs are biodegradable, non-toxic and present activity under extreme conditions of temperature, pH and salinity². They have great potential for application in different industries (cosmetic, food, agricultural, petroleum etc.)³. The objective of this study was to improve the production of glycolipidic Bs from marine environments through experimental design. *Enterobacter* sp. C11McH isolated from the Gulf of Mexico was the strain used in this work. A Box-Behnken experimental design was employed to improve culture conditions, considering the following factors: carbon source and concentration, NaCl and temperature. A Taguchi design was previously used to select the most adequate factors. The response variables were reduction of surface tension, ST (mN/m) and emulsion index, EI₂₄ (%). The results obtained showed that the interaction between sucrose and temperature at low levels had a significant effect on Bs production resulting in an EI₂₄ of 47.5 % after 216 h. Additionally, NaCl also favored Bs activity (emulsion) at lower concentrations. In terms of ST, the best results were obtained with high levels of sucrose and low levels of NaCl with a reduction from 63.0 nN/m to 40.0 mN/m after 144 h approximately. Additionally, low temperatures tended to favored the reduction in surface tension.

Keywords: Bio surfactant, Box-Behnken design, Glycolipids, Marine environment

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Pilot-scale evaluation of a phytoremediation process of mining soils

using *Jatropha curcas*, in northern Colombia



M Viña Pico*⁽¹⁾, S Burgos Nuñez⁽¹⁾, I Urango Cardenas⁽¹⁾, G Enamorado Montes⁽¹⁾, J Marrugo Negrete⁽¹⁾

⁽¹⁾ Universidad de Córdoba, Carrera 6 No. 76-103, Montería, Colombia

*Corresponding author: mvinapico@hotmail.es



Mining is one of the main economic activities in Colombia, however, the implementation of obsolete mining practices and the inadequate management of waste has generated soil contamination. The use of *Jatropha curcas* (Jc) in phytoremediation processes for the recovery of soils contaminated with mercury (Hg) has been studied and implemented by some researchers¹. The objective of this research was to evaluate on a pilot scale the capacity of plant species Jc, to concentrate Hg in aerial tissue, taking into account factors such as soil removal, time (months) and time of year, as a sustainable strategy for recovery of a mining ground in northern Colombia. The investigation was carried out in a mining area of the department of Córdoba, northern Colombia. Two experimental units were established, one area removed by the gold activity (Zone 1) and the other one not removed (Zone 2), in each one 200 plants were established (ds = 3 m x 3 m). The soil Hg concentrations were analyzed at the beginning of the trial and monthly in the leaf tissue of the culture. In addition, the physicochemical characteristics of the soil in each zone were determined and the growth and development of the Jc plantation was monitored. The Hg concentrations in the soil were 108.49 and 105.55 µg / Kg for zones 1 and 2, the leaf tissue showed averages of 60.67 and 35.75 µg / Kg in the rainy and dry periods respectively. No statistically significant differences were found ($p > 0.05$) between sowing zones, however, the time factor of the crop and season (rainy and dry) showed significant effects on the Hg concentration in the leaf tissue ($p < 0.05$) through the months of study. The soil-leaf tissue bioaccumulation factor (BAF) was statistically different ($p < 0.05$) between zones. In the plantation of zone 1, symptoms of toxicity were observed during the development of some plants, presenting chlorosis, necrotic spots, and deformation of some seedlings. The translocation of Hg to the foliar tissue of Jc, was not influenced by the removal of the soil but varies according to the rainfall supply throughout the year. In addition, the BAF varies with respect to the physiological response of the plant between times of the year. In general, the behavior of Jc under the conditions studied makes it a great strategic and sustainable alternative for the phytoremediation of soils contaminated with Hg.

Keywords: Phytoremediation, *Jatropha c.*, Mining, Soils.



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IV Congreso Nacional de Tecnologías y Ciencias Ambientales

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Biodegradation of Aromatic Polycyclic Hydrocarbons by Endophytic

Fungi of Ecuadorian Amazon Rainforest



Marín F.*⁽¹⁾, Portero C.⁽¹⁾, Narváez A.⁽¹⁾

⁽¹⁾ Laboratorio de Biotecnología Vegetal, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre, N° 1076, Quito, Ecuador.

*Autor correspondiente: fjmarin@puce.edu.ec



The capacity of 133 endophytes fungal isolates for degrading petroleum hydrocarbons was evaluated. The endophytes were isolated from leaf and stem tissues from 23 plants collected in a natural habitat contaminated with crude oil in southwestern Ecuador. Their capacity for hydrocarbons biodegradation was tested by in vitro colorimetric qualitative test for 10 days, using the Minimal Salt Medium and crude oil as carbon source. The taxonomic identification of the isolates that showed bioactivity in the qualitative test was carried out by the analysis of the ITS gene of the region 18S of the rDNA. The endophytes that showed the best results in the previous qualitative test were selected for a quantitative in vitro test for 30 days. Residual hydrocarbons were tracked by infrared spectroscopy and gas chromatography with a flame ionization detector. The maximum removal rates of total petroleum hydrocarbons were 99.6% and 99.8%, corresponding to fungi of the genus *Verticillium* sp. and *Aspergillus* sp. respectively. This is the first report of biodegradation of crude oil hydrocarbons by endophytic fungi, the results suggest these fungal isolates are potential hydrocarbon biodegraders that could be used in bioremediation processes.

Keywords: Endophyte, biodegradation, bioremediation, hydrocarbon.



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Chapter 4 Solid and Hazardous Waste Management and Treatment



Y. Villegas Peralta⁽¹⁾, R.G. Sánchez Duarte*⁽¹⁾, J. López Cervantes⁽¹⁾, D. I. Sánchez Machado⁽¹⁾,
M. A. Correa-Murrieta⁽¹⁾, M.R Martínez Macías⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 5 de Febrero, 808 Sur, Colonia Centro, Ciudad Obregón,
Sonora México, cp. 85000

*Corresponding author: reyna.sanchez@itson.edu.mx



The nanotechnology has attracted increasing attention for its functionality in many areas of science. This field also includes the production of carbon nano-onions. Several methods have been reported for the synthesis of carbon nano-onions, however, these methods are complex because of the use of high temperatures, inert atmospheres, high pressures or vacuum, voltage, and current¹. The substrates frequently used to produce carbon nano-onions are carbon soot, methane, propane and plastic wastes, and also natural sources as chitosan extracted from chitin and lycopene extracted from tomatoes. Chitosan is a biopolymer obtained from the deacetylation of chitin, it has properties that are improved with the addition of functional groups and it is considered as a carbon source. The aim of this work was the synthesis and characterization of nano-onions as a new production method using chitosan raw material without the application of high temperatures for its future use as an adsorbent of pollutants in water solution. Chitosan nano-onions (CSNO) were prepared following the ionic gelation method of Hu et al, 2006² with some modifications. The chitosan nano-onions were characterized by Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS). FTIR technique, revealed the interactions between functional groups of the lipid (lecithin) and the chitosan. Once CSNO were formed, a displacement of the amino groups of chitosan was observed from 1656.99 cm⁻¹ to 1641.31cm⁻¹ and from 1596.83 to 1546.32 cm⁻¹. New bands were observed at 1752.12 cm⁻¹ due to the stretching of the carbonyl groups of the fatty acids and phosphate groups of lecithin appeared at 1156.88 cm⁻¹. The XRD analysis resulted in an amorphous structure. SEM images, presented a globular morphology with concave surfaces. By TEM, was shown the nano-onions congregated in a unique structure including nanotubes. The CSNO average diameter by DLS was 442.5 nm with a hexagonal arrangement, an index of polydispersity (PDI) of 0.532 and a zeta potential of 31.9. In conclusion, the production of CSNO by using a simple method was possible. The average size of nano-onions, the PDI and the value of zeta potential were indicators of a stable dispersion. Nano-onions would be used as adsorbents of pollutants in water solution in further experiments.

Keywords: Chitosan, nano-onions, SEM, shrimp waste, TEM

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Urease and Carbonic anhydrase activities of ureolytic bacteria in an enriched and unenriched concrete pore solution



M.J. Castro Alonso⁽¹⁾, L.E. Montañez Hernández⁽¹⁾, M. A. Sánchez Muñoz, R. Narayanasamy⁽²⁾, M.P. Luévanos Escareño⁽¹⁾, N. Balagurusamy⁽¹⁾

¹ Laboratorio de Biorremediación, Facultad de Ciencias Biológicas, Universidad Autónoma de Coahuila, Carretera Torreón-Matamoros Km 7.5, Torreón, México.

² Facultad de Ingeniería, Ciencias y Arquitectura de la Universidad Juárez del Estado de Durango.

*Corresponding author: bnagamani@uadec.edu.mx



In recent years, Civil engineering and biotechnology fields have been implementing various strategies to reduce the negative environmental impacts caused by construction sector. Bioconcrete has become one of the most promising sustainable technologies in comparison with other traditional and modern methods (1). This biomaterial is based on the biomineralization of calcium carbonate in concrete matrix by a series of complex microbial reactions driven by Urease and Carbonic Anhydrase enzymes. In this study, 6 of the 24 different ureolytic bacterial strains isolated from the alkaline soils of the Laguna region of Mexico were selected for enzyme studies in synthetic culture medium (Urea-CaCl₂ medium) and as well as in simulated concrete pore solution. In Urea-CaCl₂ medium, ACRN11 strain showed the highest urease activity of 259 U/ml in comparison with other tested strains at 96 hrs. In the case of carbonic anhydrase activity, ACRN5 and ACRN11 strains recorded the highest specific activity of 1.3431 U/min/mg of intracellular protein and 0.9649 U/min/mg of extracellular protein respectively at 120 hrs. This study showed the survival of ureolytic strains in simulated concrete pore solution (pH 14) for a period of 120 hrs. However, the bacterial cell protein content in simulated concrete pore solution showed around 10-fold decrease for all the six strains tested. It was also observed that the urease and carbonic anhydrase activity were decreased around 90 to 95% in comparison with Urea-CaCl₂ medium. SDS-PAGE studies on the protein profile of the bacterial strains in Urea-CaCl₂ medium showed the presence of intra- and extra-cellular proteins in the range of 17-130 kDa. Nevertheless, there is a slight loss of high molecular weight proteins of the bacterial strains in the simulated concrete pore solution. The presence of 17-95 kDa and 21-72 kDa molecular weight were observed in intracellular and extracellular proteins. It is reported in the literature the subunit C of urease is in the range of 60 – 66 kDa and carbonic anhydrase is of 17 - 36 kDa and our results showed the presence of bacterial proteins in these ranges at 120 hrs. This clearly demonstrated the presence and activity of these bacterial strains under stress conditions of extreme pH, although their activities were found to be reduced. These results along with growth and activity of urease and carbonic anhydrase of bacterial strains and their zymogram profile in enriched concrete pore solution will be presented.

Keywords: Bioconcrete, Carbonic Anhydrase, Concrete pore solution, Urease.

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remediation



C. Duca⁽¹⁾, L. Guz^(1,2), **S. Estévez-Areco⁽¹⁾**, J. Cimadoro⁽¹⁾, S. Goyanes^{*(1)}, R. Candal^{**2)}

⁽¹⁾ Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Física, Laboratorio de Polímeros y Materiales Compuestos (LPM&C), Instituto de Física de Buenos Aires (IFIBA-CONICET), Av Cantilo S/N, Buenos Aires, Argentina.

⁽²⁾ Instituto de Investigación e Ingeniería Ambiental (3ia), CONICET, Universidad Nacional de San Martín, Campus Miguelete, Av 25 de Mayo y Francia, Buenos Aires, Argentina.

*Corresponding authors: * goyanes@df.uba.ar ; ** rcandal@gmail.com



The preparation of hybrid materials that combine the mechanical and structural properties of polymer nanofibers with the functional groups presented in bacteria membranes leads to the generation of versatile and polyfunctional systems for several applications (supported biosorbents and filters¹, biosynthesis by microorganisms, bacteria cryo-preservation², among others). A new hybrid biomaterial consisting of polyvinyl alcohol (PVA) nanofibers mats obtained by electrospinning with bacteria entrapped inside was developed. The viable bacteria were entrapped inside by submerging the material in PBS with *Escherichia coli* for 24 hs at 37 °C to allow bacterial adhesion. The aim of this work was to study the interaction between bacteria and the electrospun mats, and its possible application as a cryo-preserved support for bacteria and as a hybrid biofilter.

The bacteria were found to be inside the nanofibers network holes, as observed by fluorescent microscopy. No bacteria were observed on the mats surface, as determined by FE-SEM. This hybrid membrane was able to preserve bacteria alive even after been preserved at -20 °C for one month. The present development could be used, for example, as a bioreactor, in medical applications, or as a filtering membrane for water treatment. To demonstrate its applicability as a heavy metal removal membrane, the hybrid material was successfully used to remove copper from water without releasing bacteria into the aqueous media. The present material opens the door to membranes in which different species of bacteria could be combined with electrospun mats for specific environmental applications.

Keywords: Bacteria Preservation; Biofilter; Electrospinning; Metal Adsorption.

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Filtration of aqueous systems using green-electrospun PVA membranes

J. Cimadoro⁽¹⁾, L. Ribba⁽¹⁾, **S. Estevez Areco**⁽¹⁾, L. Guz⁽¹⁾⁽²⁾, R. Candal^{*(2)}, S. Goyanes^{*(1)}

⁽¹⁾ *Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Física, Laboratorio de Polímeros y Materiales Compuestos (LPM&C), Instituto de Física de Buenos Aires (IFIBA-CONICET), Av Cantilo S/N, Buenos Aires, Argentina.*

⁽²⁾ *Instituto de Investigación e Ingeniería Ambiental (3ia), CONICET, Universidad Nacional de San Martín, Campus Miguelete, Av 25 de Mayo y Francia, Buenos Aires, Argentina.*

*Corresponding authors: rjandal@gmail.com, sgoyanes@df.uba.ar



The availability of clean water is one of the main technological, social and economic issues of the 21st century. The removal of suspended particles by filtration is a critical step on the way to ensuring clean water from natural water sources, as well as to recycling wastewater for reuse. Electrospun nanofibrous membranes are a very attractive and a plausible solution in filtration technology due to their unique properties, including their high porosity (typically around 80%), controllable pore size distribution, high permeability and fully interconnected pore network. Depending on the pore size of the membrane, different type of impurities can be removed from the aqueous solution. Due to the size exclusion of the pollution, as the pore size decreases, the rejection ratio increases.

In this work, PVA membranes crosslinked with citric acid were developed in a green and scalable process by electrospinning. The obtained membranes, with initial pores of the order of microns or more, can, after a short swelling transitory time, retain particles of nanometric size. The morphology of the mesh varies as a function of time in contact with water, as observed by AFM. The filtration performance of the mesh was characterized using water and TiO₂ nanoparticle suspensions, showing an increase in the rejection rate over time, which exceeds 99% after a post-transitional period.

Keywords: Electrospinning, Nanoparticles filtration, Water filtration.



eutectic solvent



M. I. García-Benítez⁽¹⁾, A.D. Cañas-Jardón⁽¹⁾, W. Sánchez-Ortiz⁽¹⁾, I. Mejía-Caballero⁽²⁾, J. I. Aldana-González⁽²⁾, Tu Le Manh⁽³⁾, E. M. Arce-Estrada⁽³⁾, M. Palomar-Pardavé⁽²⁾

⁽¹⁾ Universidad Mexiquense del Bicentenario, UES-Tultitlán, Tultitlán, Edo. de México, CP 54910.

⁽²⁾ Universidad Autónoma Metropolitana, Unidad-Azcapotzalco, Azcapotzalco, CDMX, CP 02200.

⁽³⁾ Instituto Politécnico Nacional, ESQIE, Gustavo A. Madero, CDMX, CP 07738, MÉXICO.

*Corresponding authors: martin.ismael@outlook.com

It is known that chrome electrodeposition on an industrial scale has been made from aqueous media. However, this process is not efficient and toxic. Faced with this problem, and in order to replace aqueous media by others that allow to have advantages over these, such as: stability, wide window of potential, hydrogen-free formation during electrodeposition, good performance and deposit morphology, Abbott et al. [1,2] has generated new "green" solvents, deep eutectic solvents (DES). This work presents a thorough study on the electrochemical nucleation of chromium from the anhydrous chromium salt using a choline chloride. The DES was formed by the mixture of choline chloride- urea (1:2 molar), the chromium salt (CrCl_3) was dissolved in the DES under magnet stirring during 12 h at 100°C . The electrochemical studies were carried out using cyclic voltammetry and chronoamperometry, at 70° , onto glassy carbon (GC) as a working electrode of a conventional three electrode cell, with a graphite bar as the counter electrode and a silver wire as the pseudoreference electrode. The evidence of chromium nucleation was observed by means of scanning electron microscopy (SEM) JEOL SEM 6700F equipment as well as by the EDS technique coupled with the SEM. The voltammograms recorded (Fig. 1) onto GC in the system $\text{Cr}^{3+}/\text{DES}$ show, in the forward scan direction, the presence of two peaks: the first one is located around -1.4 V associated with reduction of Cr^{+3} to Cr^{2+} and the second one around -2.1 V associated with reduction of Cr^{+2} to Cr^0 . This observation, for the first time, was verified using chronoamperometry technique the formation of species. Allowing to conclude that the electrodeposite of chromium from an anhydrous salt is possible, and the water content of the medium is negligible.

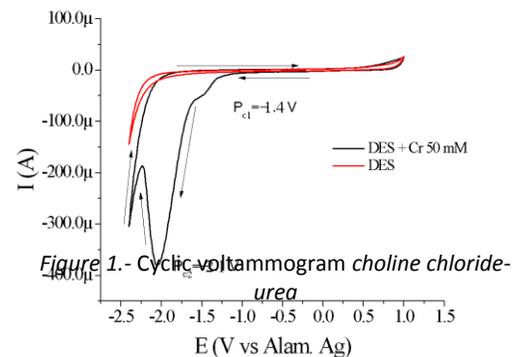


Figure 1.- Cyclic voltammogram choline chloride-urea

Keywords: Deep eutectic solvent; chromium nucleation; trivalent chromium;

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The inhibition of CO₂ corrosion via sustainable geminal zwitterion

compounds: effect of the length of the hydrocarbon chain from amines.



E. Gómez Juárez^{*(1,2)}, R. Hernández Altamirano^(1,2), V. Y. Mena Cervantes^(1,2), Jorge G. Vázquez Arenas^(1,2)

⁽¹⁾ Instituto Mexicano para la producción más limpia, Av. Acueducto S/N, Gustavo A. Madero, Ticomán, Ciudad de México, 07340, México.

⁽²⁾ Instituto Politécnico Nacional, Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad de Biocombustibles (LaNDACBio), Ciudad de México, 07340, México

*Corresponding author: egomezj5@gmail.com



The chemical synthesis of five molecules of beta-amino acid type (zwitterion) was carried out, taking into account five parameters of green chemistry to determine the eco-efficiency of the synthesis, in order to evaluate their applications as corrosion inhibitors for carbon steel (AISI - 1018). The zwitterions come from amines (of hydrocarbon chains) with variable length, achieving mass reaction efficiencies higher than 80%, without the generation of waste. The molecular structures were characterized by infrared spectroscopic methods (FT-IR) and nuclear magnetic resonance (NMR). In addition, a synthesis route was developed to obtain corrosion inhibitors. The electrochemical characterization consisted of potentiodynamic sweeps in acid media (carbon dioxide - CO₂) and high salinity; to determine the optimal concentrations in which the inhibitors decrease the corrosion rate (CR) of the steel; likewise, the technique of electrochemical impedance spectroscopy (EIS) was used to determine a more systematic evaluation of the corrosion mechanism that takes place in steel. Likewise; AISI - 1018 steel coupons were used to evaluate the corrosion of all the inhibitors (10, 25, 50, 75 and 100 ppm) in an electrolyte whose concentration was 1.0 M sodium chloride (NaCl) saturated with CO₂ to pH approximately of 3.5 and a temperature of 70°C. The best performance against corrosion at the lowest concentrations was obtained for the LZW-B12 molecule at a concentration of 75 ppm, whose values of current density (i_{corr}), corrosion potential (V), corrosion rate (CR) and Inhibition efficiency (% EI) were: $1.5 \times 10^{-5} \text{ A} \cdot \text{cm}^{-2}$, -0.615 V, 0.174 mmpy and 91.66% respectively, likewise for the LZW-B16 molecule at a concentration of 25 ppm, the same parameters were: $1.0 \times 10^{-7} \text{ A} \cdot \text{cm}^{-2}$, -0.66 V, 0.001 mmpy and 99.94%, respectively According to the results of the polarization curves. The Nyquist spectra for these inhibitors revealed that the capacitive contributions are more important in the system than the resistive processes, which highlights the importance of the inhibitor phenomena of the inhibitor in the corrosion process. The impedance components grow several orders of magnitude in the presence of LZW-B12 at 75 ppm and LZW-B16 at 25 ppm. Compared; with the experiments performed in the absence of the corrosion inhibitor, it suggests that the inhibitors are effective in retarding the corrosion of steel in these media. During this evaluation, it was observed that the EIS diagrams increased according to immersion time, which suggests that the continuous and stable adsorption of the inhibitors improved their resistance to corrosion.

Keywords: Corrosion, CO₂, zwitterionics corrosion inhibitors, Corrosion Rate.



Evaluation of alternative nitrogen sources in the production of polyhydroxybutyrate by *Halomonas boliviensis*



D.E. Marsiglia López^{*(1)}, N.A. Gómez Vanegas⁽¹⁾, A.P. Villegas Quiceno⁽²⁾

⁽¹⁾ Grupo de Bioprocesos, Universidad de Antioquia, calle 67, N°53-108, Medellín, Colombia

⁽²⁾ TERMOMEC, Universidad Cooperativa de Colombia, Calle 50 No 41-34, Medellín, Colombia

*Corresponding author: dalma.marsiglia@udea.edu.co



Biodegradable polymers have shown to be an excellent alternative to replace petrochemical plastics considering their good mechanical and thermal properties, taking advantage of the ability of several microorganisms to synthesize biopolymers like polyhydroxybutyrate (PHB) that have good mechanical and thermal properties². However, the major drawback for PHB is its high production costs; therefore, this research proposes to use an efficient microorganism supplied with an economical nutrient source to induce higher amounts of biomass and biopolymer to accomplish a competitive commercial cost. The halophile bacteria *Halomonas boliviensis* is a high PHB (up to 80%) producer microorganism in response to a physiological stress by nitrogen and oxygen supply¹, only three amino acids, i.e., aspartic acid, glycine, and glutamine induce its growth. Considering this, the effect on cell growth and PHB production by *H. boliviensis* was evaluated using Soluble Isolated Soy Protein (SISP), Wheat Gluten Protein (WGP), and Whey Protein (WP). An enzymatic hydrolysis of proteins was performed using a Savinase 16L protease to break the peptide bonds and solubilize the protein in the culture media. PHB production was carried out at flask scale at 30 °C, 200 rpm and 10:1 C/N ratio during 48h. Results show high concentrations of biomass 3.8, 4.9, 3.2, g/L and biopolymer production of 2.6, 3.9, 2.3 g/L by SISP, WGP and WP, respectively. These results are promising as the addition of cheap nutrient sources in culture media can make the production of PHB at industrial scale economically feasible and thus become the substitute of conventional plastics.

Keywords: Economical substrates, *Halomonas boliviensis*, Nitrogen source, PHB production.

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Production of poly- β -hydroxybutyrate from whey at different C:N

relations



A.B. Holguin-Loya⁽¹⁾, S.B. Perez-Vega⁽¹⁾, C. E. Molina-Guerrero, I. Pérez-Reyes⁽¹⁾, V. H. Ramos Sanchez⁽¹⁾, I. Salmerón^{*(1)}

⁽¹⁾ Universidad Autónoma de Chihuahua, Facultad de Ciencias Químicas, Circuito Universitario s/n, Campus II, Chihuahua, México.

*Corresponding author: isalmeron@uach.mx



High production of non-renewable plastic materials has led to numerous contamination concerns hence the production of biodegradable plastics such as polyhydroxyalkanoates are of great interest⁽¹⁾. During the scale-up of a bioprocess the fermenting media has a considerable effect on over-all production costs⁽²⁾. In the present investigation we studied different C:N relations and media components during the formulation of a low-cost whey media for polyhydroxybutyrate (PHB) production by *Bacillus* 2355 spp. Fermentation experiments were carried out at different carbon-nitrogen ratios (C:N) where 10:1 and 1:10 was studied varying the source of carbon with glucose and lactose, and the peptide lactalbumin was the nitrogen source. Fermentations were performed in 150 mL sealed Erlenmeyer flasks, at 37 °C and with an agitation of 200 rpm. A methylation of the PHB produced by the bacteria was carried out in order to quantify it by gas chromatography. A one-way ANOVA statistical analysis was used to analyze data. PHB production was significant different ($P < .05$) obtaining higher values with glucose-lactalbumin at a C:N of 1:10 when compared to lactose-lactoalbumin at a C:N of 1:10 with 30.9 mg/L and 23.5 mg/L of PHB respectively. For the C:N of 10:1 lactalbumin-glucose 29.9 mg/L of PHB was obtained. Since the use of glucose as the main carbohydrate will increase the cost of the biopolymer production the use of lactose from whey will be implemented and the C:N ratio to 1:10 can be adjusted with urea as previously demonstrated⁽³⁾.

Keywords: Biomaterials, green solvent extraction, PHB, whey fermentation.

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Efecto de la adición de subproductos orgánicos e inorgánicos en propiedades fisicoquímicas de abono orgánico



A. Graciano-Obeso^{*(1)}, Pollorena-López G.⁽¹⁾, Báez-Hernández G. E.⁽¹⁾ y Humarán-Sarmiento V.⁽¹⁾
⁽¹⁾ Instituto Tecnológico Superior de Guasave, carretera a Brecha, sin número, Ejido El Burriocito, Guasave, Sinaloa, México.

*Corresponding author: adalidgraciano@hotmail.com

La generación de residuos sólidos orgánicos en las actividades urbanas, agropecuarias e industriales, está estrechamente relacionado con el modelo productivo actual y representa una de las principales formas de deterioro del medio ambiente al no existir un aprovechamiento racional o un reciclaje sistemático de los mismos¹. Sin embargo, en años recientes se han desarrollado métodos para el manejo y transformación de desechos encaminados no solo a disminuir el problema de contaminación ambiental, sino a la obtención de abonos orgánicos, cuyas características físicas, químicas y biológicas inciden directamente en el mejoramiento del suelo y el crecimiento de las plantas². El objetivo de la presente investigación fue evaluar el efecto de subproductos orgánicos e inorgánicos en propiedades fisicoquímicas de abono orgánico producido a base de lombricultura. Se realizaron 6 mezclas de fermentos de diferentes subproductos orgánicos; pescado, camarón, cítricos, melaza y guano con lixiviado de lombriz, se mezcló con sulfato de potasio el cual es un producto inorgánico, a los tratamientos se les determinaron las propiedades fisicoquímicas de acuerdo con la norma³. Los datos fueron analizados bajo un diseño completamente al azar y las diferencias entre las medias fueron estimadas por medio de la prueba de Tukey con un nivel de significancia del 95%. De acuerdo a los resultados, fue posible identificar al T3 como el más adecuado para la aplicación en cultivo, ya que presentó un pH de 6.7, una CE de 16.02 mmhos/cm y MO de 0.7 %, estadísticamente diferente ($P \leq 0.05$) a los demás tratamientos, en lo que respecta a las concentraciones de N fue el más elevado de 0.85% estadísticamente diferente ($P \leq 0.05$) del T1 y el T2 que fueron de 0.75% y 0.8% respectivamente, sin embargo fue el que presentó menor concentración de P=671 ppm ($P \leq 0.05$). El aprovechamiento de subproductos orgánicos derivados de distintas actividades económicas, al ser combinados con productos inorgánicos, permiten obtener un abono orgánico líquido con elevadas concentraciones de macro elementos, un valor óptimo de pH para el suelo y una conductividad eléctrica con concentración de iones necesarios para facilitar el desarrollo de cultivos y mejorar los suelos.

Palabras claves: abono, inorgánico, orgánico, subproducto.

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Microbial synthesis of bionanobioparticles using wastes from an OFMSW

biorefinery



Leticia Romero-Cedillo*⁽¹⁾; Héctor Mario Poggi-Varaldo⁽²⁾; Yasuhiro Matsumoto Kuwabara⁽¹⁾; Miguel García-Rocha⁽¹⁾; Jaime Santoyo Salazar⁽¹⁾; Teresa Ponce Noyola⁽²⁾; Luz Bretón-Deval⁽³⁾; Carlos Escamilla Alvarado⁽⁴⁾

⁽¹⁾ Nanoscience and Nanotechnology Program, CINVESTAV, P.O.Box 14-740, CP 07000, Mexico City, Mexico

⁽²⁾ CINVESTAV, Environmental Biotechnology and Renewable Energies Group; P.O.Box 14-740, CP 07000, Mexico City, Mexico

⁽³⁾ The Institute of Biotechnology, Universidad Nacional Autónoma de México, Av. Universidad 2001, Chamilpa, CP 62210 Cuernavaca, Morelos

⁽⁴⁾ Faculty of Chemical Science, Universidad Autónoma de Nuevo León, Av. Universidad s/n, Colonia Universidad 66455, San Nicolás de los Garza, Nuevo León

*Corresponding author: leticia.romero@cinvestav.mx

Iron nanoparticles (Fe-NP) have been extensively used in bioremediation of soils and aquifers contaminated with a variety of pollutants. Fe-NP are usually produced by use of chemical agents, toxic compounds and high energy consumption. An alternative route that allows to obtain Fe-NP, particularly magnetite, is based on the metabolism of iron reducing bacteria (IRB). The purpose of the present work was to synthesize Fe-NP using anaerobic consortia from the anaerobic fluidized bed reactors (AnFBR) that are part of a biohydrogen-based biorefineries. Two lab-scale, mesophilic, AnFBR were fed with hydrolyzates from the organic fraction of municipal solid waste (OFMSW). One AnFBR was dedicated to bioH₂ production (AnFBR-H) whereas a second one produced CH₄ (AnFBR-M). The AnFBRs were loaded with granular activated carbon (GAC) as carrier medium. GAC particles were colonized by the corresponding anaerobic consortia leading to beds of bioparticles (BP) inside the AnFBRs. After 90 d, BPs were sampled and transferred to flasks containing either (i) ferric and ferrous chloride 0.25 M and 0.5 M, respectively or (ii) ferric citrate 2mM, plus hydrolyzate sufficient to give initial 10 g/L or 16g/L reducing sugars, and incubated at 37°C for 7 d. This intended to decorate the BPs with Fe-NP; herein after the nano-decorated bioparticles are called bio-nano bioparticles (BNBP). The BNBP were sampled and subjected to characterization. According to XRD pattern of BNBPH and BNBPM, the most prominent peaks for magnetite XRD were for planes (311) (400) (422) (440) and (442) at 35.4°, 43°, 53.4°, 62.5° and 67° 2θ respectively. SEM analysis indicated an amorphous distribution of nanoparticles over the bioparticle. Analysis by EDS (Energy Dispersive Spectroscopy) showed consistent iron concentration in BNBPM extracted from the AFBR-M (54-64% iron), while iron concentration in BNBPH (extracted from AFBR-H) was lower in average (range 7.8-62%, depending on iron source.) Our results are promising, because iron NP are commonly used in wastewater treatment of several polluting agents, such as the removal of chlorinated organic compounds. Finally, the biological production of BNBP coupled to a biorefinery could become an environmentally friendly platform for nanomaterial synthesis as well as an additional source of revenues.

Keywords: anaerobic fluidized bed reactors, bionano bioparticles, bioparticles, bioremediation, iron reducing bacteria.



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Biological synthesis of iron nanoparticles: an overview



Leticia Romero-Cedillo*⁽¹⁾, Héctor Mario Poggi-Varaldo⁽²⁾, Luz Bretón-Deval⁽³⁾, Vania Shuhua Robles-González⁽⁴⁾

⁽¹⁾ Nanoscience and Nanotechnology Program, CINVESTAV, Av. Instituto Politécnico Nacional 2508, CP 07360, Mexico City, Mexico

⁽²⁾ Department of Biotechnology and Bioengineering, CINVESTAV, Av. Instituto Politécnico Nacional 2508, CP 07360 Mexico City, Mexico

⁽³⁾ The Institute of Biotechnology, Universidad Nacional Autónoma de México, Av. Universidad 2001, Chamilpa, CP 62210 Cuernavaca, Morelos

⁽⁴⁾ Institute of Agroindustries, Universidad Tecnológica de la Mixteca, Carretera a Acatlima Km 2.5, CP 69000, Huajuapán de León, Oaxaca, Mexico

*Corresponding author: leticia.romero@cinvestav.mx



Engineered nanomaterials have received great attention in recent years due to their diversity of applications, *i.e.*, bioenergies, bioremediation, healthcare and medicine, electronics, and solar energy, among others. Typically, metallic nanoparticles (NP) have been synthesized by physical and chemical methods whose main advantage is the production of large quantities of NP. However, chemical methods are expensive, toxic, flammable, harmful and produce hazardous wastes. A promising alternative to the known conventional synthesis is the biological production of metallic NP. The aim of this work is to critically review several topics on Fe-NPs: (i) the biological pathways or mechanisms involved on iron reduction; (ii) the enzymes that play a key role in microbial synthesis of iron NP; (iii) live biocatalysts involved in iron NP production; (iv) the potential of several organic wastes as electron donors and capping agents in biological synthesis; (v) the application and production of nanoparticles in biorefinery and bioenergy processes with focus on bioH₂-based processes; (vi) progress on evaluation of bio-nanoparticles technology by using tools of system analysis such as life cycle assessment, environmental impact assessment, ecological footprint, etc. Metal NP, specially magnetite, can be synthesized by bacteria, fungi, plants, algae and actinomycetes. Microbially facilitated magnetite synthesis may have numerous advantages, for instance magnetotactic bacteria can produce magnetic nanoparticles with well controlled-shape, size and morphology, and can be easily separated from the matrix by magnets. A wide array of substances such as starch, glucose, ascorbic acid, wood derived, sugar, tannic, *etc.*, have been evaluated regarding their effect on the stability and dispersion of biologically-synthesized NP. The addition of these capping agents on the surface of NP can also avoid deleterious oxidative reactions. Thus, biological processes can effectively produce ferrite particles of stable, active, well-defined nanometer size and fine scaled crystallography. So far, information on evaluation of results of bio-nanoparticles based on system analysis is still disperse and relatively scarce. So, efforts should be made to increase this type of studies because the challenge is not only just to technically optimize particular biological NP methods, but also to validate the whole systems/processes and to determine their effects on the environment, health, and resources as well as the overall sustainability.



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Keywords: bionanoparticles, biorefinery, biosynthesis, iron reducing bacteria, life cycle assessment, sustainability.

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Protonated cross-linked chitosan to remove Cr (VI) from liquid residue

generated in COD analysis



Correa-Murrieta, M.A.^(*), Sánchez-Duarte, R.G.⁽¹⁾, Martínez-Macías, M.R.⁽¹⁾, Villegas-Peralta, Y.⁽¹⁾,
Villa-Coronado, K.A.⁽¹⁾, Dévora-Isiordia, G.E.⁽¹⁾, Álvarez-Sánchez, J.⁽¹⁾

⁽¹⁾ Departamento de Ciencias del Agua y Medio Ambiente, Instituto Tecnológico de Sonora, 5 de
Febrero 818 Sur, Centro, Cd. Obregón, Sonora, 85000, México.

*Corresponding author: maria.correa@itson.edu.mx



The pollution generated in the laboratories of water analysis of the universities is a focus of attention. The COD (Chemical Oxygen Demand) test generates a toxic effluent due to its high content of chromium, mercury, silver and acidity. The objective of this investigation was to remove Cr (VI) from liquid waste generated in COD analysis, using beads of chitosan crosslinked with glutaraldehyde and protonated in acidic medium, in a batch system. Chitosan was obtained by alkaline hydrolysis from shrimp waste. Response surface methodology (RSM) was used to determine the adsorption optimal conditions (amount of adsorbent and contact time). Adsorption isotherm and kinetic essays were carried out, and the data were modeled using different equations. The RSM estimated that 5 g of wet beads, and 12.9 h of contact time are the optimal conditions to reach the highest percentage of adsorption de Cr (VI) on modified chitosan. The pseudo-second order kinetic was the model that best fitted the experimental data, which indicates that chemical adsorption could be the limiting step that controls the adsorption process. Equilibrium data for chromium (VI) removal was best represented by Langmuir isotherm. The maximum adsorption capacities estimated from the Langmuir model were 384.6 mg Cr (VI)/g protonated cross-linked chitosan. From the results obtained, it is concluded that the chitosan modified with glutaraldehyde and protonated in acidic medium, is a sustainable alternative to remove Cr (VI) from the liquid residue generated in COD analysis.

Keywords: Adsorption, CDO waste, chitosan, chromium



Optimizing biohydrogen and volatile fatty acids by dark fermentation of sugarcane vinasse



Felipe Eng*, Lucas Tadeo Fuess and Marcelo Zaiat

Centro de Pesquisa, Desenvolvimento e Inovação em Engenharia Ambiental, Escola de Engenharia de São Carlos - EESC, Universidade de São Paulo – USP, Av. João Dagnone, 1100 - Santa Angelina, 13.563-120 - São Carlos, SP – Brasil

*Corresponding author: felipe.eng@usp.br



Sugarcane vinasse is the main wastewater from ethanol industry. Historically, vinasse has been considered an undesirable byproduct of ethanol distillation and still remains in many producing countries, as wastewater that generates secondary effects such as the contamination of rivers, groundwater sources and seas. One of the alternative treatments of this wastewater is the application of anaerobic digestion, with the consequent production of hydrogen (H₂) and volatile fatty acids (VFA) as intermediates and biogas as final product (1). In this work are presented the results of the optimization of the production of VFA (acetic, butyric and propionic acids mainly) and H₂ by the cultures under conditions of anaerobioses of three vinasses as substrates with an acidogenic inoculum. The treatment was made in bottles of 500 mL, at an initial COD of 10 g.L⁻¹, a pH between 4.5-9.9, with temperature control between 33-60°C and a agitation velocity of 130 r.min⁻¹. The studies were done using first a Box-Behnken design (factorial 33) and then a central composite design (22). Both the AOV and H₂ production were favored using the initial pH of the culture medium 7.5-8.8 and with one of the vinasse tested. However, the production of AOV occurred mainly in the mesophilic range (40°C), but H₂ production was favored in the thermophilic range (50-60°C).

Keywords: hydrogen, volatile fatty acids, anaerobic digestion, sugarcane vinasse

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Application of cellulose of three pine species with recycled fibers to manufacture corrugated paperboard components



Teófilo Escoto García ⁽¹⁾, Janet Jiménez Castro Elvira ⁽²⁾, Héctor Jesús Contreras Quiñones ⁽¹⁾ and Raul Vega ⁽¹⁾

⁽¹⁾ Nogales. Department Predio of las wood Agujas. cellulose Zapopan and paper Jalisco (CUCEI) México., of C.P the 45020. University

⁽²⁾ Faculty of Guadalajara, of chemical km 15.5 engineering road to of the Universidad de Guadalajara., Blvd. Marcelino García Barragán No. 142, Guadalajara Jalisco., México. C.P 44430.

*Corresponding author: tescoto@dmcyp.cucei.udg.mx



The studies concerning the application of cellulose for corrugated paperboard from the Pinus in Mexico, have been relatively few, even when the corrugated paperboard is manufactured both from virgin cellulose fiber (kraft liner) and recycled fibers (test liner and corrugated), so it is important to find a relationship cost-benefit that includes other varieties of pinus and secondary fibers [1]. Based on previous work, it is important to develop studies aimed at evaluating other fibrous sources having feasibility and application in corrugated paperboard [2], currently the total pulps are obtained from wood (virgin pulp) and a lesser proportion of recycled paper [3]. Methodology Sampling and felled pine trees (Pinus douglasiana, Pinus oocarpa and Pinus devoniana) were carried out in field Experimental Forest School of the Department of wood, pulp and paper CUCEI – U de G. The trunks are debarked and splintered in shipper Bruks Mekaniska T 380, and chips were treated in digester Jayme BTR, under a 22 factorial model. The pulps were evaluated by refining curves by methods Tappi. Standard paper type kraft liner, liner test sheets and corrugated with 150, 200 and 100 g/m² respectively, and 60 minutes of refining. Were evaluated using physical and compression test (Tensile and burst index, RCT, CLT, CCT and CMT according to Tappi standards [4]) Result and discussion 60 minutes of refining and treatment T2 was optimal. The pulp of Pinus devoniana and Pinus douglasiana presented better rates of tensile and burst index. With respect to the kraft liner paper, species of Pinus douglasiana and Pinus devoniana were better in index of tension, explosion and lateral compression of the paper (CLT). For corrugated paper and test liner, Pinus devoniana and Pinus douglasiana were also best.

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Synthesis of a based-crude glycerol catalyst

L. Díaz-Jiménez* ⁽¹⁾, S. Carlos-Hernández ⁽¹⁾, A.E. Vega-González ⁽²⁾

⁽¹⁾ *Cinvestav Saltillo, SRNyE, LARR, Av. Industria Metalúrgica 1062, Ramos Arizpe, Coah., México*

⁽²⁾ *Instituto Tecnológico de Saltillo, Av. Venustiano Carranza, Saltillo, Coah., México*

*Corresponding author: lourdes.diaz@cinvestav.mx



Crude glycerol as a by-product of the biodiesel production is an issue that needs to be solved. Due to its low quality, it is difficult to be commercialized and then it should be disposed. When the raw material for biodiesel production is a waste, like residual fats, the problem of the crude glycerol is huge, because it has a lot of impurities. On the other hand, guishe is a lignocellulosic by-product without industrial application; it is obtained from the from the production of a natural fiber known as ixtle¹. In recent years, great attention has been given to the use of lignocellulosic residues by thermochemical technologies ². Then, the synthesis of a catalyst by pyrolysis of guishe and glycerol is proposed. This paper deals with the synthesis of a catalysts by pyrolysis using two by- products as raw material: crude glycerol and guishe in three weight ratios (3:1, 1:1 and 1:3). After that, the catalytic activity of the materials was assayed through the transesterification reaction of soybean oil. The catalysts were characterized by DRX, TGA-TDA, FTIR and elemental analysis. The catalytic activity assays were carried out using commercial soybean oil and methanol (molar ratio 6:1, 65 °C), with 3 w% catalyst. The reaction was verified in a three-necked round flask at atmospheric pressure. The acidity of the oil was determined by volumetric titration. The product analysis of the reaction was done by gas chromatography coupled to mass spectrometry. The results showed than the three catalysts were active in the transesterification reaction; however it was observed differences in yield and selectivity for biodiesel production. The activity of catalysts decrease with the ratio crude glycerol/guishe as follow 1:3>1:1>3:1. It is concluded that the catalyst obtained from crude glycerol and guishe are an alternative for the revalorization of these by-products.

Keywords: ixtle waste, crude glycerol, biochar, biodiesel.

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Effect of cellulose acetate on the mechanical properties of thermoplastic

corn starch molded by extrusion-injection



Miguel A. Pablo Calderón ⁽¹⁾, Ángel Hernández Martínez ⁽²⁾, Miguel Chávez Gutiérrez ⁽³⁾
⁽¹⁾ Instituto Politécnico Nacional; CIIDIR-Oaxaca, Santa Cruz Xoxocotlán, Oaxaca. C.P. 71230
⁽²⁾ Universidad Autónoma de México; CFATA-Juriquilla; Juriquilla, Querétaro. C.P. 76230.
⁽³⁾ Instituto Politécnico Nacional; CIIDIR-Oaxaca, Santa Cruz Xoxocotlán, Oaxaca. C.P. 71230

*Corresponding author: mpabloc@alumno.ipn.mx



Composite materials' properties are influenced by the intrinsic properties of their constituents such as the characteristics of the interface and the volume fraction¹. Cellulose acetate (CA) is an amorphous material that can influence the mechanical and thermal properties of thermoplastic corn starch (TPS). For this work, mixtures of TPS with CA were prepared in the proportions 100-0%, 80-20%, 60-40%, 40-60%, 20-80% and 0-100% by weight respectively. The extrusion was carried out in an Xplore brand equipment, with a temperature ramp of 170-175-180 °C, processing speed 400 rpm, barrel temperature 180 °C, mold temperature 100 °C, piston pressure hydraulic 10 Bar and injection time 16 seconds. The samples were characterized mechanically by stress-strain tests in a Universal machine performing stress and bending tests. Through the stress-strain tests it was found that the addition of CA in the continuous phase of TPS undergoes an increase in its properties as the modulus of elasticity increasing from 0.21 to 20.87, maximum stress at the tension of 3.10 to 84 mPa and, for the case of elongation, a decrease from 100.55% to 4.7%. That it to say that it went from a flexible material, little resistant to tension and flexion, tenacious and of viscoelastic behavior, to a material resistant to tension, rigid, fragile and with some resistant to bending, due to the lack of interfacial adhesion between phases of miscible type and possibly to the alignment of a high percentage of the chains in the mixtures of immiscible type resulting from the extrusion-injection molding. X-ray diffraction analysis showed the amorphous nature of the mixtures, this due to the loss of crystallinity caused after mechanical disruption and phase transition resulting from the shearing forces inherent to extrusion processing. Infrared spectroscopy in attenuated total reflectance mode (FTIR-ATR) showed the variation in functional groups such as carbonyl.



Biological production of graphene hydrogels for electrode applications

in bioelectrochemical systems



Prados M.B.*⁽¹⁾, Lescano M.⁽¹⁾, Barclay J.⁽¹⁾, Gasnier A.⁽²⁾, Pedano M.L.⁽³⁾, Sica M.⁽¹⁾, Curutchet G.⁽⁴⁾

⁽¹⁾ Instituto de Energía y Desarrollo Sustentable, Centro Atómico Bariloche (CAB), Comisión Nacional de Energía Atómica (CNEA), Av. E. Bustillo 9500, S. C. de Bariloche, Argentina

⁽²⁾ Gerencia de Investigación Aplicada, CAB, CNEA, S. C. de Bariloche, Argentina

⁽³⁾ Laboratorio de Fotónica y Optoelectrónica, CAB, CNEA, S. C. de Bariloche, Argentina

⁽⁴⁾ Instituto de Investigaciones e Ingeniería Ambiental y Escuela de Ciencia y Tecnología, Univ. Nac. De San Martín, Buenos Aires, Argentina.

*Corresponding author: mbprados@cab.cnea.gov.ar



Bioelectrochemical systems (BES) represent a promising technology to turn organic pollutants present in wastewater into a renewable source of energy. These systems are based on electrogenic microorganisms. Currently, the development of this technology requires the optimisation of the electrodes. New materials such as graphene, a single-atom thick 2D carbon nanostructure, are being investigated for BES electrode applications. We have recently demonstrated that graphene hydrogel (GH), prepared by self-assembly of graphene oxide (GO) through chemical reduction, are suitable to develop dense and conductive biofilms of *Geobacter sulfurreducens*. In addition, we observed that this bacterium was capable of further reducing the graphene. Therefore, with the aim of developing a sustainable method for the production of GH, in this work, we tested the capability of a pure culture of *G. sulfurreducens* and an electrogenic bacterial consortium to reduce GO, inducing the formation of a hydrogel. The bacterial consortium was previously isolated from the Reconquista river (Buenos Aires, Argentina) sediment. Bacterial cultures were inoculated (10%) in anaerobic culture media (N₂:CO₂, 80:20) with acetate, as electron donor, and incubated at constant temperature (30^o C). Different conditions of electron acceptor were tested: GO 1 mg/ml or GO 1 mg/ml plus fumarate 50 mM. We followed the reduction process by visual inspection, to avoid disruption of the forming hydrogel. After 1 month, the hydrogels were characterized by scanning electron microscopy (SEM), Raman and Fourier Transformed Infrared Spectroscopy (FTIR). At 72 h, the bacterial consortium was reducing GO (the suspension changed its colour from brown to black). The formation of a hydrogel was evidenced at 10 days, when the consortium was grown in the presence of fumarate. In the absence of fumarate, a colloidal reduced GO was observed even after 30 days of culture. *G. sulfurreducens* was capable of inducing the formation of a hydrogel only after 90 days of culture. SEM images of the GH showed a 3D-architecture with interconnected macroporous, similar to the chemically produced GH. The Raman and FTIR spectrum confirmed the reduction of GO by bacteria. In conclusion, GH production can be effectively achieved with electrogenic bacteria. Importantly, the fastest rate observed is similar to the time required for chemical production of hydrogels. The GH is mechanically stable and easy to handle, an important requisite to consider the material for electrode applications.

Keywords: Bioenergy, Bioremediation, Electrogenic microorganisms, Graphene Oxide.



Fluorescent protein from *Stomolophus* sp. 2: an extreme pH indicator protein

Martínez-Pérez, R.B.⁽¹⁾, Rodríguez, J.⁽²⁾, Rentería-Mexía, A.M.⁽¹⁾, Díaz-Tenorio, L.M.^{(1)*}

⁽¹⁾ Laboratorio de Biotecnología de Organismos Acuáticos, Instituto Tecnológico de Sonora (ITSON), Av. Antonio Caso 2266, 85137, Cd. Obregón, Sonora, México.

⁽²⁾ Laboratorio de Biocatálisis, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C (CIATEJ), Camino Arenero 1227, El Bajío, 45019 Zapopan, Jalisco, México.

*Corresponding author: lourdes.diaz@itson.edu.mx



Medusae are a diverse group of planktonic predators, they live in virtually all marine habitats, they are invertebrates and osmoconformers. Since a few years some blooms of these organisms were happening in the world, and that can be a problem because they are predators from fish, crustacean, and mollusk eggs and larvae, so it is important to catch, and to approach them. Some researchers have suggested that their proteins might be used for different biotechnological proposes (as biosensors, catalytic agents, toxins). In Mexico, cannonball jellyfishes are processed to be sold as food in Asian countries. This species and its proteins are not well-know, thus their study is necessary. The aim of this work was to obtain a native fluorescent protein from *Stomolophus* sp. 2 to evaluate the stability and pH response. Fluorescent protein was obtained from water waste of thawed whole body organism. Protein extract from jellyfish waste was precipitated with an acetone solution, after precipitation, the protein was re-solubilized with buffer MOPS, then hydrophobic interchange chromatography was used as purification step. Pure fluorescent protein (λ_{em} : 380 nm, λ_{ex} : 280 nm) can fluoresce with more intensity at extreme acid and alkaline pH (2 and 12), 6782 and 6183 RFU (Relative Fluorescence Units) at 25 °C, respectively. After incubation (24 h, 25 °C) the fluorescence was decreased in almost 65%, but still it can be detected. The extreme characteristic of fluorescent protein from *Stomolophus* sp. 2 can be used as bio-indicator for the rapid analysis of industrial wastewater from paper, sulfuric acid, among other industries with alkaline o acidic residues.

Keywords: Biosensor, fluorescent protein, jellyfish, wastewater indicator.

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Physical-Chemical Characterization Sabila Biopolymer (Aloe vera)



Gálvez Chan R .A* ⁽¹⁾, Alvarez Sánchez J ⁽¹⁾, Silva Encinas G. O ⁽¹⁾, López Cuero I ⁽¹⁾, Paredes Gálvez P. A ⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, Av. Antonio Caso, N° 2266, Ciudad Obregón, México.

*Corresponding author: rosario.galvez@itson.edu.mx



Nowadays, search for new and better alternatives to obtaining different polymers that benefit to humanity but also the environment. Recycling is an option, but it is not a very effective alternative for all plastics, however, biopolymers are a solution from the source of the problem because the vast majority of them their biodegradation time is much less than a common plastic. The biopolymers, which mostly come from renewable resources, becomes in an interesting alternative for the plastics industry. The objective of the present investigation, is the Chemical-Physical characterization of a sabila based biopolymer (aloe vera) to determine its possible applications (1). The tests carried out for the characterization were: permeability, solubility, resistance, thickness, drop test and chemical analysis with the infrared spectrophotometer, giving favorable results for industrial use. The physical-chemical properties of the biofilm of aloe, are similar to synthetic plastics, being a viable alternative to replace them, thus contributing to society with a better a quality of life by being friendly to environment.

Keywords: Aloe Vera, Biodegradation, Biopolymer, Characterization.

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Adsorption of Cu, Fe and Zn by an extruded matrix of biodegradable polymers



M. Martínez^(1*), J. Rangel⁽²⁾, M. Gimeno⁽³⁾, K. Shirai.^(1*)

^(1*) Universidad Autónoma Metropolitana, Biotechnology Department, Av. San Rafael Atlixco, No. 186, Mexico City, Mexico

⁽²⁾ Instituto Potosino de Investigación Científica y Tecnológica A.C., Division of Environmental Sciences, Camino a la Presa San José, No 2055, San Luis Potosí, Mexico

⁽³⁾ Universidad Nacional Autónoma de México, Department of Food and Biotechnology, Investigación Científica, No 70, Mexico City, Mexico

*Corresponding author: smk@xanum.uam.mx



Heavy metals as water contaminants can hardly be biodegraded and will gradually accumulate in living organisms which may cause serious environmental and health problems. The elimination of toxic metal ions from contaminated water before discharge is consequently in great demand¹. Chitosan (Ch) is a natural copolymer consisting of N-acetyl-glucosamine and D-glucosamine. The presence of a significant number of amine groups distributed along its polymeric chain substantiates Ch in the form of a polyanion to neutralize and remove various metallic ions from effluents². The lactic-acid fermentation of shrimp waste followed by acid and alkali treatments to remove residual ash, proteins, and acetyl groups employed for Ch production.³ Ch powder were mixed with a thermoplastic material and extruded as cylindrical pellets. Potentiometric titrations, FTIR and XRD, characterized the extruded materials (MCP). Batch adsorption experiments were carried out in 10mL flasks with Cu, Fe and Zn solutions at several concentrations, adding 0.0125g of the MCP. The adsorption data followed a Pseudo second order and fitted to the Freundlich model. The maximum adsorption capacity of Cu, Fe, and Zn onto MCP was 4 mg/g, 10.72 mg/g, and 165 mg/g respectively. The adsorption efficiencies rise up to 40% (Cu), 48.7% (Fe) and 49.5% (Zn).

Keywords: Chitosan, Heavy Metals, Adsorption, Wastewater

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Influence of nitrate reductase enzyme over the biosynthesis of silver nanoparticles by white rot fungi *Bjerkandera* sp. Anamorph R1

J. Osorio Echavarría^{*(1)}, N.A. Gómez Venegas⁽¹⁾, J. Osorio Echavarría⁽¹⁾, C.P. Ossa Orozco⁽²⁾,
⁽¹⁾ Grupo de Bioprocesos, Universidad de Antioquia, Calle 67 Número 53-108, Medellín, Colombia
⁽²⁾ Grupo de Biomateriales, Universidad de Antioquia, Calle 67 Número 53-108, Medellín, Colombia
* Corresponding author: jeronimo.osorio@udea.edu.co.



In recent years, silver nanoparticles (AgNPs) have shown to be an important tool in the development of biomedical devices. For this reason, the study of their production methods is of special interest. In this way, this research proposes a synthesis of nanometals from microorganisms as a new method, taking advantage of the metal ion reducing capacity through their enzymatic system. In this work, the synthesis of AgNPs was evaluated using the bio-reduction potential of the Nitrate Reductase (NR) enzyme from the white rot fungi *Bjerkandera* sp. Anamorph R1 towards their implementation in biomedical applications. During this evaluation, at the end of the stationary phase the production of the NR enzyme was detected, and exhibited its maximum production rate on the 8th day of fungal growth. By using the UV-Vis and Scanning Electron Micrograph spectra, it was possible to corroborate that the best enzymatic conditions yielded the best synthesis conditions. It was also possible to confirm that the mechanism of reduction of the Ag⁺ ion in Ag⁰ nanoparticles is carried out by this enzymatic way. These results are promising as the addition of this type of biological AgNPs on polymeric matrices can enhance the antimicrobial efficacy of these compounds, which makes them an ideal substitute in the fabrication of wound dressings.

Keywords: Silver Nanoparticles (AgNPs), Nitrate Reductase (NR), White Rot Fungi, *Bjerkandera* sp. Anamorph R1.



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Chapter 5 Microbial Ecology and Molecular Biology



Microbial community composition of biofilm developed on a zeolite fixed bed anaerobic bioreactor to treat nickel contaminated water

K. Calderón^{1*}, G. Bustamante-Noriega², V.M Reyes-Martínez², R.G Martinez-Meza², F.J Almdariz-Tapia² and M.T Certucha-Barragán²

⁽¹⁾ Department of Scientific and Technological Research (DICTUS), University of Sonora. Edificio 7G, Blvd. Luis Donaldo Colosio s/n. Col. Centro, C.P. 83000 Hermosillo, Sonora, Mexico

⁽²⁾ Department of Chemical and Metallurgic Engineering. University of Sonora. Edificio 5C, Blvd. Luis Donaldo Colosio s/n. Col. Centro, C.P. 83000 Hermosillo, Sonora, Mexico

*Corresponding author: kadiya.calderon@unison.mx



The electroplating industry is one of the most important heavy metal sources which discharges and contaminates environmental systems. The high concentrations of nickel in water may cause several damages on all types of living organisms ^[1]. Anaerobic wastewater treatment offers an excellent option to remove heavy metals as nickel at the same time that offers the recovery energy advantage of methane production ^[2]. The aim of this work was to evaluate the microbial community composition of developed biofilm as bio-adsorbent associated to zeolite fixed bed anaerobic bioreactor to treat 500 ppm of Nickel (Ni²⁺) contained in real industrial wastewater. Microorganisms that colonized zeolite demonstrated an optimal performance in a pH interval of 6.4 to 7.0; COD was evaluated and 50 % of organic matter was removed. As well, 99 % of nickel removal and high methane production (200 mL/day) were achieved, representing an important amount considering the lab-scale bioreactor capacity. High-throughput sequencing analyses demonstrated differences on the microbial community composition developed throughout the anaerobic bioreactor treatment, revealing shifts in microbial dynamics between *Thermotogae* and *Bacilli* classes. This result could suggest that the microbial community developed on zeolite plays an important role for heavy metals removal as Ni²⁺, allowing high methane recovery. Moreover, SEM-EDS analyses were performed showing clearly the biofilm structure and demonstrating that Ni²⁺ may be metabolized and not bio-accumulated by the biofilm microbial community.

Keywords: microbial community, nickel removal, anaerobic bioreactor, zeolite.

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***Pedobacter* sp.3.14.7 a keratinolytic bacteria that produces a feather**

protein hydrolysate with high antioxidant activity



B Bezus⁽¹⁾, F Ruscasso⁽¹⁾, G Garmendia⁽²⁾, S Vero⁽²⁾, **G Curutchet**⁽³⁾, S Cavalitto⁽¹⁾, I Cavello^{(1)*}.
⁽¹⁾ CINDEFI (UNLP, CONICET – La Plata). 50 y 115, La Plata, Bs As - Argentina.

⁽²⁾ Cátedra de Microbiología, Departamento de Biociencias, Facultad de Química, Universidad de la República, Montevideo, Uruguay.

⁽³⁾ Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, Buenos Aires, 1650, Argentina and CONICET

*Corresponding author: icavello@biotec.quimica.unlp.edu.ar



Animal wastes generated in the meat industry contain considerable amounts of insoluble and hard-to-degrade structural proteins like collagen, elastin and keratin, which are major constituents of bones, organs and hard tissues. These byproducts are often rich sources of protein, which can be extracted and hydrolyzed to be used as feed or functional ingredients. The large amount of waste feathers generated each year by commercial poultry plants creates a serious environmental problem. Disposal strategies like burning in incineration plants and landfilling are restricted or banned in many countries. Currently, some industries produce feather meal from waste feathers by steam pressure cooking, which require high-energy input. The resulted feather meal is deficient in methionine and histidine which limit its use in animal feed. Nutritional enhancement of feathers can be achieved by hydrolysis with feather-degrading microorganisms. Hydrolysis of keratinous wastes by microorganisms possessing keratinolytic activity represented an attractive alternative method for their efficient bioconversion and improving their nutritional value. The objective of this study was to investigate the capability of *Pedobacter* sp. 3.14.7 – a novel Antarctic keratinolytic bacterium- to produce a feather protein hydrolysate (FPH) with high antioxidant activity. The antioxidant potential of feather protein hydrolysate were evaluated using in vitro antioxidant assays, such as (ABTS) radical scavenging activity and reducing power. The strain 3.14.7 showed the ability to degrade raw feather to FPH under optimal conditions of 30 g/l chicken feathers, pH 6.0 and 72 h of fermentation at 20°C. At these conditions, quenching of the ABTS radical by FPH reached approximately 90%, with an IC₅₀ value of 0.7±0.01 mg/ml. Reducing power of the FPH demonstrated to be concentration dependent. Its value increased with the higher FPH concentration as it was reported by Mukseh-Kumar et al., 2012. They reported that 2 mg/ml of FPH produced an increase of 1.5 of absorbance at 700 nm while *Pedobacter* sp.'s FPH produced an increase of 2.6 of absorbance showing high content of aminoacids and peptides. These results indicate that FPH may be useful as supplementary protein and antioxidants in animal feed formulations.

Keywords: Antioxidant-Feather Protein Hydrolysate-Keratinase



Heavy metal and petroleum hydrocarbons removal efficiency of chitin

powder and flakes



M.E. Pérez-Cruz⁽¹⁾, A.C. De León-Cisneros⁽¹⁾, S. García-López⁽¹⁾, I.E. Jacobo-Herrera⁽¹⁾, A.C. Campa Mada⁽²⁾, A.R. Toledo⁽²⁾, M.A.G. Corella Madueño⁽³⁾, **K.G. Martinez- Robinson⁽³⁾***

⁽¹⁾ BIOSAN, 29247 San Cristóbal de las Casas, Chiapas, México.

⁽²⁾ Centro de Investigación en Alimentación y Desarrollo, AC. Carretera a La Victoria Km. 0.6.83304 Hermosillo, Sonora, Mexico

⁽³⁾ Universidad de Sonora. Blvd. Luis Encinas y Rosales s/n. 83000 Hermosillo, Sonora, Mexico.

*Corresponding author: karlaqm@ciad.mx



In this work, the effectiveness of chitin (Qn) powder and flakes for the removal of heavy metals (lead (Pb), copper (Cu) and zinc (Zn)) and petroleum hydrocarbons was compared. A sieve-type work was carried out for both heavy metals and hydrocarbons; the flake size greater than 5 mm was considered and for the ground and sieved Qn the particle size was less than 3 mm. For the removal of heavy metals, a synthetic sample prepared from standard solutions with an approximate concentration of 25 mg/L \pm 2 mg/L, pH between 5.9-6.2, and certified by the National Metrology Center of Mexico (CENAM) was used. For the removal of the hydrocarbons, a lubricating oil for gasoline engine SAE 40 was firstly analyzed, and later samples of Arenque light crude oil and Mana medium crude oil. For chitin powder (QnP), the percentages of heavy metal removal were 92.31% for Pb, 90.63% for Cu and 38.45% for Zn. In the case of Qn flakes (QnH), the metal with the highest percentage of absorption was zinc (98.80%), followed by Pb (98.19%) and Cu (92.96%). The analysis of variance shows that there is no significant difference ($p \leq 0.05$) between QnP and QnH for Cu and Pb. Regarding the results of the removal of hydrocarbons, it was observed that in a greater amount (10 g) of oil SAE 40 the removal percentage was less (32.05%) than in a smaller amount (1 g) of oil SAE 40 (55.5%). In aqueous solution, QnH removed up to 93.25% of 1 g of oil SAE 40, while QnP removed up to 92.67% of Arenque oil (5g) and 57.95% of Mana oil (5g). It can be concluded that both chitin powder and flakes were found to be effective in removing hydrocarbons from petroleum as well as heavy metals (Pb), which causes pollution and damage in our environment.

Keywords: Chitin, heavy metals, petroleum hydrocarbon, oil.



Recovery of nickel cadmium and cobalt from lixiviates of Disposal

batteries through electrodeposit in a parallel Plate reactor



Liliana Altamirano-Garcia^{*(1)}, Vianey González Romero⁽¹⁾, Edgar Pérez Diaz⁽¹⁾, Rosa María Luna Sánchez⁽²⁾

⁽¹⁾ Universidad Mexiquense del Bicentenario, Unidad de Estudios Superiores Tultitlán, Av. Ex-Hacienda de Portales s/n, Col. Villa Esmeralda, C.P. 54910, Tultitlán, Estado de México.

⁽²⁾ Universidad Autónoma Metropolitana Azcapotzalco. Depto. Energía. Av. San Pablo, No. 180, 02200, México, D.F., México.

*Corresponding author: l.altamirano@umb.mx



In this paper we present the recovery study of metals (Ni, Co, Cd) from spent Ni-Cd batteries using an electrochemical press filter type reactor. In the literature, several phenomena have been reported that can affect the moment in which these metals are deposited [1], which are mainly: a) parasitic reactions; b) formation of oxides and hydroxides that pass through the surface of the cathode, due to the change of interfacial pH; and c) change of ph in the bulk of the solution. This has a considerable influence on the efficiency of the current, and for economic reasons, which is an important factor in determining the time necessary to exhaust the metallic species, the aforementioned drawbacks must be minimized. The results indicate that the utility of a two-compartment reactor separated by an anionic membrane, which has probably been reduced to the speed of water stream surface pH. Another important factor is the decrease in the pH fluctuation of the bulk, due to the separation of the cathodic and anodic reactions, thus contributing to the reduction of the reagent consumption that must be updated to the bath to maintain the pH constant. The results show that cadmium completely inhibit the deposition of nickel, thus obtaining deposits of selective metals.

Keywords: electrodeposition, cadmium, nickel

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Effect of Pesticides and Chemical Fertilizers in Soil

Attia. R ⁽²⁾, Chourabi.H ⁽²⁾, Sifaoui.K ⁽²⁾, Hajlaoui.H ⁽¹⁾

⁽¹⁾ *Coopération Tuniso Amande GIZ rue Alain Savary Tunis , Tunisie*

⁽²⁾ *Direction des Ressources en Sols DGAFTA, 17 rue Hédi Karray Ariana 2080 Tunis, Tunisie*

*Corresponding author:: attiarafra@yahoo.fr



Tunisian agriculture has a duty of food security and a crucial role in stability, social cohesion and economic development. It will have to produce more and better in a context strongly constrained by climate change, the scarcity and increasing degradation of natural resources (soil, water and biodiversity), socio-economic difficulties and soil pollution by chemical fertilizers. The notion of Sustainable Agriculture aims at setting up a viable production system while preserving and valuing natural resources, limiting the impact of the use of products (pesticides, mineral fertilizers, etc.) on soil resources. Environmental protection encourages the reduction of the use of chemical fertilizers and synthetic biocides. So Organic agriculture represents, first and foremost, a weapon for the environment with first rank, the preservation of biodiversity. In an arid climate it is also a mode of production that allows both to save water and not to pollute it thus to preserve the soil and the atmosphere. The objective of the project is i) optimization of fertilization from farm manure and composts to minimize environmental risks, better integrate biological components that favorably affect the functioning of cultivated ecosystems ii) adoption of good practices in production systems in order to identify constraints related to this theme; iii) assess soil management procedures through observations, surveys and analyzes. By renouncing synthetic inputs (phytosanitary and veterinary products) the Farmer is exposed to a variability of yields, due to increased technical risks. The technical framework is a major stake allowing the adaptability of the organic farmer to the local variations: types of soil, speculation, climate, water ... It would be necessary to help the farmers to have a better knowledge of the needs of the practice and the use fertilizers related to productivity. The importance of addressing the cumulative effect of pesticides and chemical fertilizers on soil, water and soil resources Material and methods: the study is based on the current situation of the soils in the 7 governorates of the PAD by means of an observation and a reading of the landscape by governorate, the different types of soil, the Good agricultural practices (BPA) already applied by the farmers and to discuss the project idea and planned activities, land-related constraints, soil management methods and input use in relation to productivity and farm outcomes. - the needs of farms in training, field schools and awareness days etc. To expose the current state and challenges of soil fertility by governorate (7 chiefs of district soil). Determination of the fertilization program according to the specificities of water and soil, dissemination of information organization of technical or information days. Actions for experimentation and technical itinerary development are recommended on the base of Setting up pilot farm: a demonstration tool(reference). Dissemination of technical documents, and educational support. Various attempts to amend different types of organic matter (bovine manure, sheep manure + pruning wood, pruning wood, crop residues) on different farming systems were tested. The organic matter rate rose for the four experimental plots after three years in the Nefza AM1 Governorate from 2 to 4.07%, AM2 1.68 to 2.89%, AM3 2.11 to 3.64%, 1.79 at 3.09%. More results are obtained and recommended for famers depending various situation.

Keywords: fertility, organic management, good practices



boards (WPCBs) through bioleaching

A. Gonzalez Baez* ⁽¹⁾, D. Purchase ⁽¹⁾, H. Garelick ⁽¹⁾, L. Pantoja Muñoz⁽¹⁾

⁽¹⁾ Middlesex University, The Burroughs, London, UK

*Corresponding author: a.gonzalezbaez@mdx.ac.uk

Waste electric and electronic equipment (WEEE) is one of the fastest growing waste streams in the world, every year more than 40 million tonnes are generated and only a minor part is legally collected and recycled. Printed circuit boards (PCBs) are part of everyday items like mobile phones and computers, and they are a significant proportion of e-waste. PCBs contain hazardous components but also valuable and critical materials (e.g. copper, gold, silver, rare earth elements). Rare earth elements REEs (e.g. lanthanide, neodymium, cerium) are crucial to modern life hardware and due to their increasing demand and high supply risk, they are now considered one of the most critical elements in the world. The aim of this research is to investigate the recovery of REEs from waste PCBs (WPCBs) using an environmentally-friendly alternative, such as bioleaching, which exploits the ability of microorganisms to recover metal ions from the waste matrix. Recovery of REEs from secondary sources like electronic waste has the potential of minimising environmental impacts from traditional mining technologies, supporting preservation of primary sources and avoiding future scarcity problems. WPCBs were supplied by a local recycling company after crushing process. Physical characterisation of the material is done, as well as metal analysis by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Mass Spectrometry (ICP-MS). Fungi isolates will be used in the bioleaching experiments. Analysis of microbial mechanisms involved in the process includes High-performance Liquid Chromatography (HPLC). Response surface methodology will be applied to identify the most important parameters in REEs bioleaching and to optimize the process. After physical separation of the waste material, metal characterization showed that REEs like Lanthanum, Yttrium and Neodymium, are mainly present in the smaller particle sizes (<0.5mm – 1mm), where other metals like copper, gold and silver are less concentrated. This is particularly important as bioleaching has been proved more effective in small particle sizes and lower amounts of toxic elements like copper and silver are preferred. This research project would improve the understanding of microbial mechanisms regulating REE bioleaching from e-waste and the critical variables involved in the process, which will be essential for future work assessing the viability of this sustainable approach on a large scale.

Keywords: Bioleaching, Rare earths, PCBs, Recycling.



Jatropha seed cakes by Solid State Fermentation



Y. Maiga*⁽¹⁾, S. Roussos⁽²⁾, H. Macarie⁽²⁾, A.S. Ouattara⁽¹⁾

⁽¹⁾ Laboratory of Microbiology and Microbial Biotechnology, University Ouaga 1 Pr Joseph Ki-Zerbo, 03 BP 7021 Ouagadougou 03, Burkina Faso

⁽²⁾ Aix Marseille Univ, Univ. Avignon, C NRS, IRD, IMBE, Marseille

*Corresponding author: youssef.maiga@gmail.com



Because of energy deficit, Burkina Faso is experimenting biofuel production from *Jatropha curcas* (MMQE, 2009). This activity generates biomass waste made of hulls, and cakes that need to be eliminated (Devappa et al. 2010). *Jatropha* seed cakes (500 to 800 kg per ton of seeds) cannot be used as dietary supplement for livestock because of the presence of antinutritional (phytic acid, cursines etc.) and toxic compounds like phorbol esters. In order to safely eliminate and/or valorize this biomass residue, a study was conducted to isolate fungal strains from food (strains GRAS) and environmental samples, and test their capabilities to resist to the toxicity of the cakes. Nine strains were isolated from food (cheese) samples and seven from environmental samples (*Jatropha* seed cake, cotton seeds, cotton seed cakes, wheat bran and litter). The apical growth was determined for each strain as well as the production of spores using commercial Potato Dextrose Agar. The phenotypic characteristics were also determined through microscopic observation of the mycelium and the spores. The GRAS strains belonged to the genus *Penicillium* while *Rhizopus* like and *Aspergillus* were isolated from the environmental samples. All the strains were able to grow on synthetic medium made of *Jatropha* seed cake and agar highlighting their ability to resist to its toxicity. Besides, when *Jatropha* seed cake was supplemented with sugarcane bagasse (80/20) and used as a substrate in Solid State Fermentation (SSF), all the strains were able to grow well and produce spores. Therefore, the tested strains can be used for SSF of *Jatropha* seed cakes for its valorization in livestock feeding or the production of valuable substances.

Keywords: Fungi, *Jatropha* seed cakes, Solid state Fermentation.

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Chapter 6 Applications to Environmental Problems



Isolation of metallotolerant microorganisms from the San Pedro River,

Cananea, Sonora, for use as Cu and Mn biosorbents



A Alvarez Villa^{*(1)}, F J Almendariz Tapia⁽¹⁾, A M Pat Espadas⁽²⁾, M Plascencia Jatomea⁽³⁾, M T Certucha Barragán⁽¹⁾

⁽¹⁾ Departamento de Ingeniería Química y Metalurgia, Universidad de Sonora, Blvd Luis Encinas y Rosales Col. Centro, CP 83000, Hermosillo, México

⁽²⁾ Estación Regional del Noroeste, Instituto de Geología UNAM, Blvd Luis Donaldo Colosio s/n Col. Los Arcos, CP 83250, Hermosillo, México

⁽³⁾ Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Blvd Luis Encinas y Rosales Col. Centro, CP 83000, Hermosillo, México

*Corresponding author: alvarezvillaa@gmail.com



Due to the presence of heavy metals in bodies of water in Mexico, it is important to carry out studies that allow their removal. Metallotolerant microorganisms have properties for biosorbing heavy metals and can be found in acidic environments with high concentrations of metals. In the present study, the microorganisms that showed greater tolerance to Cu and Mn from three zones of the San Pedro River near mining activities in Cananea Sonora were isolated and the range of tolerance to Cu and Mn was determined based on the minimum inhibitory concentration (MIC). Three samples of river sediment were taken in sterile 500 mL containers to inoculate in solid culture medium enriched with Cu and Mn, the microorganisms that were developed at the highest concentrations were selected for identification. The MIC range for Cu and Mn in solid culture medium was obtained based on the count of the colony forming units (CFU) in media with 0, 200, 400, 600, 800 and 1000 mg L⁻¹ of metal to determine the concentrations where there was no visible growth. The metallotolerant microorganisms of each zone were identified as: *Pseudomonas rhodesiae* primer 16S 100% identity, *Bacillus cereus* primer 16S 99% identity and *Rhodotorula taiwanensis* primer 26S 99% identity. The MIC range for *Pseudomonas rhodesiae* was 600-800 mg L⁻¹ of Cu and 400-600 mg L⁻¹ of Mn, for *Bacillus cereus* was <200 mg L⁻¹ of Cu and 400-600 mg L⁻¹ of Mn, for *Rhodotorula taiwanensis* was 600-800 mg L⁻¹ of Cu and 800-1000 mg L⁻¹ of Mn. The three isolated microorganisms showed tolerance to high concentrations of Cu and Mn, so they are viable to be used in a system constituted by biomass of tolerant microorganisms for the removal of Cu and Mn present in contaminated waters. With the results of the MIC, the ranges were determined where the concentrations in which the isolated microorganisms are not able to develop are found, which provide an idea on how the concentration of Cu and Mn affects the microbial ecology.

Keywords: heavy metals, metallotolerant microorganisms, minimum inhibitory concentration (MIC)



**Microbial diversity associated with soil in the wild chiltepin (*Capsicum
annuum* L. Var. *Glabriusculum*) in the state of Sonora.**



M. Clark¹, K. Calderón⁽¹⁾, L.F García-Ortega⁽²⁾, L. Mondragón-Camarillo⁽²⁾, L.A. Medina-Juárez⁽¹⁾, R. Vilchez-Vargas⁴ and A.C. Hayano-Kanashiro⁽¹⁾

⁽¹⁾ *Department of Scientific and Technological Research (DICTUS), University of Sonora. Edificio 7G, Blvd. Luis Donaldo Colosio s/n. Col. Centro, C.P. 83000 Hermosillo, Sonora, Mexico.*

⁽²⁾ *Division of Molecular Biology, Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, Mexico.*

⁽³⁾ *UBIBPRO, Facultad de Estudios Superiores Iztacala, UNAM. Mexico*

⁽⁴⁾ *Department of Gastroenterology, Hepatology and Infectiology. University Hospital Magdeburg.*

Germany

*Corresponding author: kadiya.calderon@unison.mx

The Chiltepin (*Capsicum annum* L. var. *glabriusculum*) is considered as semi-domesticated or wild species and as progenitor of the cultivated chili peppers. It is a chili of prominent genetic, biological and commercial value in the State of Sonora. The aim of this study was to analyze the microbial diversity of the soil associated to wild chiltepin and their cultivation, according to its location and season of the year. Soil samples were collected during the dry, rainy and the post-rainy season of three Sonoran municipalities; San Pedro el Saucito, Banámichi and Moctezuma. Preliminary results based on the analysis of the phenotypic characteristics of the soil cultivable microbial communities showed colony forming units (CFU) making up a core group of microorganisms within the soil samples. In addition, different phenotypical colonies were observed, demonstrating significant differences based on certain groups of microorganisms depending on location and season performed. Moreover, pH and humidity of soil were analyzed. San Pedro el Saucito demonstrated a clear trend towards a strongly alkaline soil, on the other hand, Banámichi and Moctezuma were characterized by a slightly acidic trend. As well, quantification of inorganic nitrogen pool in soil were evaluated, detecting differences in ammonia, nitrate and nitrite between each evaluated season, showing a significant difference that suggests differential effects on the nitrogen cycle in soils associated to chiltepin harvests, in conclusion, this work will help to know the composition of the soil microbiota associated with the chiltepin plant since they have a fundamental role in the processes of the ecosystems that drive the biogeochemical cycles.

Keywords: CFU, chiltepin, nitrogen cycle, soil



Composition and structure of the rhizospheric bacterial communities

associated to wild and greenhouse “sweet biznagas”



ME de la Torre-Hernández ⁽¹⁾, LI Salinas-Virgen⁽¹⁾, *, JF Aguirre-Garrido⁽²⁾, HC Ramírez-Saad⁽¹⁾.

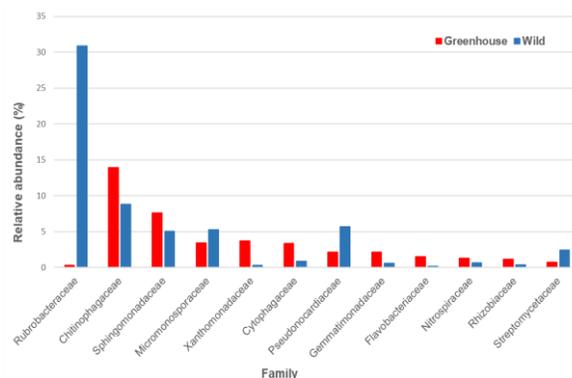
⁽¹⁾ Universidad Autónoma Metropolitana-Xochimilco, Calzada del Hueso 1100, 04960, CdMx, México

⁽²⁾ Universidad Autónoma Metropolitana-Lerma, Av. Hidalgo Pte. 46, Col. Lerma de Villada, 52006, Edo. de Mex., México

* Corresponding author: mdelatorre@correo.xoc.uam.mx

In the semi-desert of Querétaro, around a third of the local cactus species are in some category of threat to their survival, as is the case of the sweet biznaga *Echinocactus platyacanthus*, that has been overexploited to produce the popular sweet “acitrón”. Furthermore, the propagation of this plant is difficult due to its slow growth and low establishment rate when it is reintroduced into its natural habitat. A useful measure to overcome this limitation is the use of plant growth promoting rhizobacteria (PGPR), which may contribute to its development and reproduction. The objective of this work was to assess under a metataxonomic approach, the composition and structure of the bacterial communities associated to the rhizosphere of *E. platyacanthus*, growing in wild and greenhouse conditions. Metagenomic DNA was extracted from the rhizosphere of small *E. platyacanthus* plants (5 to 30 cm diameter), growing in the wild and cultivated in greenhouse. Massive sequencing of the V6-V8 region of the 16S rRNA gene was performed on the Illumina MiSeq platform. A total of 330,552 sequences were obtained, after the quality analysis and removal of chimeric sequences, the final number of pair-ended sequences in the two analyzed groups was 65,707. The sequences were classified at 97% similarity in 8,013 OTUs, distributed in 130 families. At this taxa level, the abundance and composition of both bacterial communities showed statistical differences (Figure 1).

The most pronounced difference was in the relative abundance of members of the family Rubrobacteraceae, however, at the phylum level, Actinobacteria accounted for >40% in the rhizosphere of wild plants, while they were <10% in the greenhouse plants. This could be related to their well-known association as PGPR, although the strong differences in the respective soils could also have an influence.



Keywords: cactus species; endangered; metataxonomic approach; PGPR.

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Functional diversity of soil microbial communities in response to grazing

intensity in a Mediterranean silvopastoral system



P. García-Gonzalo^{*(1)}, E. Arroyo⁽¹⁾, M.M. Gil-Díaz⁽¹⁾, M.C. Lobo⁽¹⁾, T. Martínez⁽¹⁾
⁽¹⁾ IMIDRA, Finca "El Encín" A-2, Km 38,2 28800 Alcalá de Henares (Madrid), Spain.
* Corresponding author: pilar.garcia.gonzalo@madrid.org



The health of grassland ecosystems strongly depends on the grassland management strategies, such as grazing and grazing exclusion. Degraded grasslands, principally attributed to overgrazing, can potentially affect the C-climate feedback via changes in soil microbial activity. However there are still uncertainties about the grazing effects on soil microbial community changes. A four-year field experiment was performed in a Mediterranean silvopastoral system, to investigate the grazing impact over changes in soil microbial community structure, functional diversity and enzymatic soil activities. Five plots were selected according to their different ecological and grazing management and five permanent fences of 20 x 20 m were constructed as excluded plots. Microbial functional diversity was determined using Biolog Ecoplates. Structure and diversity of bacterial communities were studied in a PCR-DGGE analysis of *amoA* and *NirS* functional genes. Soil enzymatic activities including β -glucosidase, β -galactosidase, acid and alkaline phosphatase, urease and arylsulphatase activities were determined. The results showed that grazing management affects functional diversity. Comparison of the Shannon diversity index reflects an increased functional diversity in grazing excluded plots. The clustering analysis of DGGE profiles concerning denitrifying (*NirS* gene) and ammonia-oxidizing bacteria (*amoA* gene) revealed that major differences in bacterial community structure were related to ecological habitat followed by grazing management. Diversity analysis through Shannon index showed a higher diversity in denitrifying bacterial communities from grazed plots samples. Conversely, the highest diversity in ammonia-oxidizing bacterial communities was found in grazing excluded plots samples. Comparing enzymatic activities, differences were found according to the grazing management. The high-intensity grazed treatment promoted microbial physiological activities. However the activities of all enzymes except β -galactosidase increased in grazed excluded plots. Results indicates that after four years excluding grazing, soil microbial community changes are largely dependent on grazing intensity. The critical level of diversity necessary to maintain the sustainability and functionality of this silvopastoral system will depend on a light or moderate grazing intensity.

Keywords: bacterial communities, diversity, grazing intensity, PCR-DGGE



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Identification of chitinase genes in halophilic microorganisms for the treatment of shrimp waste



Gutiérrez Sámano Pamela Susana, Cira Chavez Luis Alberto*, Estrada Alvarado Maria Isabel, De los Santos Villalobos Sergio.

Instituto tecnológico de Sonora, 5 de Febrero, 818, Ciudad Obregón, Sonora, México

*Corresponding author: luis.cira@itson.edu.mx

SAGARPA reported an increase in national shrimp production in 2013, from 127,517 tons to 211,096 tons in 2016. In turn, there is an increase in the amount of waste produced by this industry, due to the fact that only a portion of the This crustacean is used as food and fences of 30 to 45% of its weight, is discarded as waste, which could be treated to obtain byproducts such as chitin and chitosan that are known to have antitumor, antibacterial and antifungal activities. On the other hand, traditional methods of obtaining chitin consist of chemical treatments with acids and concentrated bases, which, in addition to the high economic impact, are contaminants¹. Therefore, biotechnological methods, using enzymes, are presented as an attractive and environmentally sustainable alternative, although the range of chitinases available is limited, especially as regards the range of operating conditions². So the objective of this work is the identification of extremity microorganisms, these organisms have evolved to exist in extreme environments and fall in a number of different categories, including halophiles that grow in salt concentrations from 0 to 30% p/v. As a result, these microorganisms produce unique enzymes and metabolites capable of developing biological activity under conditions in which their counterparts could not be functional³. In the present study, a pair of primers was designed and tested for the identification of chitinase genes, both type exo and endo chitinases in halophilic microorganisms, by PCR endpoint. As a result, 282 bp fragments were obtained, which correspond to the transcription of the genes coding for chitinolytic enzymes in the halophilic microorganisms studied. The above suggests that these primers are effective in the universal detection of the genes involved in the production of chitinase enzymes.

Keywords: Chitin, extremophile, PCR end point and primers.

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The metal reduction and bioelectricity production is controlled by the GSU1771 regulator in *Geobacter sulfurreducens*

Sergio A. Martínez Bahena, Víctor Almaza Rebollar, Yoatzin Domínguez Fernández, Katy Juárez López and José Alberto Hernández Eligio*.

CONACYT, Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, UNAM. Av. Universidad 2001, Col. Chamilpa, C.P. 62210 Cuernavaca, Morelos, México.

*Corresponding author: alherel@ibt.unam.mx



Geobacter sulfurreducens is bacteria capable to couple the anaerobic respiration to the reduction of metals. In addition, this bacterium can generate bioelectricity from the oxidation of organic compounds, transferring the resulting electrons to electrodes. In *G. sulfurreducens*, the extracellular transfer of electrons is directed by more than 100 *c*-type cytochromes and a conductive structure type Pili. Recently it was reported that a strain generated by directed evolution, which has several mutations along the genome, reduces more efficiently insoluble Fe(III) oxides¹. Among the mutations that this strain presents is the insertion of an IS element into the coding region of the *gsu1771* gene. The *gsu1771* gene codes for a protein member of the transcriptional regulators SARP-type described in *Streptomyces* species. In this work, the participation of the GSU1771 regulator in the expression of the important genes in the extracellular transfer of electrons was evaluated. The Δ GSU1771 mutant strain exhibits a growth delay in fumarate as electron acceptor (NBAF medium). However, it can reduce Fe(III) soluble to Fe(II) faster than the wild-type strain in citrate ferric medium. Interestingly, Δ GSU1771 mutant strain produces more *c*-type cytochromes than the wild-type strain, as was observed through the fractional extraction of internal membrane, periplasm and outer membrane proteins, and heme-staining. By Western-blot analysis we determined that Δ GSU1771 mutant strain produces more PilA protein (structural protein of the Pili) than the wild-type strain. Reverse transcription and quantitative PCR in Δ GSU1771 mutant strain reveals that *omcB*, *omcC*, *pilA*, *omcE*, *omcS* and *omcZ* genes were upregulated while *dcuB* and *frdCAB* genes were downregulated. With DNA-protein binding assay we found that GSU1771 protein binds to promoter regions of *pilA*, *omcE*, *omcS*, *omcZ*, *dcuB* and *frdCAB* genes. These results point out that GSU1771 regulatory protein represses the transcription of the *pilA* gene and some *c*-type cytochrome involved in soluble Fe(III) reduction and on the other hand activated a fumarate metabolism genes, positioning the Δ GSU1771 mutant strain as potential to be used in bioremediation and bioelectricity generation processes.

Keywords: *c*-type cytochromes, electron transfer, *Geobacter sulfurreducens*, pili.

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Medium chain length polyhydroxyalkanoates biosynthesis in

Azotobacter vinelandii OP from sucrose

G. Morales-Flores⁽¹⁾, J. Guzmán-Aparicio⁽¹⁾, M.S. Moreno-Leon⁽¹⁾, A.R. Reyes-González⁽¹⁾, C.F. Peña-Malacara⁽²⁾, C. Flores-Ocampo⁽²⁾, D.G. Segura-González*⁽¹⁾

⁽¹⁾ Departamento de Microbiología Molecular,

⁽²⁾ Departamento de Ingeniería Celular y Biotecnología, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, México.

*Corresponding autor: daniel@ibt.unam.mx



Polyhydroxyalkanoates (PHA) are a family of bio-polyesters, representing a carbon and energy-storage polymer. PHA have properties allowing to use them as a biodegradable plastics. The physical and thermal properties of PHA depend on the type of hydroxyalkanoate monomers present; for instance, there are short-chain-length (scl-PHA), with monomers of 4-5 carbon atoms, and medium-chain-length PHA (mcl-PHA), with monomers having more than 6 carbon atoms. mcl-PHA are more flexible, elastic and have a higher elongation at break than scl-PHA. The metabolic routes present in the bacterium affect the composition of the PHA produced. The *A. vinelandii* bacterium produces a scl-PHA called polyhydroxybutyrate (PHB). In some *Pseudomonas spp* a 3-hydroxyacyl-ACP thioesterase (encoded by *phaG*) and mcl-CoA ligase (gene *PA3924*) allow the synthesis of mcl-PHA from carbohydrates, by linking the *de novo* fatty acid biosynthesis with the synthesis of the precursors for the mcl-PHA synthesis. Therefore, the objective of this project was to produce mcl-PHA in *A. vinelandii* OP growing on sucrose by expressing the *Pseudomonas phaG* y *PA3924* genes. We constructed a recombinant strain that bears *phaG* and *PA3924* genes from *Pseudomonas* in *A. vinelandii* OP under the *scrX* promotor, which is inducible by sucrose. This strain was called OPpAGMF. To determine the effects of the expression of these genes, the wild-type and recombinant strains were grown on glucose and sucrose was added at 36h to induce the expression of the heterologous genes. The net production of PHA and growth were quantified. The OPpAGMF showed a similar content of PHA compared to the wild-type strain. The specific production [PHB]/[proteins] was similar in the recombinant strain (0.0231mg PHB/mg proteins) than in the wild-type (0.0275 mg PHB/mg proteins). The data of PHA composition showed the incorporation of mcl monomers. Conclusions: The synthesis of mcl-PHA in *A. vinelandii* was possible by expressing the *Pseudomonas phaG* and *PA3924* genes, allowing to produce a high amounts of a more flexible and elastic biodegradable plastics in this bacterium.

Keywords: Biodegradable biopolymers, biocompatibility, polyhydroxyalkanoates



Analysis of the expression of Heavy Metal Associated (HMA) domain containing proteins in certain Cucurbitaceae plants under arsenic stress.

C. V. Ochoa Bañuelos⁽¹⁾, A. R. Almeida Robles⁽¹⁾, C. E. López Ortiz⁽²⁾, L. E. Montañez Hernández⁽¹⁾, M. P. Luévanos Escareño⁽¹⁾, P. Nimmakayala⁽²⁾, U. Reddy^{*(2)}, N. Balagurusamy^{*(1)}.

⁽¹⁾Laboratorio de Biorremediación, Facultad de Ciencias Biológicas, Universidad Autónoma de Coahuila, Carretera Torreón-Matamoros Km 7.5, Torreón, México

⁽²⁾Department of Biology, West Virginia State University, Hamblin Hall, Institute, West Virginia, U.

*Corresponding author: bnagamani@uadec.edu.mx; ureddy@wvstateu.edu



Comarca Lagunera, North-East of Mexico has been reported to have arsenic concentration of 2.7-78.6 mg As.Kg⁻¹ in soil and 12-650 µg As.L⁻¹ in water. This region is the first largest producer of melon in Mexico and the second largest in the case of watermelon. Arsenic detoxification mechanisms of these plants have not been elucidated yet. The Heavy Metal Associated (HMA) domain is found in many transport proteins and two of its conserved cys residues provide thiol groups to bind heavy metals, and hence could be involved in arsenic uptake and detoxification. Genomes of cucumber, melon and watermelon were obtained from ICUGI database and Pfam was used to identify peptide sequences with HMA. The study revealed 57, 52 and 49 genes related to HMA domain in cucumber, melon and watermelon respectively. These genes were classified in families such as heavy metal transport-detoxification proteins, ATPases, chaperons and superoxide dismutases. Further in this study, cucumber, melon and watermelon were grown under non-controlled greenhouse conditions. The plants were irrigated daily with arsenic at different concentrations, viz., 0.07, 0.2, 0.67, 1.33, 2.34 µM of As (III) and a control was maintained without arsenic. It was observed that the effect all concentrations tested affected the growth of the cucumber and watermelon, but there was significant difference between the lower (0.07, 0.2 µM) and higher (1.33, 2.34 µM) concentrations. However, in melon, lower concentrations of 0.07 µM and 0.2 µM did not show any negative impact over control. On the contrary, they were found to beneficially promote the growth of melon. Interestingly, the flowering process in cucumber was first observed in 1.33 µM concentration on 35th day, while melon and watermelon registered at the lowest concentration. Lack of fruit formation in all the crops tested could be due to the reason that As (III) decreased boron and silica uptake and which are essential for fruit development. It was observed that the toxic effect of As (III) was observed in terms of the reduction in rate of photosynthesis and water absorption in all crops and possible increment of phosphate uptake only in melon. Results on the presence of HMA domains, genes and their phylogenetic relationship in the crops tested will be presented.

Keywords: Arsenic, Cucurbits Detoxification, HMA domain.

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community of *Echinocactus platyacanthus*



LI Salinas-Virgen¹, ME de la Torre-Hernández^{1*}, JF Aguirre-Garrido², HC Ramírez-Saad¹.
(¹) Universidad Autónoma Metropolitana-Xochimilco, Calzada del Hueso 1100, 04960, CdMx, México
(²) Universidad Autónoma Metropolitana-Lerma, Av. Hidalgo Pte. 46, Col. Lerma de Villada, 52006, Edo. de Mex., México

* Corresponding author: mdelatorre@correo.xoc.uam.mx



About 65% of the Mexican landscape is occupied by arid and semi-arid regions. Plant species within the Cactaceae family are highly represented in these regions, and some of them are threatened by various factors, mainly human activities. In the semi-arid region at Queretaro state, around a third of the local cactus species are endangered in some category; this is the case of *Echinocactus platyacanthus*. Propagation of this plant is difficult due to its slow growth and low survival rate when is reintroduced into its natural habitat. A useful approach to overcome this limitation is the use of PGPR, which contributes to their development, reproduction and survival. The objective of this work is to study, under a microbiological approach, the composition of the bacterial communities associated with *E. platyacanthus* and characterizing *in vitro* the PGPR activities of the rhizospheric isolates. A total of 268 bacterial morphotypes associated with *E. platyacanthus* were isolated and grouped into 45 different 16S rRNA gene-restriction assay patterns. Representative bacteria of each pattern were selected, their 16S rRNA gene amplified and sequenced to make the phylogenetic analysis. The more abundant genera were *Bacillus* and *Pseudomonas*, although *Stenotrophomonas*, *Staphylococcus* and *Chitinophaga* were also found but less abundant. The *in vitro* characterization of the PGPR traits of *E. platyacanthus* isolates focused on: growth inhibition of the phytopathogenic fungi *S. reilianum*, *U. maydis* and *F. solani*; quantification of indoleacetic acid (IAA) production; qualitative detection of siderophore production and ability to solubilize phosphates (rate 1–1.46). According to these results, the strains that showed the highest values on the diverse PGPR traits were: *Stenotrophomonas* (P31) on siderophore production (rate 1.7 in LB-CAS-FeCl₃); *Chitinophaga* (P12), *Pseudomonas* (P1) and *Staphylococcus* (P6) on IAA production (98, 94 and 93 µg/ml, respectively); *Brevibacterium* (P8) with a rate of 1.31 in NBRIP medium for phosphate solubilization. After in planta tests, these strains alone or in combination could be postulated as inoculants for the endangered cactus *E. platyacanthus*.

Keywords: cactus species; endangered; IAA; siderophore production.

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Screening for moderately halophilic bacteria producing keratinolytic exoenzymes using by-products of chicken feathers

Ana Maria Felix-Velis⁽¹⁾, Juan Carlos Coronado-Corral⁽¹⁾, Maria Isabel Estrada-Alvarado⁽¹⁾, Luis Alberto Cira-Chavez⁽¹⁾

⁽¹⁾Departamento de Biotecnología y Ciencias Alimentarias. Instituto Tecnológico de Sonora. 5 de febrero 818 sur. Ciudad Obregón, Sonora, 85000, México

* Corresponding author: luis.cira@itson.edu.mx



Nowadays, the chicken feather waste is not properly managed by the poultry industry. Consequently, a large amount of pollutants is expelled to the environment, compromising the quality of the air, water, and soil. The chicken feather is composed of approximately 90% keratin, which is rich in essential amino acids such as cysteine, methionine, glycine, serine, and alanine. It can be an interesting option for the poultry industries to take advantage of the chicken feather for biotechnological processes. Many industrial treatments transform the feather meal into a by-product. However, most of them are thermo-energetic or include chemical treatments. On the other hand, halophilic bacteria secrete some different types of enzymes, such as keratinases. Furthermore, they are willing to resist different conditions that can meet in the industrial production, such as salinity, temperature, and pH adversities. The present research consists of adding the by-product chicken feather as a substrate inside a medium, to prove the possible production of keratinolytic enzymes. A casein screening was made with moderately halophilic bacteria from 30 selected strains, (casein has a disulfide bridge between the cysteine which is found in the protein of chicken feather.), The presence of a massive halo in the medium was confirmed on BLLSAM1058, PPCAM1063, BLRMAM1065, BLCCAM1063, PPSE3AM1057, BLLSRAM1068, BLLCLAM1061, BLLRAM1063, YRAM1062, YRAM1064, BLRMAM1069 and BLLS3 strains. The positive strains have been selected as possible producers of keratinases. Moderately halophilic bacteria have the potential as enzyme producers for industrial processes to treat a poultry common waste.

Keywords: By-product, halophiles, keratin, keratinases



Genetic analysis of a halophilic bacteria with potential for the bioremediation of soils and solid waste

Rodríguez-Franco D.A.⁽¹⁾, Cira-Chávez L.A.*⁽¹⁾, Estrada-Alvarado M.I.⁽¹⁾, de los Santos-Villalobos S.⁽¹⁾, Gasso-Ortega L.E.⁽¹⁾ and Shirai-Matsumoto C.K.⁽²⁾

⁽¹⁾ Instituto Tecnológico de Sonora, Departamento de Biotecnología y Ciencias Alimentarias, Calle 5 de febrero 818 sur, Col. Centro, C.P. 85000, Ciudad Obregón, Sonora

⁽²⁾ Universidad Autónoma Metropolitana unidad Iztaalapa, Departamento de Biotecnología, Av. San Rafael Atlixco 186, Col. Vicentina, C.P. 09340, México, D.F.

* Corresponding author: luis.cira@itsom.edu.mx



In recent years, studies on the management of waste by biological methods have increased, because they are a friendly alternative to the environment and in some cases cheaper than the treatments currently used. In addition, it is seeking to add value to the waste generated in various productive activities, converting them into the raw material of other processes, thus avoiding contaminating soils and water bodies. One example is the degradation of chitinous residues by enzymes produced by halophilic bacteria, resulting in the production of chitooligosaccharides and N-acetylglucosamine, with applications in the agricultural, pharmaceutical, cosmetic industries, among others. The objective of this work is to know the genetic machinery of the halophilic strain YRAM72 to hydrolyze chitin and to withstand extreme conditions. DNA extraction was carried out by organic solvents, the genome was sequenced, assembled and scored. Enzymatic and electrophoretic assays were performed. We found only one 16S ribosomal gene with which the strain was identified as *Vibrio antiquarius*, which has a robust chitinolytic machinery, exhibiting genes that code for at least three endochitinases (EC 3.2.1.14) and two β -N-acetylhexosaminidases (EC 3.2.1.52); which was verified by enzymatic and electrophoretic tests. We also found resistance genes for heavy metals (chromium, zinc, cadmium, cobalt, copper and arsenic), antibiotics (tetracycline, beta-lactamases and fluoroquinolones) and adverse environmental conditions (osmotic, oxidative and periplasmic stress, heat and cold shock, carbon starvation and detoxification). In conclusion, the genomic analysis revealed that the strain YRAM72 identified as *V. antiquarius* can be an excellent option for the treatment of chitinous residues at different physicochemical conditions and in the presence of compounds that would be toxic to other organisms, producing biomolecules of industrial interest.

Keywords: Halophiles, genome, chitinolytic enzymes



Removal of pharmaceuticals with a constructed wetland of subsurface flow and characterization of the bacterial communities at the rhizosphere

Zapata Morales A⁽¹⁾, Alfaro de la Torre C.^{(1)*}, Garcia de la Cruz R.⁽¹⁾, Hernandez Morales A.⁽¹⁾

⁽¹⁾ Universidad Autónoma de San Luis Potosí, Manuel Nava 6, Zona Universitaria, CP 78210, San Luis Potosí, México

*Corresponding author: alfaroca@uaslp.mx



Pharmaceutical compounds have become a problem of concern since they can bioaccumulate in organisms in polluted aquatic systems. The conventional processes applied to treat municipal wastewaters are not efficient in the elimination of those substances. In this context, constructed wetlands are alternatives that could be efficient and, of low cost. This work evaluated the capacity of a constructed wetland of subsurface flow with plants of *Typha* spp. to remove diclofenac and naproxen in water, as well as to identify the microorganisms associated with the roots of the plants developed in the wetland, exposed or not to the drugs. Batch tests (n=10) were done to evaluate the kinetic of the pharmaceuticals removal, the results indicated a removal efficiency of 87% for diclofenac (3 mg/L) and 94% for naproxen (5 mg/L) after five days of exposure. Synthetic solutions were feed in constructed wetlands (2:1m²) one per each pharmaceutical tested) during 105 days (~3.5 months) reaching a removal efficiency of 98% for diclofenac (3mg/L) and 99% for naproxen (5 mg/L), with a Hydraulic Residence Time (TRH) of 5 days and a flow of 0.6 L/h. The plants did not show negative effects such as chlorosis. At the end of the wetlands operation, the bacterial consortia developed in the root of *Typha* spp. were isolated, bacterial colonies were obtained and showed tolerance to concentrations higher than 1000 mg/L of diclofenac and naproxen when exposed in controlled conditions in the laboratory; colonies belong to the *Pseudomonas* genus (>90%). These isolates have the ability to hydrolyze 1 to 3 carbon sources (carboxymethylcellulose, pectin and starch), which proves the versatility of the *Typha* spp. for the use of alternate sources of carbon. Bacterial isolates of *Typha* spp, are able to produce indoleic acid contributing to the development of the plants in the wetlands with diclofenac and naproxen.

Keywords: Pharmaceuticals compounds, Constructed Wetlands, Bacterial consortium.



Medium chain length polyhydroxyalkanoates biosynthesis in *Azotobacter vinelandii* OP from sucrose

G. Morales-Flores⁽¹⁾, J. Guzmán-Aparicio⁽¹⁾, M.S. Moreno-Leon⁽¹⁾, A.R. Reyes-González⁽¹⁾, C.F. Peña-Malacara⁽²⁾, C. Flores-Ocampo⁽²⁾, D.G. Segura-González*⁽¹⁾

⁽¹⁾ Departamento de Microbiología Molecular,

⁽²⁾ Departamento de Ingeniería Celular y Biotecnología, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, México.

*Corresponding autor: daniel@ibt.unam.mx



Polyhydroxyalkanoates (PHA) are a family of bio-polyesters, representing a carbon and energy-storage polymer produced by numerous bacteria. PHA have various properties allowing to use them as a biodegradable plastic. Moreover, their biocompatibility allows PHA to be used for medical and therapeutic applications. PHA can differ in physical and chemical properties depending on the hydroxyalkanoate monomers content; for instance, there are short-chain-length (scl-PHA), with 4-5 carbon atoms, and medium-chain-length PHA (mcl-PHA), with more than 6 carbon atoms. The mcl-PHA are flexible, elastic and have a higher elongation at break than scl-PHA. The features that determine the monomer composition of each type of PHA are the carbon source, the metabolic routes involved in the synthesis of the monomer precursor and the type of PHA synthase that leads the synthesis in the organism. The *A. vinelandii* bacterium produces a scl-PHA called polyhydroxybutyrate (PHB). In *Pseudomonas* spp a 3-hydroxyacyl acyl carrier protein thioesterase (encoded by *phaG*) and mcl-CoA ligase (gene PA3924) participate linking the de novo fatty acid biosynthesis with PHA synthesis providing monomer precursors for the mcl-PHA synthesis. Therefore, the objective of this project is to incorporate the *phaG* y PA3924 genes from *Pseudomonas* in *A. vinelandii* OP chromosome for biosynthesis of different types of mcl-PHA growing on sucrose. A recombinant strain was constructed that bears *phaG* and PA3924 genes under the *scrX* promotor, which is induced by sucrose. This strain was called OPpAGMF. To determine the effects of the expression of these genes, the wild- type and recombinant strains were grown on glucose. Sucrose was added at 36h to induce the expression of the heterologous genes. The net production of PHA and growth were quantified. The OPpAGMF showed a similar content of PHA compared to the wild-type strain. The specific production [PHB]/[proteins] was similar in the recombinant strain (23.1mg PHB/mg proteins) than in the wild-type (27.5 mg PHB/mg proteins). The data of PHA composition showed the incorporation of mcl monomers. The specificity of the *A. vinelandii* PHA synthase enzyme will be discussed, together with the effect of *phaG* y PA3924 genes expression on the PHA composition analyzed through gas chromatography.

Keywords: *Azotobacter vinelandii*, polyhydroxyalkanoates, biocompatibility, sucrose



Antibiotic resistance genes identification in bacteria isolated from Ciudad Obregon, Sonora, Mexico hospitals

Fregoso-Gómez J.A.⁽¹⁾, Hernandez-Bautista F.⁽²⁾, Frías-Alcalá L.A.⁽¹⁾, Ulloa-Mercado R.G., Gortares-Moroyoqui P.^{(1)*}

⁽¹⁾Departamento de Biotecnología y Ciencias Alimentarias. Instituto Tecnológico de Sonora. 5 de Febrero 818 sur. C. P. 85000 Obregón, Sonora, México.

⁽²⁾ Instituto Mexicano Del Seguro Social

*Corresponding Author: pablo.gortares@itson.edu.mx.



Nosocomial infections caused by carbapenem-resistant *Acinetobacter* spp are a global health problem. *A. baumannii* is the most clinically relevant specie of this gender. Acquired resistance to carbapenem is mediated by several genes. VIM (Verona integron-encoded metallo- β -lactamase) and IMP (imipenemase) are two of these genes responsible of carbapenem resistance. The objective of this work was to identify antibiotic resistance genes VIM and IMP in 18 strains of *Acinetobacter baumannii* provided by three hospitals of Ciudad Obregón Sonora, México using end-point PCR. The primers used in this work were particularly designed. The primers sequence for VIM were: forward 5'-GATGGTGTGGTCGCATA and reverse 5'CGAATGCGCAGCACCCAG to obtain a 390 pairs bases fragment. While for IMP were forward 5'CTACCGCAGCAGAGTCTTTAC and reverse AACCCAGTTTTGCCTTACCAT 3' to obtain a 587 pair bases fragment. To DNA extraction Dneasy Blood & Tissue Kit. was used Thermocycler conditions were for denaturation, 94 °C and 40 sec; for alignment 60 °C and 40 sec, for elongation and 72 °C and 45 sec. A total of 35 cycles were carried out. 2.5 units of Boline MyTaq enzyme was used with 30 ng of DNA per reaction, Volume of PCR sample was 25 μ l per reaction, MgCl₂ was 2.5 mM, 0.427 mM dNTPs, and a concentration of primers 0.28 μ M, as a base pair marker was used 1Kb bioline ladder. Electrophoresis was performed in 1% agarose, stained with ethyl bromide. Samples of *A. baumannii* were identified using VITEK2. Results showed that all samples presented a band of 390 base pairs, which indicates that the VIM gene is present in all studied strains. However, no band was obtained for the IMP gene. The VIM gene is a highly disseminated and there is no relationship in the presence or absence between both genes.

Keywords: antibiotic resistance gene, disseminated gene, VIM, IMP.



Pitting and general corrosion generated by acetic and butyric acids

produced by *Clostridium celerecrescens* on the api xl 52 steel surface



O.A. Ramos Monroy^{*(1)}, N. Ruiz Ordaz^{*(1)}, C. Juárez Ramírez⁽¹⁾, J. Galíndez Mayer⁽¹⁾, M.J. Hernández Gayosso⁽²⁾.

⁽¹⁾ Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prolongación de Carpio y Plan de Ayala S/N, Col. Santo Tomás, C.P. 11340, México, CDMX.

⁽²⁾ Instituto Mexicano del Petróleo, Grupo de Corrosión, Eje Central Lázaro Cárdenas 152, Col. San Bartolo Atepehuacan, C.P. 07730, México, CDMX.

*Corresponding authors: ibq_os@yahoo.com.mx ; noraruizordaz@yahoo.com.mx



Based on reports about microbial-influenced corrosion processes, the role played by sulphate-reducing bacteria has been widely studied and analyzed¹. However, the damage caused by acid-producing bacteria to metal structures, although less extensively studied, is also severe. In an earlier study², two electrochemical techniques were used to evaluate the corrosion caused by *Clostridium celerecrescens* on API XL 52 steel. The polarization resistance and the electrochemical impedance spectroscopy allowed to know the corrosion process and estimate the corrosion rate; however, the role played by *Clostridium celerecrescens* in the corrosion process was not considered. The main objective of this study was to understand the influence of *Clostridium celerecrescens* on the corrosion process. In this work, in an interval of one year, two spore-forming bacteria were isolated from a gas pipeline. Both bacteria were identified as *C. celerecrescens*. Their corrosive effect on the API XL 52 steel was evaluated. The identification was carried out by biochemical techniques and by 16S rDNA amplification, sequencing and comparison with the NCBI database. The strains were also compared between them using molecular techniques as the Amplified Ribosomal DNA Restriction Analysis (ARDRA) and the Random Amplified Polymorphic DNA Analysis (RAPD). The results showed that both strains corresponded to *C. celerecrescens* with a 99% similarity according to the sequence reported on the NCBI database. No plasmids were found in any of the bacterial strains. The ARDRA and RAPD electrophoresis profiles showed that both strains were identical; so, it was concluded that *C. celerecrescens* was a persistent bacterium in the gas pipeline. Because of the strain nature, its contribution to the corrosion process could be due to the organic acids production; thus, the acids production was evaluated during the corrosion process. The concentration of acetic and butyric acids in liquid medium was 18 mg L⁻¹ and 24 mg L⁻¹ respectively. Meaning that the acid concentration on the pipe surface, under the attached biofilm, must be higher. The corrosion rate was gravimetrically measured in three conditions, a) in sterile conditions (reference system), b) with added organic acids (accelerated corrosion) and c) in the presence of *C. celerecrescens*. The values obtained were 1.5±0.05 mpy, 2.7±0.07 mpy and 5.4±0.07 mpy, respectively. The scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) supported the analysis of local damage caused by bacteria caused on steel. It was concluded that the spore-forming bacterium *C. celerecrescens* is an aggressive agent on gas pipelines.

Keywords: API XL steel, biochemical and molecular techniques, corrosion, EDS and SEM.

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Biofertilization with NFB to restore a contaminated soil of Tlaxcala



- K. Flores-Vazquez⁽¹⁾, G. Calva-Calva⁽²⁾, S. E. Viguera Carmona⁽¹⁾, **J. Pérez Vargas**^{*(1)}
⁽¹⁾ *Tecnológico de Estudios Superiores de Ecatepec, División de Química y Bioquímica, Posgrado en Ingeniería Bioquímica, Av. Tecnológico s/n, Col. Valle de Anáhuac, Ecatepec Estado de México, México.*
⁽²⁾ *CINVESTAV-IPN Departamento de Biotecnología y Biotecnología, Ingeniería Metabólica, Av. IPN 2508, Col. San Pedro Zacatenco, Cd. De México, México.*
*Corresponding author: djperezvargas@hotmail.com



For many years have been worked with different bioremediation techniques for the elimination of toxic compounds. Large contaminated areas are a problem for the application of technologies when farmers with low resources want to use them, because the costs are not so accessible to them. The Atoyac River of Tlaxcala is highly contaminated due to the industries around the river like are the food, textile, chemical, petrochemical, and other services industries. This implies a potential damage to the health of the inhabitants of the area. The waters have contaminated the soil due to the practice of agriculture in the surrounding villages that use the arms of the river. The objective was to study some alternatives to improve soil area, using biofertilization with free-living nitrogen fixing bacteria (BFNA) in the remediation of contaminated soil. Water, soil and heavy metal analyzes were carried out in accordance with the regulations¹ to determine the soil conditions that would be bioremediated. After 6 months of biotreatment germination study It had been performed with peas, pumpkin and mustard, optimization germination from 40 to 95 to 100% respectively. The conclusion of the studies carried out indicates that the treatment with BFNA applied to a soil improves the soil conditions in a period of 6 months.

Keywords: Bioremediation, biofertilization, contaminated soil, germination.

1.- NORMA OFICIAL MEXICANA NOM-021-RECNAT-2000. Que establece las especificaciones de fertilidad, salinidad y clasificación de suelos, estudio de muestreo y análisis



Molecular characterization of microbial consortia from selected stages in a biorefinery using a novel technique for primer design

Carlos Alfredo Amaro-Aponte¹; Leticia Romero-Cedillo²; Héctor Mario Poggi-Varaldo^{1*}; Daniel A. Estrada-Bárceñas⁽³⁾; Édgar Baldemar Sepúlveda-García⁽⁴⁾; Dulce María Delgadillo-Álvarez⁽⁵⁾; Teresa Ponce Noyola⁽⁴⁾; José Tapia Ramírez⁽⁵⁾

⁽¹⁾ Environmental Biotechnology and Renewable Energies Group, CINVESTAV; P.O.Box 14-740, CP 07000, Mexico City, Mexico

⁽²⁾ Nanoscience and Nanotechnology Program, CINVESTAV, P.O. Box 14-740, CP 07000, Mexico City, Mexico

⁽³⁾ National Collection of Microbial Strains and Cell Cultures, CINVESTAV P.O. Box 14-740, CP 07000, Mexico City, Mexico

⁽⁴⁾ Department of Biotechnology and Bioengineering, CINVESTAV, P.O. Box 14-740, CP 07000, Mexico City, Mexico

⁽⁵⁾ LANSE-CINVESTAV; P.O. Box 14-740, CP 07000, Mexico City, Mexico

*Corresponding author:: r4cepe@yahoo.com; leticia.romero@cinvestav.mx

Molecular techniques used to characterize microbial communities in bioprocesses include denaturing or temperature gradient gel electrophoresis (DGGE or TGGE, respectively), Restriction Fragment Length Polymorphism (RFLP), among others. All of them require primer design with target regions that allow to amplify DNA fragments as well as to differentiate species. The objective of this work was to characterize and to identify the microbial species in consortia acting in the stages H-M-N from an HMZSN biorefinery. An important consideration in TGGE is to design primers that would facilitate the implementation of nested PCR. So, the design of primers should exhibit novel features. Among the latter we highlight that the target of the first set of primers was the 16S gene in order to simultaneously include the domains archaea and bacteria, and that of the second pair of primers was to increase specificity in the V3 hypervariable region. Primers in this work were representative of the two groups: (i) methanogens, and (ii) H₂-producing bacteria. Isolation of strains was carried out in liquid culture media M₁ and M₂, one for promoting H₂ production and the second for methanization. Inocula were sampled from hydrogenogenic and methanogenic fluidized bed reactors, and a semi-continuous, complete mix Seed Methanogenic Reactor. Considering the 3 inocula, a total of 40 strains were isolated; 75% corresponded to methanogenic archaea (MA). From these, 12 strains (40% of the MA) grew in culture medium M₁ (glucose plus trace metals and vitamins) whereas 18 (60%) grew in M₂ (also glucose, although poorer in trace metals and vitamins, spiked with NaHCO₃). Up to 10 of the total isolated strains (25%) were H₂-producing bacteria; half of them were able to grow in both culture media. Interestingly, inoculum from the hydrogenogenic fluidized reactor only had H₂-producing bacteria. On the other hand, inoculum from the methanogenic reactors only exhibited CH₄-producing microorganisms. Bacterial genres of some isolated strains corresponded to *Citrobacter*, *Rhodobacter*, *Rummelibacillus* and *Planomicrobium*. Work is on going to implement the method with TGGE and nested PCR to validate biological Fe(III) reduction for bionanoparticle synthesis and reductive dehalogenation of PCE using the primers.

Key words: 16S gene, Hydrogen producing bacteria, Methanogens, Nested PCR, Temperature Gradient Gel Electrophoresis.



Halocin and antibiotic resistance encoding genes in halophilic archaea

isolated from Saltern of Uyuni, Bolivia



Guachalla Alarcón Andrea ⁽¹⁾, Gutiérrez Llave Francisca ⁽¹⁾, Cárdenas Alegría Oscar ⁽²⁾, Medrano Medrano Beatriz ⁽¹⁾, Laura Mejía Lizeth ⁽¹⁾, Aliaga Rodríguez Jonatan ⁽¹⁾, Hurtado Gil Camila ⁽¹⁾, Romero Calle Danitza ⁽²⁾, Sánchez Montaña Rolando ⁽¹⁾, Álvarez Aliaga María Teresa ⁽²⁾

⁽¹⁾ Unidad de Biología Molecular, Carrera de Bioquímica – Universidad Mayor de San Andrés, Av. Saavedra 2224, La Paz, Bolivia

⁽²⁾ Unidad de Bioquímica Molecular, Instituto de Investigaciones Farmacéuticas y Bioquímicas – Universidad Mayor de San Andrés, Av. Saavedra 2224, La Paz, Bolivia

*Corresponding author: andreagilu@hotmail.com



Bolivia hosts a great diversity of extreme ecosystems, including hypersaline niches; the purpose of this work was to isolate halophilic archaea from Salar de Uyuni (SU) containing halocin encoding genes and to explore antibiotic resistance genes (ARG) distribution in them. Halophilic archaea can be applied in biotechnological processes for their production of metabolites such as carotenoids, enzymes, and halocins. Moreover, halocins, which are antibiotics resistant to high saline concentrations and temperature, have different mechanisms of action and biotechnological potentials that have not been elucidated yet. On the other hand, the presence of antibiotic resistance genes in these microorganisms is described, addressing the presence of ARG in hypersaline extreme niches that are not in direct contact with pharmaceutical industries' wastes or anthropogenic contaminants. Samples from the SU were collected in September 2016 and cultured in the Modified Growth Medium (MGM) at 43 ° C for 4-6 weeks until the appearance of reddish colonies. Afterwards, their genomic DNA was tested with 16S rRNA primers: 27F and 958R and sequenced by Sanger method. Subsequently, the presence of 3 genes encoding halocins (C8, S8 and H4) and 16 ARG was tested by conventional PCR. In the study, two strains belonging to the Halobacteriales family were identified as Haloarcula spp. One of the strains held the 3 genes encoding halocins; and the second only the C8 encoding gene. Both strains held 7 ARGs, among them those coding for resistance to beta-lactams, quinolones, sulfonamides, macrolides, tetracyclines, gentamicin and chloramphenicol.

Keywords: Salar de Uyuni, halophilic archaea, halocins, antibiotic resistance genes (ARG).



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Chapter 7 Wastewater Treatment



Effect of enrofloxacin on the growth and biochemical composition of *Chlorella* sp

Aguilar Alvarez, A. M. ⁽¹⁾, Burboa Charis, V. A. ⁽¹⁾, Ulloa Mercado, R. G.*⁽¹⁾, Gortáres Moroyoqui, P. ⁽¹⁾, Rentería Mexía, A.M. ⁽¹⁾, Leyva Soto, L. A. ⁽¹⁾, Hernández Chávez, J. F. ⁽²⁾

⁽¹⁾ Dpto. Biotecnología y Ciencias Alimentarias; Instituto Tecnológico de Sonora, Av. Antonio Caso 2266, Cd Obregón, Sonora, México

⁽²⁾ Dpto. Ciencias Agronómicas y Veterinarias; Instituto Tecnológico de Sonora, Av. Antonio Caso 2266, Cd Obregón, Sonora, México

*Corresponding author: ruth.ulloa@itson.edu.mx



The increase in production by pig farms has generated an excessive use of veterinary antibiotics, increasing the concentration of these in the wastewater generated by these farms¹. These residues found in the environment represent a great threat to local ecosystems² and human health³. The objective of this project was to evaluate the growth and biochemical composition of the microalgae wise, in the 0.5 mg/L ENR, there was an increase of 5% of carbohydrates in respect to the control. In the 2 mg/L ENR, there was a higher concen*Chlorella* sp. in bioreactors with different concentrations of enrofloxacin (ENR) in bold basal medium. To determine the effects of ENR, bioassays with concentrations of 0.5, 1 and 2 mg/L was performed; growth was evaluated for 12 days at 25± 2 °C with cycles of 12 h light and 12 h dark. The growth rate (μ) and biochemical composition was determined. Antibiotic concentration in the liquid phase was determined by capillary electrophoresis⁴. Also, the biochemical composition of each trial was done. After 15 days, the bioassays with 0.5, 1 and 2 mg/L of ENR showed higher growth inhibition than the control with 15%, 74% and 89% respectively. An increase of proteins of 49% in respect to the control was observed in the 1 mg/L ENR bioassays. Liketration of chlorophyll a in comparison to the control. It has been shown that there is a negative effect on the growth of *Chlorella* sp. in the presence of enrofloxacin at least above 1 mg/L and there was a change in the biochemical composition. This should be considered for the ENR treatment with *Chlorella* sp.

Keywords: Antibiotics, Biochemical composition, Microalgae, Piggery wastewater

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Isolation and application of a microalgae on a microbial fuel cell

employing aquaculture effluent as substratum



Morando-Grijalva, C. A. ⁽¹⁾, *Vázquez-Larios, A. L. ⁽¹⁾, Alcántara-Hernández, R. ⁽³⁾, Ortega-Clemente, L. A. ⁽²⁾, Robledo-Narvaéz, P. N. ⁽¹⁾.

⁽¹⁾ Instituto Tecnológico Superior de Tierra Blanca, Veracruz, S/N, Tierra Blanca, México.

⁽²⁾ Instituto Tecnológico de Boca del Río, Carr. Veracruz-Córdoba 12, S/N, Boca del Río, México.

⁽³⁾ Universidad Autónoma de México, Instituto de investigación, S/N, Ciudad de México, México.

*Corresponding author: linevazquez@yahoo.com.mx

The objective of the present study was applied *Chlamydomonas* (*Ch*) as biocathode on a microbial fuel cell (MFC) to generate bioelectricity and fatty acids using aquaculture wastewater. The work consisted in the isolation of a microalgae¹ and the obtain of anaerobe inoculum². The MFC used two-chamber, each holding an operation volume of 900 mL, separated by a proton exchange membrane (PEM), characterized for polarization curve³. Once operated the MFC, extracted the lipids the accumulated biomass in the cathode using a Soxhlet with a mix chloroform/methanol (1:2 v/v), subsequently derivatization was carried by method Lepage & Roy (1984) for their analysis on a gas chromatography (Perkin Elmer, Model Autosystem, Flame ionization), with an Elite-Wax capillary column (30 m in length × 0.250 mm in diameter)⁴. In the polarization curve was obtained an internal resistance of 22 and maximum power density of 73.67 mW m⁻², high to reported by Lan *et al.* (2013)⁵. The removal percentage of DQO showed a 96.8%, similar to reported by Cucu *et al.* (2016)⁶. The accumulated biomass was 72.8 mg L⁻¹, presented a 74% of palmitic acid on total lipids. It was managed to obtain of electric energy starting Tilapia wastewater and the application of *Chlamydomonas* as biocathode on to MFC with a power density 73.67 mW m⁻². The lipids profile is a convenient for biodiesel elaboration.

Keywords: bioenergy, fatty acids, microalgae, wastewater.

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Anaerobic digestate as a substrate for the semi-continuous culture of *Arthrospira* sp.: Protein production

Álvarez Montero Xavier Guillermo⁽¹⁾, Otero Casal Ana María⁽²⁾

⁽¹⁾ Universidad de Guayaquil, Facultad de Ciencias Naturales, Laboratorio de Química Ambiental, Av. Raúl Gómez Lince s/n y Av. Juan Tanca Marengo, Guayaquil, Ecuador

⁽²⁾ Universidade de Santiago de Compostela, Facultade de Bioloxía, Centro de Investigación Biolóxica (CIBUS), Departamento de Microbioloxía y Parasitología, Rúa de Lope Gómez de Marzoa s/n, Santiago de Compostela, España

*Corresponding author: xavier.alvarezmo@ug.edu.ec



The feasibility of semi-continuous culture of *Arthrospira* (*Spirulina*) sp. in tubular photobioreactor was performed, using the anaerobic digestate from wastewater of a fish processing plant as a nutrient source. Semi-continuous cultures were carried out in aerated tubular cultures units of 250 mL, adapting the percentage of the anaerobic digestate in the culture medium (10-20%) with a dilution rate of 0.1 d⁻¹. Growth, ammonia removal and production of phycobiliprotein and protein by *Arthrospira* sp. were assessed. The removal efficiency of ammonia for *Arthrospira* sp. with a higher ammonium concentration (11.3 mM) was 55.9%, although stripping was higher. Results indicate that the cultures could support much lower residence time since all the ammonium was removed in during the first 24 hours. High concentration of protein (2.5 ± 0.2 mg mL⁻¹), phycobiliprotein (allophycocyanin: 84.3 ± 10.2 mg g⁻¹; phycocyanin: 73.7 ± 7.7 mg g⁻¹) were obtained. The results indicate the possibility of the use of filamentous cyanobacteria for the treatment of industrial effluents rich on ammonium, with different possibilities of valorization of the biomass produced.

Keywords: Ammonium removal efficiency, anaerobic digestate, cyanobacteria



Technical feasibility of artificial wetlands inoculated with pesticide

tolerant bacteria for the treatment of agroindustrial effluents: A Review



Cedillo-Herrera, C.I.G.⁽¹⁾, Amabilis-Sosa, L.E.⁽²⁾, Rangel-Peraza, J.G.⁽¹⁾, Rodríguez-Mata, A.⁽²⁾, Mendivil-García, K.⁽¹⁾

⁽¹⁾ *Instituto Tecnológico Nacional de México-Instituto Tecnológico de Culiacán, Juan de Dios Batiz 310, Culiacán, México*

⁽²⁾ *CONACYT-Instituto Tecnológico de Culiacán, Juan de Dios Batiz 310, Culiacán, México*

*Corresponding autor: leoamabilis@yahoo.com.mx; cinthiaisabel.cedillo@gmail.com



Agriculture is one of the main economic activities in Sinaloa and generates agricultural wastewaters (AIWW) with low biodegradability and large nutrient concentrations. Flow of AIWW into surface water bodies is a potential risk for human health and the ecosystem. There are options to AIWW treatment, among which those related to adsorption such as activated carbon, activated silica, organic waste such as coconut shells, starch and chitosan distinguish. advanced oxidation processes (AOPs) as photocatalysis, photofenton, ultraviolet light/hydrogen peroxide (UV/H₂O₂), electrocoagulation and electrochemistry also stand out. However, the former do not have design parameters for their implementation on a real scale and their lifetimes are unknown. Besides its significant cost, it could not carry AOPs out on a pilot scale. Facing this, versatile municipal wastewater treatment technologies appear, such as constructed wetlands (CW), involving both heterotrophic, and photosynthetic organisms and packing medium with adsorbent capacity. In parallel, several studies have identified bacterial consortia capable of tolerate and use pesticides as a carbon source. In addition, macrophytes confer advantages at the micro and mesocosm level due to the supply of oxygen and other carbon sources, generating synergy with bacteria, mainly with those tolerant and/or pesticides degraders (TB/PD). The above suggests implementing CW inoculated with TB/PD. Present scientific review addresses degradation of the compounds containing in AIWW, through different case studies and physical-chemical-biological foundations on the role played by bacterial species, the packaging medium and its porosity, and different species of macrophyte. Accentuating design and operation parameters to implement CW inoculated with TB/PD in agricultural drainages treatment. Conclusions mention principal operation and design parameters to apply CW inoculated with TB/PD in agricultural drainage, including biologic aspects such as bacterial consortia conformation, plants distribution on the superficial space, hydraulic characteristics and economic costs.

Keywords: Agroindustrial wastewater, constructed wetlands, treatment, tolerant bacteria, pesticides biodegradation.



Quantification and removal of polyphenols in anaerobic biochemical systems

J. A. Contreras-Contreras⁽¹⁾, A. Martínez-Cruz⁽¹⁾, Dra. M. Bernal-González⁽¹⁾, Dra. M. C. Durán-Domínguez-de-Bazúa⁽¹⁾

⁽¹⁾ Universidad Nacional Autónoma de México UNAM, Av. Universidad N° 3000, Ciudad de México, México

*Corresponding author e-mail: mcduran@unam.mx



In Mexico, the production of sugarcane and the sugar industry are very important for the economic and social development of the country. In the production of sugar, residual byproducts are generated, one of them are the vinasses. The vinasses have high contents of polyphenols that are produced during the caramelization of sugar. Polyphenols are substances formed by multiple units of phenol, are highly recalcitrant, persist in the soil and are toxic for some microorganisms. They also give a dark brown color to the vinasses, which prevents the penetration of light into the surface water bodies¹. To recycle vinasses three upflow anaerobic sludge blanket reactors (UASB) were built at laboratory scale operating at temperatures of 45, 55 and 65°C. Here, a transformation of organic and inorganic matter measured as chemical oxygen demand (COD) occurs. There is also a decrease in sulphates and an increase in sulfides. One of the objectives of the research was to determine if there was removal of polyphenols. For this purpose, these compounds were quantified in the influents and effluents of the reactors using the Folin-Ciocateu's method, and by means of a statistical analysis, it was concluded that so far there is no removal of polyphenols in the treatment. For this reason, a removal process using activated carbon from bagasse was proposed. The adsorption data were evaluated using commercial activated carbon to know how the removal of these compounds directly influences the COD of the vinasses.

Keywords: Anaerobic biochemical systems, polyphenols, vinasses

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Effect of the chemical oxygen demand:sulfates ratio (COD/SO₄²⁻ ratio) of the flotation liquid effluent from a mining plant during its biological treatment

R. Cuellar-Briseño⁽¹⁾, L. M. Barrera-Mena⁽¹⁾, J. A. Contreras-Contreras⁽¹⁾, M. Bernal-González^{(1)*}, María-del-Carmen Durán-Domínguez⁽¹⁾, E. Rodolfo Bazúa-Rueda⁽¹⁾
⁽¹⁾ UNAM, Facultad de Química, Departamento de Ingeniería Química, Laboratorios 301, 302, 303. Laboratorios de Ingeniería Química Ambiental y de Química Ambiental, 04510 Ciudad de México. México

*Corresponding author e-mail: marisela_bernal2000@yahoo.com.mx



In this research, the effluent of the flotation process from a mining plant where zinc, copper and lead sulfide concentrates are produced was studied. In order to achieve the concentration of metal sulfides with a maximum efficiency during the flotation process, especially through saving of influent water, the ability of sulfate-reducing microorganisms (SRM) present in an anaerobic reactor to convert the sulfates present in the flotation effluent into sulfides was taken into consideration. Experiments at laboratory scale were carried out using an upflow anaerobic sludge blanket reactor, UASB, with working volume of 2.2. L. The influent was pumped using a peristaltic pump intermittently every 24 hours. Parameters considered were: pH value, alkalinity factor (α), temperature (T), chemical oxygen demand (COD), sulfates (SO₄²⁻), sulfides (S²⁻), volatile fatty acids (VFA), and biochemical oxygen demand (BOD). This investigation was divided into two stages. In the first stage, the reactor was fed with flotation process water direct from a cooperating Mexican mine. During the second stage it was combined with synthetic wastewater simulating sewage (Orduña-Bustamante *et al.*, 2012)^[1]. The objective was to vary the specific proportion of organic compounds, measured as COD, with respect to SO₄²⁻ content evaluating its effect on the elimination of this dissolved matter. Results showed that a COD/SO₄²⁻ ratio of 0.79 and 4237 mg COD L⁻¹, COD elimination was maintained up to 86.7%. At a COD/SO₄²⁻ ratio of 0.03 and 258 mg COD L⁻¹, COD elimination was 13.58%.

Keywords: Chemical oxygen demand (COD), sulfates (SO₄²⁻), sulfate-reducing microorganisms (SRM), sulfides (S²⁻)

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Metal bioremediation performed by native algae isolated from the Reconquista River

Ferraro G⁽¹⁾, Areco MM⁽²⁾, Passucci V⁽²⁾, Bagnato C⁽¹⁾, Toranzo R⁽¹⁾, Castiglioni D⁽¹⁾, Curutchet G⁽²⁾

⁽¹⁾ Instituto de Energía y Desarrollo Sustentable (IEDS-CNEA), Av Bustillo 9500, Bariloche, Argentina

⁽²⁾ Instituto de Investigación e Ingeniería Ambiental, 31a, UNSAM, 25 de Mayo y Francia, San Martín, Buenos Aires, Argentina.

*Corresponding author: gisela.ferraro@cab.cnea.gov.ar; giselaf@gmail.com



The contamination of freshwater systems with thousands of industrial and natural chemical compounds is one of the major environmental and human health problems worldwide. Alternative technologies for cleaning industrial effluents and water courses are needed. Algal biomass possesses high metal binding ability, particularly at low metal concentrations. Moreover, algae strains that grow in contaminated sites have shown better remediation performances than the same culture collection species (1). The Reconquista River is one of the most polluted watercourses in Argentina. In this work a bioprospection of the algae present in the Reconquista River is performed to identify and isolate algal strains with potential for metal remediation. Different algae were identified: *Chlorophytes* (*Chlorellaceae*, *Radiococcaceae*, *Scenedesmaceae*); *Euglenophytes*; *Bacillariophyta* (centric and pennate diatoms) and *Cyanobacteria* (*Nostocaceae*, *Merismopediaceae*, *Oscillatoriaceae*). Metal (25 mg/L Zn²⁺) removal efficiency (E%) for 9 individual strains belonging to the *Chlorellaceae* and *Scenedesmaceae* family showed values of 65-98%. Changes in cell's morphology after metal adsorption and the identification of the main cell wall group's involved in the metal remediation process were analyzed by SEM and FT-IR, respectively. The most efficient strains were tested for the remediation of a poly-metallic solution (Zn²⁺, Cu²⁺ and Cr³⁺). Results demonstrate the algae's ability to remediate effluents and will allow to design the scale up of this process.

Keywords: algae, bioremediation, isolation, metal

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vulgaris from synthetic wastewater



Celestino García-Gómez*⁽¹⁾, Karla Sofía Piedra-Alegría⁽¹⁾, Georgina Guadalupe Zamarripa-Segovia⁽¹⁾, Sanjuana Cecilia Galicia-Campos⁽¹⁾, Juan Antonio Vidales-Contreras⁽¹⁾

⁽¹⁾ Universidad Autónoma de Nuevo León, Facultad de Agronomía, Francisco Villa S/N, C. P. 66050 General Escobedo, Nuevo León, México

*Corresponding author: celestino.garciaqm@uanl.edu.mx

The absorption capacity of nutrients by microalgae has been reported with good performance, for this reason, their uses for municipal wastewater treatment have shown increasing interest in research community. Municipal wastewaters have high organic content and are rich with inorganics compounds which are essential for microalgae cultivation, and are considered to have economic and environmental potentials for producing biomass and valuable compounds, recovering nutrients, and treatment of wastewater¹. In this work, biomass, chlorophyll, lipids, phycobilin, carbohydrates production, and removal of ammoniacal nitrogen and ortho phosphate by the microalgae *Chlorella vulgaris* sp., cultivated in synthetic wastewater (SWW), was studied under different light intensity (3000-10000 lux), Ph (7.5-9.5) and daily illumination time (8-16 h), at 25 ± 1°C with an air-bubbling of 0.5 vvm. The optimum conditions for microalgal autotrophic cultivation were predicted by using response surface methodology (RSM) and validated through laboratory experiments. The results showed that the adaptation of microalgae for this nutrient source was effective. The optimum conditions for the cultivation of *Chlorella vulgaris* sp., in synthetic municipal wastewater were the range of 9-9.5 for pH, the light intensity of 10000 lx in the daily illumination time for 18 h. Under the optimum conditions, the yield of microalgal biomass, chlorophyll, lipids, phycobilin and carbohydrates production was 722.5 mg/L, 11.5 mg/g, 33.2%, 0.025 mg/g and 18%, respectively. Nitrogen and phosphorus removal efficiencies from SWW were 76% and 79% in autotrophic culture. The mixotrophic cultivations enhanced the microalgal production, nutrients removal and valuable compounds. Moreover, the addition of 400 mg/L glucose in mixotrophic cultivation, as a low-cost alternative, was found to be a suitable medium for effective enhancement of our response variables. These strategies can be a good reference for the enhancement of microalgal production and their application for the large-scale wastewater treatment under a controlled environment.

Keywords: microalgae, *Chlorella vulgaris*, wastewater, biomass.

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Benzotriazole mineralization by denitrifying sludge in presence of acetate

García-Camacho J. ⁽¹⁾, Romo-Gómez C. ⁽¹⁾, Texier A.C. ⁽²⁾, Cuervo-López F. ^{*(2)}

⁽¹⁾ Universidad Autónoma del Estado de Hidalgo, Carretera Pachuca-Tulancingo km. 4.5, Mineral de la Reforma, Hidalgo, México

⁽²⁾ Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco, 186, Ciudad de México, México

*Corresponding author: fmcl@xanum.uam.mx



1H-Benzotriazole (BTri) is a heterocyclic compound used as additive aircraft liquid, flame retardant, anticorrosive and pharmaceutical precursor¹. Biological treatments have been proposed for BTri elimination; however, there is not enough information about BTri mineralization. Denitrification is an alternative treatment process with the capability of mineralizing aromatic compounds. It has been reported that the addition of acetate increased specific consumption rate of aromatic pollutants². The aim of this work is to assess the enhancement in BTri consumption by adding acetate as alternative electron donor. Denitrification reference, control and abiotic assays, were conducted in 125 mL serological bottles with 100 mL work volume. Mineral medium² was added with 5 mg L⁻¹ BTri-C, 100 or 200 mg L⁻¹ acetate-C and 150 or 250 mg L⁻¹ NO₃⁻-N. Sludge behavior was evaluated by: substrate consumption efficiencies (% E), HCO₃⁻ and N₂ production yielding (Y) and specific consumption or production rate (q), as response variables. Abiotic assays evidenced that the losses of BTri by volatilization, chemical reaction or adsorption were negligible. Results indicated that BTri was not used as sole electron source in the denitrification process. When acetate was added (0.72 and 0.80 C/N) BTri was totally consumed and mineralized ($Y_{\text{HCO}_3} = 0.97 \pm 0.02$) within 96 h; thus, acetate addition enhanced BTri consumption and its mineralization. Other works indicate recalcitrance or low BTri consumption under denitrifying conditions, but no mention on mineralization or denitrification was done³.

Keywords: Benzotriazole; Co-substrate; Denitrification; Mineralization.

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Radish production in soils amended with treated sewage sludge

Mancho C^{(1)*}, Pinilla P⁽¹⁾, Gil-Díaz M⁽¹⁾, Sastre-Conde I⁽²⁾, Alonso J⁽¹⁾, Gutiérrez A⁽¹⁾, Lobo MC⁽¹⁾
⁽¹⁾ IMIDRA. Finca "El Encín" A-2, km 38,2. 28800 Alcalá de Henares (Madrid). Spain.
⁽²⁾ SEMILLA-INAGEA, Babieca, 2. 07918, Son Ferriol, Palma de Mallorca (Balearic Island). Spain

*Corresponding author: carolina.mancho@madrid.org

Environmental policies promote the use of wastes as organic amendment as an alternative to landfill disposal or incineration due to the beneficial effect of their composition on soil fertility. In addition, the use of these organic amendments could replace total or partially the inorganic fertilization, avoiding the risks of nitrate lixiviation and consequently the groundwater eutrophization. A greenhouse experiment was carried out to evaluate the effect of sewage sludge composted with pruning wastes (CP) at 20 and 40 Mg/ha and thermal dried sewage sludge (ST) at 3 and 6 Mg/ha in two successive crops of *Raphanus sativus* on a calcareous soil (Haplic Calcisol) in 70L pots. A treatment with inorganic fertilization (NPK at 0,5 Mg/ha) and soil without treatment were used to compare the plant production. After each harvest, radish production was evaluated as well as the effect of the treatments on plant and soil according to Spanish official methodology¹. In the first crop the highest yield was observed at the dose of 20 t/ha. In the second crop, the highest yields were observed in CP-treatments, being the increases dose dependent. The application of ST treatments led to yields similar to those obtained with the inorganic fertilization. No differences were observed in nutrient and metal content in plant regardless treatment or crop. Regarding the effect on soil characteristics, the use of the CP treatments increased significantly soil organic matter, being the increase dose dependent. This effect was not observed in ST treatments. Heavy metal concentration increased in soil treated with treated sewage sludges, but in all cases, the values were below the legislative limits. In general, the CP treatment at the lower dose could substitute a mineral fertilization by achieving similar or even higher production of a radish crop under the assayed conditions in addition to promoting the soil fertility.

Keywords: compost, *Raphanus sativus*, sewage sludge, soil.

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Evaluation of the consumption of glucose of *Chlorella* and *Scenedesmus*

immobilized in alginate beads in synthetic wastewater



J. M. Jordán Esquer⁽¹⁾, L. A. Leyva Soto^{*(1,2)}

⁽¹⁾ ITSON, Department of Biotechnology and Food Sciences, Obregón, Mexico

⁽²⁾ CONACYT ICC1, Mexico City

*Corresponding author: minuethjordan@hotmail.com



The porcine farms generate wastewater with high loads of organic matter, inorganic nutrients and micro pollutants¹. The microalgae are excellent candidates for the treatment of these waters². However, the biomass recovery systems are inefficient and generate high costs³; the immobilization of microalgae in alginate beads supposes a great profitability to this problem⁴. The efficiency and viability of immobilized microalgae have not been studied in the treatment of effluents from porcine farms; that is why the objective of this investigation was to establish a biological system with microalgae immobilized in alginate beads, for the complementary elimination of organic load of synthetic wastewater. *Chlorella sp.* and *Scenedesmus obliquus* microalgae were immobilized in alginate beads, using three different concentrations of glucose in order to generate: 10,000, 20,000 and 30,000 mg/L of COD. COD consumption was measured for three days using the Miller DNS technique (for reducing sugar quantification). The microalgae growth was evaluated by direct counting in microscope using a Neubauer Chamber. Each experiment was performed in triplicate in 250 mL Erlenmeyer flasks twice. The most high removal rate was in 24 h for both microalgae. *Scenedesmus* removed 8.18, 44.49 and 66.66% and *Chlorella* 44.26, 71.04 and 83.39% for treatment with 10,000, 20,000 and 30,000 mg/L of COD respectively. The results show that both microalgae have capacity for glucose removal and is a promising alternative to incorporate in the porcine farms wastewater treatment.

Keywords: COD removal, immobilized microalgae, porcine farms, wastewater treatment

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Use of a submerged membrane bioreactor (sMBR) for synthetic fish meal

processing wastewater treatment



G. López Avilés⁽¹⁾, F. J. Almendariz Tapia^{*(1)}, G. Lesage⁽²⁾, S. Perez Fabiel⁽³⁾, O. Monge Amaya⁽¹⁾, M. T. Certucha Barragán⁽¹⁾, M. Plascencia Jatomea⁽¹⁾, E. R. Meza Escalante⁽⁴⁾

⁽¹⁾ Universidad de Sonora, Blvd. Luis Encinas, Col. Centro, 83000 Hermosillo, Sonora, México.

⁽²⁾ 2IEM, Univ Montpellier, CNRS, ENSCM, Place Eugène Bataillon, 34095 Montpellier cedex 5, France

⁽³⁾ Universidad Politécnica de Chiapas, Eduardo J. Selvas s/n y Avenida Manuel de J. Cancino. Col. Magisterial, 29082. Tuxtla Gutiérrez, Chiapas, México

⁽⁴⁾ Instituto Tecnológico de Sonora. 5 de febrero 818 Sur, Col. Centro, 85000, Ciudad Obregón, Sonora, México.

*Corresponding author: fjalmdariz@iq.uson.mx

Fish meal industry generates wastewater characterized by a high concentration of nitrogen, organic matter and salt, in addition to various toxic micropollutants (1). In most cases, those wastewaters are discharged into the sea without any treatment. This is the case of the fish meal industries of Guaymas, in the state of Sonora (Mexico), where it is estimated that 1495 m³/d of sewage is discharged into the sea (2). An alternative solution is to use submerged membrane bioreactors (sMBR) for its cleaning process (3). The aim of this work was to study organic matter removal, nitrification and biomass growth in a submerged flat-sheet membrane bioreactor, fed with synthetic wastewater, of similar composition to the effluents generated in a fish meal industry. Experiments were carried out on a lab-scale submerged membrane bioreactor with an operating volume of 30 L, aeration was maintained above 4 mg O₂/L, temperatures in the 15-25°C range, with pH varying between 7 and 7.8. The sMBR was operated with 5 days of hydraulic retention time (HRT) and 40 days of sludge retention time (SRT). Glucose and ammonium chloride were used as organic matter and ammonia source respectively and NaCl was added to mimic the salinity of a fish meal industry effluent. After biomass acclimatization with saline conditions of 12 gNaCl/L and COD/N ratio of 15 in the bioreactor, results showed that organic matter removal was higher than 90%, for all organic loading rates (0.8, 1, 1.33 and 2 gCOD/L·d) and nitrogen loading rates (0.053, 0.067, 0.089 and 0.133 gN/L·d) tested during the study. However, nitrification was only carried out with the lowest OLR (0.8 gCOD/L·d) and NLR (0.053 gN/L·d), since an excessive organic matter concentration in the wastewater appears as a limiting factor to this process' operating conditions, where nitrification values of 65% were reached, including nitrogen assimilation to produce biomass. The results obtained through the study demonstrate the feasibility of using aerobic membrane bioreactors to treat wastewater containing organic matter, nitrogen and salt.

Keywords: Aerobic treatment; fish meal wastewater; membrane bioreactor; nitrification.

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Acid mine drainage remediation using sulfate reduction and sulfide

oxidation



C.D. Loreto Muñoz^{*(1)}, O. Monge Amaya⁽¹⁾, F.J. Almendariz⁽¹⁾, V. Ochoa-Herrera⁽²⁾, R. Sierra⁽³⁾

⁽¹⁾ University of Sonora, Rosales and Luis Encinas Blvd., Hermosillo, MX

⁽²⁾ Universidad San Francisco de Quito, Diego Robles and Via Interoceánica, Quito, EC

⁽³⁾ University of Arizona, P.O. Box 210011, Tucson, USA

*Corresponding author: cynthiadenisse_0905@hotmail.com.



Direct discharge of acid mine drainage (AMD) generates negative impacts in water resources, due to its high acidity, elevated sulfate concentrations, and presence of metals and other toxic compounds. Different technologies have been developed for AMD treatment based on sulfate reduction and sulfide oxidation. Sulfide, produced from sulfate reduction, is a toxic compound, but could be used to precipitate metals. Sulfide that doesn't react can be re-oxidized to sulfate or elemental sulfur and could be easily separated and used in the production of sulfuric acid or as fertilizer^{1,2}. Anaerobic reactors have been used for biological removal of sulfate but are considered as pretreatments since anaerobic effluents contain high quantities of soluble organic matter. To attain standard discharge levels a secondary aerobic treatment should be added. Anaerobic-aerobic systems are easy to operate, produce less sludge, and consume less energy². Integrated anaerobic-aerobic reactors (IAAB) allow the treatment of large volumes of wastewater, and are used for AMD treatment³. Integrating sulfate conversion and sulfide removal as elemental sulfur in a single reactor has practical interest. This review focusses in AMD treatment using sulfate reduction and sulfide-oxidation processes. IAAB systems represent an alternative for space, odor, and sludge production restraints, allowing the treatment of a great variety of industrial and domestic effluents.

Keywords: acid mine drainage, sulfate reduction, sulfide oxidation.

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2-Chlorophenol consumption by cometabolism in nitrifying SBR reactors

Miguel Martínez-Jardines⁽¹⁾, Sergio Martínez-Hernández⁽²⁾, Anne-Claire Texier⁽¹⁾, Flor Cuervo-López^{*(1)}.

⁽¹⁾ Department of Biotechnology, Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco 186, CDMX 09340, Mexico,

⁽²⁾ Institute of Biotechnology and Applied Ecology, Universidad Veracruzana. Av. de las Culturas Veracruzanas 101, Xalapa 91090, Veracruz, Mexico.

*Corresponding author: e-mail. fmcl@xanum.uam.mx



Cometabolism has been defined as the capacity of microorganisms to transform a substrate that is not used as growth support in the obligatory presence of a growth substrate or easily biodegradable compound under aerobic or anaerobic conditions¹. Under nitrifying conditions, the rate of degradation of trichlorethylene increased with the increase in ammonium concentration, which was used as growth substrate². It has been suggested a cometabolic consumption of 2-chlorophenol (2-CP) using nitrifying sludge³. In this work, the cometabolic consumption of 2-chlorophenol (2-CP) by adding different initial ammonium concentrations was evaluated in SBR systems inoculated with a nitrifying sludge. Cometabolic consumption of 2-CP was evaluated in two SBR reactors fed with 60 mg 2-CP-C/L and different initial ammonium concentrations (100, 200, 300, 400, and 500 mg NH₄⁺-N/L) by means of specific consumption rates (q). The SBRs were operated during 13 cycles: filling 0.02 d, reaction time 14-21 d, settle 0.02 d and draw 0.083 d. Irrespectively to the increase in ammonium concentration and throughout the operational cycles, the sludge achieved a complete nitrification in 14 days, accounting for ammonium consumption efficiencies close to 99% and nitrate production yields between 0.93 and 0.99. Likewise, the sludge was able to completely consume 2-CP within 7 days. The increase in ammonium concentration provoked an increment in the specific rates of both ammonium (qNH₄⁺-N) and 2-CP (q2-CP-C) consumption up to 5.2 and 3.1 times, respectively. The cometabolic consumption of 2-CP associated to the increase in ammonium concentration was supported by a direct and significant relationship between qNH₄⁺-N and q2-CP-C (r=0.83). According to these results, the use of nitrifying sludge and high ammonium concentrations in SBR systems can be a suitable alternative for increasing the cometabolic consumption of recalcitrant compounds like 2-CP.

Keywords: 2-Chlorophenol, Cometabolism, Nitrification, SBR reactor.



FCER reactor



Moreno Romá E.J.⁽¹⁾, Burboa Charis V.A.⁽²⁾, Garcia Gomez C.*⁽³⁾

⁽¹⁾ Instituto Tecnológico Superior de Cajeme, Carretera Internacional a Nogales Km 2, Amaneceres, 85024 Cd Obregón, Sonora, México

⁽²⁾ Instituto Tecnológico de Sonora, 5 de Febrero 818, Cd Obregón, Son., México

⁽³⁾ Universidad Autónoma de Nuevo León, Francisco Villa s/n Col. ExHacienda el Canada, General Escobedo, N.L. México

*Corresponding author: celestino.garcia@hotmail.com



The generation of heavy metals such as Zn^{2+} and Cd^{2+} are one of the main problems of pollution of water resources in Mexico, decreasing the quality and quantity of water available to the population. Since metals can not be metabolized, the body tends to accumulate, in fatty tissues of animals. In plants this phenomenon occurs in leaves, stems and roots, which cause serious problems causing the death of living beings¹. The objective of this work is to evaluate the removal of heavy metals in synthetic wastewater by electrocoagulation in a flow-column electrocoagulation reactor (FCER). The design of the continuous reactor was based on the model of Hashim², with modifications of the dimensions; the lengths of reactor are 24.5 cm long, 7.2 cm internal diameter, an operation volume of 997.52 mL. There were five electrodes with a diameter of 6.5 cm and 5×10^{-3} cm wide, each electrode consists of 10 holes for water flow with a diameter of 5×10^{-3} cm each, with two anodes (Al) and three cathodes (Al). A Composite Central Design was made with an extension of Response Surface Methodology (RSM), using Design Expert 7.0. The variables used for the design were the concentration of the contaminant in the range of 20 to 60 ppm, the electrolytic support concentration of 0.25 to 1.25 g / L, current intensity of 0.10 to 0.50 A and the treatment time of 10 to 50 minutes. An experimental matrix of 30 runs was obtained, where Cd^{2+} was the most difficult contaminant to remove due to the experimental conditions that were managed, obtaining a removal of 2 to 80%. On Zn^{2+} , removals from 52 to 99.99% were obtained. The removal of the Zn^{2+} ion, the optimal treatment conditions were not very significant compared to the Cd^{2+} ion, where all the conditions showed high degrees of significance. For the metal Zn^{2+} , the only important variable was the treatment time. In addition, the electrolytic support also showed a degree of significance at the time of removal.

Keywords: Electrocoagulation, heavy metals, Response surface methodology waste water treatment.

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Atrazine half-life determination in water

Juan Napoles-Armenta⁽¹⁾, Luis Alonso Leyva-Soto⁽²⁾, Celestino García-Gómez⁽³⁾, Edgardo Martínez-Orozco⁽¹⁾, Pablo Gortáres Moroyoqui^{*(2)}

⁽¹⁾ Instituto Tecnológico José Mario Molina Pasquel y Henríquez, Avenida José Guadalupe Tejeda Vázquez, 557, Arandas, México

⁽²⁾ Instituto Tecnológico de Sonora, 5 de febrero, 818, Ciudad Obregón, México

⁽³⁾ Universidad Autónoma de Nuevo León, Facultad de Agronomía, Francisco I. Madero, S/N, Ciudad General Escobedo, México

*Corresponding author: pablo.gortares@itson.edu.mx



Water bodies are contaminated by some pollutants found in low concentrations (ng/L or µg/L). Atrazine is an herbicide of interest because of in aquatic species can cause feminization and even toxicity¹. The objective of this work was to calculate the half-life of atrazine in water. In order to check the persistence of atrazine in the water, atrazine degradation was analyzed during 46 days. It was solubilized at a concentration of 7.5 mg/L, in methanol and then in water, atrazine reactive grade with a purity of 99.1%, brand Flukar was used. Different salt concentrations (NaCl) by triplicate were tested (0, 7.5, 15, 22.5 and 30 g/L). Test tubes with a 20 mL thread were used, adding 15 mL of initial volume. The experiment was carried out at 25 ° C, samples were taken on days 0, 1, 2, 3, 4, 5, 7, 9, 12, 15, 20, 29 and 46. The extraction was done from 1 mL sample and adding 2 mL of carbon tetrachloride; stirring and letting rest for phase separation. The aqueous phase remains on the surface, then from the lower phase one milliliter is taken and left to evaporate for 24 hours. The atrazine was resuspended in 1 mL methanol and analyzed by HPLC. The calibration curve received the same treatment as samples. Results were adjusted to first order degradation atrazine model obtaining the half-life for each of sodium chloride concentration. A half-life of 5.13 days was obtained with 0 g/L, 3.87 days with 7.5 g/L, 3.49 days 15 g/L 3.40 days with 22.5 g/L and 4.69 days with 30 g/L of salt. The half-life values are below those reported by Moore *et al.* (2000) who published that the average life of the atrazine is from 16 to 48 days in aqueous medium of wetlands. There are no significant differences among half-life of atrazine with different salt concentration.

Keywords: atrazine, half-life, pollutant, water

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Comparison between two electrochemical reactors based on their

removal efficiency of atrazine in wastewater



Juan Napoles-Armenta⁽¹⁾, Lilian Alejandra Salcedo-Gastelum⁽²⁾, Luis Alonso Leyva-Soto⁽²⁾,
Celestino García-Gómez⁽³⁾, Edgardo Martínez-Orozco⁽¹⁾, Pablo Gortáres-Moroyoqui^{*(2)}

⁽¹⁾ Instituto Tecnológico José Mario Molina Pasquel y Henríquez, Avenida José Guadalupe Tejeda
Vázquez, 557, Arandas, México

⁽²⁾ Instituto Tecnológico de Sonora, 5 de febrero, 818, Ciudad Obregón, México

⁽³⁾ Universidad Autónoma de Nuevo León, Facultad de Agronomía, Francisco I. Madero, S/N,
Ciudad General Escobedo, México

*Corresponding author: pablo.gortares@itson.edu.mx



Water is considered as the main resource for life. Atrazine is an herbicide of interest because of in aquatic species can cause feminization and even toxicity¹. It is an herbicide applied to agricultural land or near roads and railway lines, which a certain amount is entrapment enter the air, soil and nearby waters including groundwater. The objective of this research was to compare the removal of commercial atrazine in synthetic wastewater between a cylindrical reactor and a rectangular reactor, using a response surface methodology to determine the most efficient of them. Electrochemistry is a treatment related to electric currents and chemical reactions, and the conversion of chemical energy into electrical energy and in the opposite direction. Electrooxidation is one of the electrochemical oxidation methods used for the treatment of organic pollutants. Two Ti electrodes and one Ti/PbO₂ electrode were used in each reactor. Time (min), current intensity (A) and recirculation flow (mL/min) were used as independent variables to evaluate the effect over atrazine removal (%) and energy consumption (kWh/m³). Design Expert 7.0[®] program was used to establish twenty treatments per reactor that is a total of forty treatments involving independent variables. Synthetic wastewater containing 5 mg/L of atrazine was used in all experiments. Optimal values for each variable were determined maximizing removal efficiency and minimizing energy consumption. Both reactors were evaluated with the same values of intensity, time and recirculation. Same optimal values (2 A, 180 min, and 200 mL/min) were obtained, in both reactors, with which almost the same removal efficiency was achieved 77.45 % and 76.89 % in rectangular and cylindrical reactor respectively. However, energy consumption showed significant difference 137.45 kWh/m³ and 73.63 kWh/m³ respectively. Therefore, it can be stated that the cylindrical reactor is better option in terms of required energy.

Keywords: atrazine, electrooxidation, treatment, wáter

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Removal of chromium by an isolated microbial consortium of tannery effluents

B. Reyes Romero^{(1) (2)}, J.I. Chairez-Oría⁽²⁾, R. Hernández-Altamirano⁽¹⁾, R. Vázquez-Medina⁽³⁾, D.A. Vivanco Campos^{(1) (2)}, J.A. Cruz-Peláez^{(1) (2)}, S.M. García-Solares,^{(1)*}

⁽¹⁾ Centro Mexicano para la Producción más Limpia IPN, Laboratorio Nacional de Desarrollo y Aseguramiento de la Calidad de los Biocombustibles. Av. Acueducto S/N, Gustavo A. Madero, Ticomán, 07340 Ciudad de México, CDMX. Tel. 57296000, ext 52621.

⁽²⁾ Unidad Profesional Interdisciplinaria de Biología (UPIBI), Av. Acueducto, 550, Ciudad de México, México.

⁽³⁾ Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada IPN, Cerro Blanco 141, Colinas del Cimatario, 76090 Santiago de Querétaro, Qro.

*Corresponding author: smgarciasolares_25@outlook.es; sgarcias@ipn.mx

The tannery industry accomplishes a treatment of animal skins for its transformation into leather by chemical and mechanical processes. The aim is to avoid putrefaction and be able to use it as raw material in various products. This industry is considered as one of the dirtiest, since it consumes large amounts of water, 15 - 20 m³ per ton of raw skin; besides, for each ton of treated skin you get 200 kg of tanned leather, 190 to 350 kg of waste, 200 to 250 kg of tanned leather waste and 50 m³ of wastewater. The conventional processes that are generally applied for the elimination of chromium are expensive and sometimes generate other waste, the biological processes present an alternative for the removal of chromium in wastewater and present good results, since it also removes other contaminants. The aim of this work was to test a microbial consortium isolated from an effluent from the tannery process for the removal of chromium. The removal of chromium was evaluated in bioreactors under anaerobic conditions with a batch operation, the initial concentration of chromium for each reactor was 0.326 mg L⁻¹ and acetate was used as a substrate in three different stoichiometric ratios (chromium: acetate). In the reactor A the moles of excess chromium, reactor B the moles of equal chromium and acetate and reactor C the moles of excess substrate. Analyzes of Chemical Demand of Oxygen, biomass, hexavalent chromium, total chromium and trivalent chromium were carried out in each one reactors. The main results that have been obtained are the highest percentage of chromium removal in reactor C (51.4%), followed by reactor B (43.0%) and finally reactor A (39.1%). In the case of Chemical Oxygen Demand, the highest percentage of removal was obtained for reactor C (51.3%) followed by reactor A (35.6%) and finally reactor B (26.0%). These results confirm the presence of microorganisms able of remove chromium in tannery effluents, increasing their efficiency with an excess of substrate (acetate) in the bioreactors.

Keywords: Biorreactor, chromium, microbial consortium, tannery effluent.

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Optimization of the malathion degradation process by advanced oxidation using an electrochemical reactor

Manuel Antonio Reyes-Prado⁽¹⁾, Jesús Gabriel Rangel-Peraza⁽¹⁾, Yaneth Alejandra Bustos-Terrones⁽²⁾, Leonel Ernesto Amabilis Sosa⁽²⁾, Sait Omar Cristerna Sosa⁽¹⁾

⁽¹⁾ TecNM - Instituto Tecnológico De Culiacán. Av. Juan de Dios Bátiz 310 Pte. Col. Guadalupe, CP. 80220. Culiacán, México.

⁽²⁾ CONACyT-Instituto Tecnológico de Culiacán. Av. Juan de Dios Bátiz 310 Pte. Col. Guadalupe, CP. 80220. Culiacán, México.

*Corresponding author: jesus.rangel@itculiacan.edu.mx ; manuel_reyes1595@hotmail.com



In this study, a parallel electrodes reactor for the generation of OH• ions and the removal of malathion by mineralization was designed, developing the Fenton reaction. A comparison of different materials to be used as electrodes (stainless steel, aluminium and galvanized) was made and the use of reagents or catalysts, the orthogonal arrangement of the Latin square type allowed to know the optimal conditions of the treatment that allows a greater removal of the pesticide. The results showed that the use of aluminum electrodes with dimensions of 2 cm wide x 15 cm long with a thickness of 0.8 mm allows a shorter time in the electrochemical process applying a load of 30V, with these factors, the electrochemical process of 220 min could be carried out. It was also found that the use of H₂O₂ as a reagent provides an increase in the removal of malathion by removing 170 mg/L of malathion present in the samples, which demonstrated the feasibility of treatment for pesticide degradation. Based on the optimal treatment, a study was carried out on the removal kinetics to determine the order in which it belongs, and it was found that it obeys a first-order kinetics, obtaining a degradation constant of 0.0043 min⁻¹.

Keywords: Electrochemical process, degradation kinetics, latin square, malathion.



Chloramphenicol elimination in aquaculture wastewater using

electrooxidation



Romero-Soto I.C.⁽¹⁾, O. Díaz², L.A. Leyva-Soto¹, P. Drogui², G. Buelna³, L. M. Díaz-Tenorio¹, R. G. Ulloa-Mercado¹, P. Gortáres-Moroyoqui^{1,*}

⁽¹⁾Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora. 5 de Febrero 818 Sur, 85000 Ciudad Obregón, Sonora, México.

⁽²⁾Institut national de la recherche scientifique (Centre Eau, Terre et Environnement), Université du Québec, 490 rue de la Couronne, Québec, Qc, Canada, G1K 9A9.

⁽³⁾Centre de recherche industrielle du Québec (CRIQ), 333 Franquet, Québec, Qc, Canada, G1P 4C7.

*Corresponding Author. Pablo.gortares@itson.edu.mx

Nowadays, the use of antibiotics have increased and with it, the bacterial resistance towards them. Chloramphenicol (CAP) is a compound forbidden in Mexico since 2012¹. However, it is still used in animal farming and aquaculture industries. In this study, electrooxidation (EO) process using Ti/PbO₂ anode and Ti cathode was applied to investigate the CAP degradation in real aquaculture wastewater. The experimentation was conducted in a batch reactor agitated by a magnetic stirrer. The CAP measurement was performed by liquid chromatography tandem mass spectrometry (Thermo TSQ Quantum Access). The liquid chromatography separation was achieved in a HyperSil Gold C18 column (Thermo Scientific) heated to 35°C. To obtain and apply the optimal conditions in real wastewater, the CAP removal was evaluated in synthetic solution obtaining the next conditions: current intensity (I) of 0.65 A, treatment time (t) of 34 minutes, and CAP initial concentration of 0.5 mg/L. In real wastewater the antibiotic was not present and, for this reason, 0.5 mg/L of CAP were added and treated by EO using the optimal conditions. Under these conditions, a complete removal of CAP was obtained with an energy consumption of 4.65 kW/h/m³. Also, the presence of NO⁻², NO⁻³, PO₄⁻³, SO₄⁻² and NH₄⁺ were evaluated using standard methods² before and after the process, finding a slight increase of 7.39% in SO₄⁻² and of 41.4% in PO₄⁻³, which may be due to oxidation of the initial organic compounds, coming from different residual nutrients added during feeding in the aquaculture activity and transformed into inorganic forms by the treatment, that were not detected at the initial stage of the process. The NO⁻², NO⁻³ and NH₄⁺ were eliminated by 34.21%, 10.71% and 33.75%, respectively. With these results, EO could be considered as an interesting approach to remove antibiotics and other organic compounds.

Keywords: Aquaculture, chloramphenicol, electrooxidation, wastewater.

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Optimization of heterogeneous photocatalysis with ZnO as a low-cost catalyst. Case study: malathion degradation

Sait C-Sosa⁽¹⁾, Gloria Isabel Peraza⁽¹⁾; Abraham Efraim Rodríguez-Mata⁽²⁾; Jesús Gabriel Rangel-Peraza⁽¹⁾; Leonel Ernesto Amabilis-Sosa⁽²⁾

⁽¹⁾ *Tecnológico Nacional de México-Instituto Tecnológico de Culiacán, Juan de dios Bátiz 310, Culiacán, México.*

⁽²⁾ *CONACYT-Instituto Tecnológico de Culiacán, Juan de dios Bátiz 310, Culiacán, México.*

*Corresponding author: lamabilis@conacyt.mx



Main economic activity in Sinaloa is agriculture, therefore the use of pesticides is a common practice. These substances pollute water bodies that are sources of supply to population and bioaccumulate in fauna, flora and human being. This research evaluated operating parameters of a heterogeneous photocatalysis system with ZnO as a low-cost catalyst, considering the feasibility of being implemented in agricultural drainages. The study tested ZnO concentrations of 100, 200 and 300 ppm, with H₂O₂/DQO ratios of 0.48/1, 0.24/1 and 0.14/1, and irradiation times of 30, 60, 90 and 120 minutes. Synthetic water with 824 mg/L malathion was treated with a UV irradiation chamber of 80 W/m² at 254 nm. Analytical results and factorial design give optimal parameters. ZnO in heterogeneous photocatalysis were 100 ppm, 0.24 H₂O₂/DQO and 120 min of irradiation. With this combination, 96.95% of Malathion and 75% of soluble organic matter are removed. Overall results suggest carry out specific analyses of the catalyst to proceed to its scaling at pilot level and with other pollutants of interest in real agro-industrial wastewater.

Keywords: Agro-industrial wastewater, heterogeneous photocatalysis, organophosphate pesticides, ZnO



Use of *Chlamydomonas* and *Chlorella vulgaris* for the obtain of fatty acids using aquaculture wastewater

Morando-Grijalva, C. A. ⁽¹⁾, Vázquez-Larios, A. L. ^{*(1)}, Alcántara-Hernández, R. ⁽³⁾, Ortega-Clemente, L. A. ⁽²⁾, Robledo-Narváez, P. N. ⁽¹⁾.

⁽¹⁾ Instituto Tecnológico Superior de Tierra Blanca, Veracruz, S/N, Tierra Blanca, México.

⁽²⁾ Instituto Tecnológico de Boca del Río, Carr. Veracruz-Córdoba 12, S/N, Boca del Río, México.

⁽³⁾ Universidad Autónoma de México, Instituto de investigación, S/N, Ciudad de México, México.

*Corresponding author: linevazquez@yahoo.com.mx.



The aim of the present study was the application of *Chlamydomonas* (*Ch*) and *Chlorella vulgaris* (*Cv*) for treatment Aquaculture Wastewater (AW) and the obtaining of fatty acids. The AW previously filtered was characterized to determine NH_4^+ , NO_3^- , NO_2^- , PO_4^{3-} with a HANNA Multiparametric (Model HI83099), once obtained the biomass the lipids extraction was carried using a Soxhlet with a mix chloroform/methanol (1:2 v/v), subsequently derivatization was carried by method Lepage & Roy (1984) for their analysis on gas chromatography (Perkin Elmer, Model Autosystem, Flame ionization), with an Elite-Wax capillary column (30 m in length \times 0.250 mm in diameter)¹. The microalgae cultured on AW showed the removal of 75%, 32%, 95% and 98%, NH_4^+ , NO_2^- , NO_3^- and PO_4^{3-} for *Cv*, respectively. *Ch* showed removal of 57%, 85% and 96%, NO_2^- , NO_3^- and PO_4^{3-} , respectively. Similarly, at the reported by Gutwinski, P. & Cema, G. (2016)² with 36% N and 100% PO_4^{3-} ; and Khotari *et al.*, (2013)³ reported a 90 NH_4^+ , 74 NO_2^- , 90 NO_3^- , 70 PO_4^{3-} ; the AW as a culture medium contains the nutrients necessary for microalgae growth and accumulation of until 69% of lipids and 506 and 927 mg L⁻¹ of biomass for *Ch* and *Cv*, respectively. In addition, *Ch* y *Cv* showed fatty acids profile rich of palmitic acid with a 78 and 35%, respectively, similarly to the reported by Salama *et al.*, (2013)⁴, where they reported a 35% for the same fatty acid. *Chlamydomonas* can be a good alternative for the treatment of AW more due to high nutrients removal percentages, more than 90% for Nitrate (NO_3^-) and Phosphate (PO_4^{3-}). The obtained fatty acids may be used in the elaboration of biodiesel.

Keywords: fatty acids, microalgae, treatment, wastewater.

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Wastewater and acid mine drainage treatment by microbial fuel cells

L.S. Vélez-Pérez ⁽¹⁾, G. Hernández-Flores* ⁽²⁾, O. Solorza-Feria ⁽⁴⁾, O. Talavera Mendoza ⁽¹⁾, H.M. Poggi-Varaldo ⁽³⁾, J.A. López-Díaz ⁽¹⁾, C. Escamilla-Alvarado ⁽⁵⁾, Y. Romero-Ramírez

⁽¹⁾ *Escuela Superior de Ciencias de la Tierra, Universidad Autónoma de Guerrero. Ex hacienda San Juan Bautista s/n, Taxco el Viejo, Guerrero, CP 40323, México.*

⁽²⁾ *CONACYT - Escuela Superior de Ciencias de la Tierra, Universidad Autónoma de Guerrero. Ex hacienda San Juan Bautista s/n, Taxco el Viejo, Guerrero, CP 40323, México.*

⁽³⁾ *Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Environmental Biotechnology and Renewable Energies Group, P.O.Box 14-740, 07000, Mexico City, Mexico.*

⁽⁴⁾ *Dept. of Chemistry, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Delegación Gustavo A. Madero, México, C. P. 07360.*

⁽⁵⁾ *Universidad Autónoma de Nuevo León, Faculty of Chemical Sciences, Parque de Investigación e Innovación Tecnológica, km 10 Autopista al Aeropuerto Internacional Mariano Escobedo, 66629, Apodaca, Nuevo León, México.*

*Corresponding author: gjoheflo_10@hotmail.com , gghernandez@conacyt.mx

In Mexico, nearly 210 m³/s of municipal wastewaters (MWW) as well as an important volume of acid mine drainage (AMD) from mining activities are generated. Organic and inorganic pollutants are found in the MWW and must be oxidized to retrieve some of the water properties. On the other hand, in mining zones the AMD is produced by oxidation phenomena, mainly pyrite oxidation. The AMD is a very aggressive effluent, because of its very low pH, high concentrations of heavy metals, SO₄⁼, NO₃⁻, among other pollutants. Thus, the aim of this research was to evaluate the simultaneous treatment of MWW and AMD in a dual-chamber microbial fuel cell (DC-MFC). The latter was a plexiglass cylinder with a total volume 128 mL, 64 mL each chamber. Nafion® 117 was the proton exchange membrane (PEM). Graphite rods were used as anode and cathode. The MWW was sampled from the influent to a wastewater treatment plant and was used as fuel in the anode section whereas AMD from a mine was filled in the cathodic chamber. Anaerobic sludge was used as inoculum in the anodic chamber. One MFC was connected to an external resistance (R_{ext}) of 100 Ω. The second MFC was used as reference filled with the same influents. Chemical oxygen demand (COD), volatile suspended solids (VSS), pH, alkalinity and electrolytic conductivity (EC) were monitored in the influent and effluent of the anode chamber. The same parameters plus the concentrations of SO₄⁼, NO₃⁻ and heavy metals were analyzed in the influent and effluent of the cathodic chamber. COD removal was 15% in both cells. The pH of the AMD increased from 2.50 up to 4.16 and 4.80 for the active and control MFC, respectively. Also the SO₄⁼ and NO₃⁻ concentrations were reduced by 14 and >90% in both cells. Heavy metal contents decreased for some heavy metals. In spite of the short period in operation (120 h), our results show the simultaneous treatment of MWW and partial neutralization of AMD in a MFC is a promising alternative for the remediation of these effluents.

Keywords: Acid mine drainage; Bioelectricity; Heavy metals removal; Microbial fuel cells.



Debaryomyces hansenii F39A as biosorbent for textile dyes removal

F Ruscasso⁽¹⁾, B Bezus⁽¹⁾, M Acosta⁽¹⁾, G Garmendia⁽²⁾, S Vero⁽²⁾, **G Curutchet**⁽³⁾, I Cavello^{(1)*}, S Cavalitto⁽¹⁾.

⁽¹⁾ CINDEFI (UNLP, CONICET – La Plata). 50 y 115, La Plata, Bs As - Argentina.

⁽²⁾ Cátedra de Microbiología, Departamento de Biociencias, Facultad de Química, Universidad de la República, Montevideo, Uruguay.

⁽³⁾ Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, Buenos Aires, 1650, Argentina and CONICET

*Corresponding author: icavello@biotec.quimica.unlp.edu.ar



Many industries, such as textile, paper and plastics, use dyes in order to color their products and consume substantial volumes of water. As a result, they generate a considerable amount of colored wastewater. Due to increasingly stringent restrictions on the organic content of industrial effluents, it is necessary to eliminate dyes from wastewater before it is discharged. Wastewater containing dyes is very difficult to treat, since the dyes are recalcitrant organic molecules, resistant to aerobic digestion, and are stable to light, heat and oxidizing agents. Recently, numerous approaches have been studied for the development of cheaper and effective adsorbents. In this context, yeasts have been successfully employed in textile dye treatment through biosorption mechanisms. Their success is largely based on their unicellular nature, along with high growth rates and the capability to grow into inexpensive growth media. The main objective of this research was to examine the biosorptive capacity of the Antarctic yeast *Debaryomyces hansenii* F39A biomass for a series of commercially available dyes. Reactive blue 19 and Reactive red 141 were chosen due they represent different dyes groupings including double azo class, anthraquinone and reactive class. Variables including; pH, dye concentration, amount of adsorbent and contact time were studied. The equilibrium sorption capacity of the biomass increased with increasing initial dye concentration up to 300 mg/l for Reactive Red 141 and up to 350 mg/l for Reactive Blue 19, respectively. Experimental isotherms fit the Langmuir model and the maximum uptake capacity (Q^0) for the selected dyes was in the range 110-125 mg/g biomass. At initial dye concentration of 100 mg/l, 2 g/l biomass loading and $20 \pm 1^\circ\text{C}$, *D. hansenii* F39A adsorbed around of 90 % of Reactive Red 141 and 50 % of Reactive Blue 19 at pH 6.0. When biomass loading was increased (6 g/l), the uptake reached up to 90 % for Reactive Blue 19. Dye uptake process followed the pseudo-second-order kinetics for each dye-system studied. It may be concluded that a biosorption process could be adopted as a cost effective and efficient approach for decolorization of effluents and it may be an alternative to more costly materials such as activated carbon. Yeasts undoubtedly have the potential to efficiently and effectively remove dyes.

Keywords : Antarctic yeast- Biosorption-Reactive dyes.



Characterization of microorganisms in suspended and immobilized

biomasses in the anaerobic treatment of tequila vinasses



A. Serrano Meza ⁽¹⁾, M. A. Garzón Zúñiga* ⁽¹⁾, B. E. Barragán Huerta ⁽¹⁾, E. B. Estrada Arriaga ⁽²⁾, J. G. García Olivares ⁽³⁾, N. Almaraz Abarca ⁽¹⁾.

⁽¹⁾ Instituto Politécnico Nacional-Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Durango, Sigma N°119, Fracc. 20 de Noviembre II, Durango, México ^{(1) (2)}
⁽²⁾ Instituto Mexicano de Tecnología del Agua, Paseo Cuauhnáhuac No. 8532, Progreso, Jiutepec, México

*Corresponding author: marco.cuerna@gmail.com



Anaerobic treatments of tequila vinasses have been carried out in bioreactors with suspended biomass, immobilized biomass (biofilm) and a combination of both known as hybrid systems¹. The characterization of the bacteria present in a hybrid system has not been developed. Thus, a submerged anaerobic biofilter (SAB) packed with tezontle was assessed in the treatment of tequila vinasses in order to identify by metagenomics the bacterial communities present in the process. The SAB was operated discontinuously, at 32±2 °C and pH range of 6.9-7.9. During the operation of the SAB, the effects of initial concentration of vinasses (55 and 40%) and HRT (48 and 168 h) on the organic matter removal were assessed. When the SAB was operated at initial concentration of 55% and HRT of 48 h (day 80 of operation, with a volumetric organic load (VOL) of 11.2 Kg COD/m³d and organic matter removal efficiency of 70%), the suspended biomass was mainly conformed by Bacteroides and Clostridium, with a proportion of 19 and 17%, respectively. When the initial concentration was 40% and the HRT 168 h (day 209 of operation, with a VOL of 2.2 Kg COD/m³d and organic matter removal efficiency of 43%), the suspended biomass was conformed by Bacteroides (43% of proportion) and Clostridium (21% of proportion), and the biofilm was conformed by Clostridium (43%), Methanosarcina (9%), and Desulfovibrio (7%).

The results indicated that the SAB has a higher proportion of microorganisms that produce VFA (Bacteroides) and H₂ (Clostridium). In a lower proportion, it has CH₄ producers (Methanosarcina) and sulfate reducers (Desulfovibrio). In concordance with these results, the VFA and H₂ production measured in the BAS was 5,580 mg/L and 1.2 ml H₂/g COD. There was not CH₄ production observed. Based on the bacterial communities identified and the VFA and H₂ production, it was demonstrated that acidogénesis is taken place during the treatment. Methanogenesis was not observed due to the presence of sulfate reducers microorganisms which compete with methanogens for the substrate², inhibiting methane production. However, the H₂ production in the SAB is an advantage because renewable energy might be produced from this gas.

Keywords: acidogenesis, H₂ production, clostridium, sulfate reduction.

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Ampicillin biotransformation by a nitrifying consortium

J.J. Ramírez Muñoz⁽¹⁾, D.I. Bejarano Ortiz⁽²⁾, F.M. Cuervo López⁽¹⁾, A.C. Texier⁽¹⁾

⁽¹⁾ UAM-Iztapalapa, Department of Biotechnology, Av. San Rafael Atlixco, N° 186, CDMX, México

⁽²⁾ CINVESTAV-IPN, Av. IPN, N° 2508, San Pedro Zacatenco, CDMX, México

*Corresponding author: actx@xanum.uam.mx



More than half of the antibiotics produced worldwide are β -lactams and many of them have been detected in effluents from sewage treatment plants, including ampicillin (amp)¹. The pollution of water by nitrogen is mainly due to the presence of ammonium (NH_4^+) in wastewater, and its elimination in treatment plants is mainly carried out through biological processes such as nitrification and denitrification. Studies on the biotransformation of amp by nitrifying sludge and the effect of this antibiotic on nitrification are scarce. The physiological and kinetic behavior of a nitrifying consortium in the presence of amp (10, 25, and 50 mg/L) was evaluated in batch cultures. Under the experimental conditions (320 ± 8 mg bacterial protein/L, C/N = 2.4, 24 h), the sludge behavior was very similar in the controls without amp and the assays with antibiotic and there was no effect on efficiency ($E_{\text{NH}_4^+} = 99.7 \pm 4.2\%$) and yields ($Y_{\text{NO}_2^-} = 0$, $Y_{\text{NO}_3^-} = 1.0 \pm 0.1$ mg N/mg N- NH_4^+ consumed) of nitrification. Specific rates of both NH_4^+ oxidation and NO_3^- formation were similar between controls and cultures with amp, showing that amp did not cause inhibition on nitrification. The insensitivity to amp of the nitrifying bacteria may be explained through the bacteria's slow growth and other survival strategies². The elimination of amp was mainly attributed to physico-chemical processes (16.0-16.5%), biosorption (23.2-47.0%), and biotransformation (10.0-29.8%). With the increase in the initial amp concentration, there was a greater participation of the biotransformation process, associated with an increase in the specific amp consumption rate. The sludge was able to oxidize NH_4^+ to NO_3^- by nitrification and eliminate amp biologically, but without reaching full mineralization.

Keywords: Ampicillin, Biotransformation, Biosorption, Nitrification.

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Ability of MCM-41-based sorbents to remove and reduce hexavalent chromium from different water matrices

P. P. Martin⁽¹⁾, M. F. Agosto⁽¹⁾ and N. Fellenz^{*(1)}

⁽¹⁾ Centro de Investigaciones y Transferencia de Río Negro, CONICET, Rotonda Cooperación y Ruta Provincial 1, Viedma, Río Negro, Argentina

*Corresponding author: nfellenz@unrn.edu.ar



Chromium compounds are used in the leather tanning industry, the manufacture of catalysts, pigments and paints, fungicides, ceramic, in photography and for chrome alloy and chromium metal production, chrome plating, among others industrials uses. In aqueous environments chromium can be found mainly as hexavalent (Cr(VI)) and/or trivalent (Cr(III)). Between them, Cr(VI)-based compounds are many times more toxic than the Cr(III) species. Due to this, strategies for remediation of wastewater with high Cr(VI) levels involving a mixed mechanism of retrieval-reduction of Cr(VI) to the less toxic trivalent state, present a clear advantage versus those that only achieve separation without chemical transformation. Recently, we demonstrated that aminopropyl-modified MCM-41 systems have the ability to adsorb and reduce HCrO₄⁻ from distilled water at acidic conditions. However, seeking to develop sorbents for the treatment of aqueous waste similar to those generated in the aforementioned industries, it is necessary testing these kinds of solids for the treatment of complex aqueous matrices that contain, in addition to chromium, other chemical species. With this goal in mind, in this work we report the synthesis and characterization of an MCM-41-NH₂ and its performance to remove and reduce Cr(VI) in the presence of Fe, Na, Ca and Zn salts. It was found that small amounts of cations affects both the ability to reduce and to adsorb chromium, and that happens in different degrees depending on the valence of the metal and the counter ion. Zn and Ca completely inhibits the ability to reduce Cr(VI) to Cr(III), while Na and Fe decrease it. In the absence of salts, the total Cr(VI) elimination capacity was 72.7 mg per gram of MCM-41-NH₂, and decreases to 56.7, 52.2 and 41.3 due to the presence of sodium, calcium and iron, respectively. We give possible explanations for these results considering different sorbates-sorbent interactions.

Keywords: MCM-41, Hexavalent chromium, Water remediation.

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Martínez-Macias ⁽¹⁾, Y. Villegas-Peralta ⁽¹⁾, R.J. Aguilar-Ruiz ⁽¹⁾, D. I. Sánchez-Machado ⁽¹⁾, J. López-Cervantes ⁽¹⁾, M. A. Correa-Murrieta ⁽¹⁾
⁽¹⁾ Instituto Tecnológico de Sonora, Av. 5 de febrero, 818 sur, Cd. Obregón, Sonora, México.
*Corresponding author: maria.martinez@itsn.edu.mx



Acid mine drainage continues being an important water pollution problem in the worldwide mining industry. When mine processes dump acid water discharge (pH 2 to 4) into rivers and lakes, and they are uncontrolled, causes environmental problems due to high amounts of metals and metalloids. In some cases, during the leaching process, avoiding the water runoff that spreads contaminants to larger bodies of water is impossible 1. In regions where numerous mines are operating, this occurrence is a common problem. Large volumes of acidic water cause losses in biodiversity, severely damaging the economy of a region. Different technologies have been applied to remove heavy metals from acid mine drainage, including the adsorption 1. However, these methods are often ineffective and/or very expensive when are used to reduce heavy metal ions at very low concentrations. Microalgae can sequester heavy metal ions by adsorption. Selected algae have been confirmed to be effective biosorbent materials 4. Yang et al., (2015) achieved a success with *Chlorella minutissima* UTEX2341, removing $83.60 \pm 0.09\%$ and $82.38 \pm 0.07\%$ of copper to concentrations of 0.2 mM and 0.4 mM, respectively, which corroborated its application as an adsorbent. In this sense, the aim to this study was to determinate the capacity of dry biomass marine-water microalgae, *Nannochloropsis oculata*, to adsorption copper from water, and the effects of pH, contact time and biomass weight. *N. oculata* culture were carried out at tubular photobioreactors with saline water, light intensity of $166 \mu\text{E m}^{-1}$ by 24 hours and controlled temperature of 25-26 °C; the medium used was the Algal medium (4mM of N2-2s), and its was added every 72 hours. The biomass was harvested and used to determine its adsorption capacity of copper. Subsequently, the samples were analyzed by spectrometry UV-VIS, and copper was quantified by comparison with authentic standard mixtures (AOAC 2005). At first, the biomass weight and contact time was defined and the pH was varied. After, the pH was defined, the biomass weight was varied to a defined time, and finally, when the pH and biomass weight were defined, the contact time was varied. Results showed that the best conditions were at pH=5, biomass weight of 0.45 g and contact time of 2 h. Under these conditions, the adsorption capacity of the microalgae biomass was 78.14%, which represented an important finding. In conclusion, the biomass of microalga *N. oculata*, is a suitable bioadsorbent candidate to the removal of metal from wastewater.

Keywords: Microalgae-adsorption-Metal.

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Oxytetracycline biosorption and degradation by *Chlorella* sp

Burboa Charis, V. A.⁽¹⁾, Ulloa Mercado, R.G. ^{*(1)}, Hernández Chávez, J. F. ⁽³⁾, Leyva Soto, L. A.⁽¹⁾, Gortáres Moroyoqui, P. ⁽¹⁾, Serrano Palacios, D. ⁽²⁾, Meza Escalante, E.R. ⁽²⁾

⁽¹⁾ Dpto. Biotecnología y Ciencias Alimentarias,

⁽²⁾ Dpto. Ciencias del Agua y Medio Ambiente,

⁽³⁾ Dpto. Ciencias Agronómicas y Veterinarias. Instituto Tecnológico de Sonora, Av. Antonio Caso 2266, Cd Obregón, Sonora, México.

*Corresponding author: ruth.ulloa@itson.edu.mx



The increase in intensive animal production for obtaining food for human consumption [1] has led to an indiscriminate use of antibiotics, especially Oxytetracycline (OTC). The use of integrated systems with microalgae presents important advantages, since they can directly biodegrade the contaminants through heterotrophic metabolism [2], they can also improve biodegradation indirectly through symbiosis with bacteria [3]. The objective of this work was to evaluate the biodegradation, photodegradation, adsorption and desorption of OTC by *Chlorella* sp. Bioassays were carried out in Bold medium with *Chlorella* sp. at concentrations of 0.4, 0.8 and 1.6 mg/L of OTC, in 800 mL reactors, with an initial density of 2×10^6 cell/mL, for 10 days; a daily cell count was performed in the Neubauer Chamber to determine the growth rate (μ) and the consumption rate (mg OTC/mg of dry biomass). An abiotic test was performed to determine the photodegradation of the antibiotic in three different conditions, with a concentration of 1 mg/L of OTC; 10 mL of sample was taken, centrifuged and filtered with 0.45 μ m membrane for both tests; the quantification of OTC was by capillary electrophoresis. The biomass of the bioassays was collected to evaluate the adsorption and absorption by the solid phase extraction method [4]. Kinetics with inactive biomass was performed at a concentration of 2 mg/L for 72 hours to determine adsorption. The samples were filtered through 0.22 μ m pore size and analyzed on HPLC. The bioassays in bold basal medium with *Chlorella* sp., added with OTC, the microalga under study showed inhibition in the presence of different concentrations of OTC, decreasing its growth rate by increasing the concentration of the antibiotic compared with the control. In 72 hours, the presence of the antibiotic in the aqueous phase was not detected, which can not be attributed to degradation by algal activity, since it can be adsorbed and absorbed by the microorganism. However, the light showed a significant effect on degradation of the pollutant; We can establish that there is a photodegradation of the antibiotic.

Keywords: Antibiotics, Microalgae, waste-water treatment, micropollutant.

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Influence of toilet paper and food waste addition in Anaerobic digestion of domestic sewage

Devson Paulo Palma Gomes ⁽²⁾*, Manuella Lopes Figueiras ⁽¹⁾, Ana Carolina Santos Freire Bonfim ⁽²⁾, Simone Machado Santos ⁽¹⁾, Sávia Gavazza ⁽³⁾

⁽¹⁾ Federal University of Pernambuco – Technology Center, Rodovia BR-104, Km 59, s/n - Nova Caruaru, Caruaru, Brasil.

⁽²⁾ Federal University of Pernambuco – Civil Engineering Department, Recife, Brasil.

*Corresponding author: devsonp@hotmail.com



Materials like food waste (FW) and toilet paper (TP) require adaptation of the traditional wastewater treatment when disposed into sewage. The influence of flushing these materials is well known when activated sludge (aerobic process) is used as treatment technology. However, in some tropical countries anaerobic technology is mainly applied for sewage treatment. In this way, this work aimed to evaluate the influence of toilet paper and food waste in anaerobic digestion of domestic sewage in UASB reactor. Three UASB kind reactors were fed with synthetic domestic sewage, but one with addition of Toilet Paper (TPR), the other with Food Waste (FWR) and a third control reactor got sewage only. At TPR 0.42 g/L of TP was added in Phase I (PI - OLR of 0.97 kg COD.m⁻³.d⁻¹), while in Phase II the amount added was 0.81 g/L of TP (PII - OLR of 1.58kg COD.m⁻³.d⁻¹). The addition to FWR were 2.0 g/L of FW for PI and 3.6 g/L of FW for PII, resulting in OLR similar to those of TPR in each phase. Triturated organic waste was used as FW (WRAP, 2008). COD, cellulose, glucose, solids, pH, alkalinity, VFA, and methane are among the monitored parameters over the 110 days of reactors' operation. The average raw COD removal efficiency were 79±1% and 75±2%, in PI and PII, respectively for TPR, with corresponding values of 81±2% and 76±1% for FWR. Both reactors decreased in COD removal efficiency after loading the Phase II and showed less removal of organic material efficiencies when compared to the control reactor in PII (83%). Therefore, both reactors showed high organic load removal despite of different compositions of the effluents (TP and FW), making possible the release of these materials in domestic sewage.

Keywords: Toilet paper; Food waste; Anaerobic digestion; Domestic sewage.

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Removal of emerging pollutants (drugs) from hospital wastewater in organic bed biofiltration systems coupled to an electro-oxidation process

Javier Alejandro Navarro Franco ⁽¹⁾, Marco Antonio Garzón Zúñiga* ⁽¹⁾, Patrick Drogui ⁽²⁾, Pablo Gortares Moroyoqui ⁽³⁾, Gerardo Buelna ⁽²⁾.

⁽¹⁾ Instituto Politécnico Nacional, CIIDIR- Unidad Durango, Calle Sigma, 119, Durango, México.

⁽²⁾ INRS-ETE;

⁽³⁾ ITSON

*Corresponding author: marco.cuerna@gmail.com



Emerging pollutants (EPs) are chemicals, for example, drugs, which are introduced into the environment mainly by the effluents of conventional wastewater treatment plants (WWTPs) (mostly activated sludge systems) as these systems not remove EPs efficiently. The constant emission of EPs in the environment worries about the possible adverse effects that they could cause in the long term in aquatic life and human health. Among the drugs that stand out are carbamazepine (CBZ) for being highly persistent, recalcitrant and for its potential ecotoxicological risk and gemfibrozil (GFZ), a lipid regulating agent that has been reported as a toxic component with carcinogenic effects. Therefore, it is necessary to improve the removal efficiencies of the WWTPs or to propose new technology. In this sense, the objective of this work is to evaluate the removal efficiency of CBZ and GFZ from hospital wastewater (HWW) through a proposed hybrid system composed by a biofilter coupled to an electrooxidation process. In order to fulfil de objective of this work a series of task have been made: First the HWW was characterized; The biofiltration system was designed and built in order to evaluated the effect of the hydraulic surface load from 0.19 to 1.0 m³/m² d and the type of packing material, in this case volcanic rock (tezontle), plastic rings and wood chips of mesquite. Design and construction of electrooxidation cells were carried out to assess the effect over the EPs removal efficiency of the next parameters: 1) current densities between 10-200 mA/cm²; 2) hydraulic retention time between 60-240 min, 3) coverage of electrodes, the Ir-Ta and PbO₂ electrodes and 4) the configuration of the cells, one square and one cylindrical. Both EPs CBZ and GFZ will be detected and quantified by GC/MS. The analytes were chemical derivatized with pentafluorobenzyl bromide. To separate the analytes a column with composition of 5%-diphenyl-95%-dimethylpolysiloxane was used. The preliminary results of this investigation include the successful detection of the standard (10 mg/L) of both drugs. Being the retention time of each analyte 9.197 and 11.291 min for CBZ and GFZ, respectively. A sample of HWW was later injected in the GC-MS equipment and CBZ was detected, but not GFZ. The area below the peak curve of CBZ was about 30 times larger than that detected in the standard of 10 mg/L, which indicates tentatively a high concentration in the real HWW. The EPs quantification would be performed by elaborating calibration curves in a next step. The biofilters were started- up and after 90 operational days, they have behaved as expected. The biofilters with inorganic packing materials (tezontle and plastic rings) had presented since the start up an important removal efficiency of organic matter over 90% and 75% respectively. While the biofilter packed with wood chips presented a washing effect of humic and fulvic acids which add organic matter to the effluent, therefore, until day 47 of operation there was no organic matter removal but by day 90 the removal efficiency reached 15%. In terms of drug analyzes, it was possible to detect the CBZ and GFZ standards by GC-MS and CBZ in the real HWW. The next step is to quantify the effect of the packing materials in the removal efficiency of these drugs.



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Study of metal ions removal in aqueous solutions and industrial

wastewater using low cost adsorbents



P. M. Navarro Quintana ⁽¹⁾, S.I. Sáez Elgueta ⁽¹⁾, B.H. Quilodrán Toloza ⁽²⁾ *

⁽¹⁾ Environmental Engineering, Natural Resources and Environment Department, Universidad de Los Lagos, camino a Chinquihue km.6, Puerto Montt, CHILE.

⁽²⁾ Natural Resources and Environment Department, Universidad de Los Lagos, camino a Chinquihue km.6, Puerto Montt, CHILE.

*Corresponding autor: bquilod@ulagos.cl



Pollution by heavy metals, such as Cu (II), Cd (II) and total Cr, toxic to marine and human life, is mainly due to anthropogenic activities, for example, fish farming, leather industry, mining, among others. Heavy metals are not biodegradable and at high concentrations generate problems in soil fertilization and water treatment. An alternative to reduce pollution levels is the removal¹, in this study two low-cost adsorbents are proposed and their presence in the environment generates a negative impact: the activated carbon synthesized from the chacay (*Ulex europaeus*), plant that is causing damage to the soil, there is still no biological or mechanical control that has been able to finish or minimize the impacts that the invasive species and the beer bagasse, by-product of the beer manufacturing industry, as an alternative for the removal of heavy metals contained in aqueous solutions. For the experimental trials, physico chemical parameters were evaluated (pH effect, adsorbent dose, equilibrium time, isotherms), the method used was batch treatment, using a thermo-adjustable stirrer and the concentration of metal ions were measured by Atomic Absorption Spectroscopy (AAS). The results obtained for the removal of metal ions in aqueous solutions by activated carbon from chacay (CCh) and beer bagasse (BC), reached 89.9% and 90.9% for Cadmium, 77.6% and 93.8% for Copper, 65.5% and 100% for Chromium, respectively. In addition, the equilibrium data were analyzed, with BC the equilibrium begins at 15 minutes for chromium and cadmium and copper achieves it at 30 minutes, with CCh the equilibrium is reached before 30 min for the three metal ions. The Langmuir isotherm showed and adsorption capacity in CCh of 136.99 mg/g for Cu (II), 151.5 mg/g for total Cr and 78.12 mg/g of Cd (II), and in BC, the adsorption capacity for Cu (II) was 8.36 mg/g, 28.5 mg/g of total Cr and 769.23 mg/g of Cd (II). The removal of Cu (II) in Fishnet farming wastewater treated with CCh and BC, yielded 54.6% and 84.97%, respectively. The total chromium removal in Tannery wastewater treated with CCh and BC reached 61.40% and 95.17%, respectively. Overall, these results suggest that green vegetable waste derived activated carbon as a low- cost adsorbent for the removal of Cu (II), total Cr and Cd (II) will be useful for future scale-up for the tertiary treatment of wastewater.

Keywords: adsorption, wastewater, by-product, activated carbon.

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Hydrodynamic evaluation of a thin-layer photobioreactor for piggery wastewater treatment

Aguilera-Torres A.⁽¹⁾, Serrano-Palacios D.⁽¹⁾, Ulloa-Mercado G.⁽¹⁾, Almazán-Ruiz F.^(2,3), Plascencia-Jatomea R.⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 5 de Febrero, 818 sur, Ciudad Obregón, Sonora, México

⁽²⁾ Departamento de Ingeniería y Tecnología, FES Zaragoza, UNAM. C.P. 09239, Iztapalapa, CDMX.

⁽³⁾ Departamento de Química, Universidad Autónoma Metropolitana, San Rafael Atlixco 186, C.P. 09340, Iztapalapa, CDMX.

*Corresponding author: rigoberto.plascencia@itson.edu.mx

Mixing the culture medium is necessary to avoid gradients in nutrient concentration, temperature and pH. It also prevents microalgae sedimentation, increases liquid-to-cell mass transfer rates, and enhances algal photosynthetic efficiency. In fact, mixing continuously moves the algal cells between the well-lit zone and the dark zone, what reduces photolimitation and photoinhibition [1]. The aim of this work is to evaluate the hydrodynamic of the photobioreactor in order to improve its performance by diminishing channeling and stagnant zones. The mixing flow pattern was studied by means residence time distribution (RTD) experimental curves in the thin-layer photobioreactor to visualize and determine the mixing flow pattern in the liquid phase. The stimulus-response technique was employed using an inorganic salt (NaCl, 20 g/L) as model tracer [2]. Different flow rates (50-150 mL/s) were evaluated. A theoretical analysis and approximation RTD experimental curves with tanks in series (TIS), axial dispersion model (ADM), plug dispersion exchange model (PDE) and by solving the hydrodynamic equations using Computational Fluid Dynamics, were used in order to establish a better approximation of stagnant zones, channeling and by-pass (preference flow) effects present in the reactor. RTD curves show that the liquid flow pattern of the thin-layer photobioreactor exhibits channeling and stagnant zones. The PDE model represents fairly these deviations from ideal flow.

Keywords: PDE, RTD, wastewater treatment, thin-layer photobioreactor.

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Nutrient recovery and biogas generation from the anaerobic digestion of waste biomass from algal biofuel production



Pedro Ayala-Parra⁽¹⁾, Jim A. Field⁽²⁾, Reyes Sierra-Alvarez^{*(3)}

⁽¹⁾ Universidad de Sonora, Lázaro Cárdenas 100 Colonia Rosales Navojoa Sonora, México

⁽²⁾, ⁽³⁾ Department of CHEE, The University of Arizona, P.O. Box 210011, Tucson AZ, USA

*Corresponding author: rsierra@email.arizona.edu



Microalgae are gaining popularity as a source of biodiesel. Recycling fertilizer nutrients is critical to sustain large-scale biodiesel production because the global supply of surplus fertilizer is limited. This study demonstrates that anaerobic digestion of residual algal biomass from biodiesel production can provide additional nutrients and energy. Anaerobic digestion of *Chlorella sorokiniana* 1412 whole cell algae (WCA), sonicated algae (SA), and SA subjected to lipid extraction (LEA) in bench-scale batch reactors operated at $30 \pm 2^\circ\text{C}$ for 42 days released a considerable amount of the nitrogen and phosphorus in the algal cells. Digestion of WCA, SA, LEA released 48.1, 77.4, and 61.5% of the total algal nitrogen as $\text{NH}_4^+\text{-N}$, and 87.7, 99.4, and 93.6% of the total algal P as soluble P, respectively. The energy recovery from algae biomass was quantified through the methane yield. The biochemical methane potential of WCA, SA and LEA was 0.298, 0.388 and 0.253 L methane/g algal volatile solids, respectively. The conversion of LEA and WCA biomass to methane was very similar (38 and 41% on a COD basis, respectively), indicating that the energy yield was not significantly lowered by extraction of the lipid fraction (which accounted for 9% of algal dry weight). Sonication improved the access of hydrolytic enzymes to algal biopolymers (compensating in part for the energy lost due to lipid extraction). The results taken as a whole indicate that anaerobic digestion of LEA can provide considerable yields of methane and soluble nutrients.

Keywords: Methane, nutrient recovery, nitrogen, phosphorous, algal biofuels.



Removal of Hexavalent chromium by chromate tolerant halobacteria

isolated from saline soils in Sonora, Mexico



Yépiz Gonzalez Armando⁽¹⁾, Estrada Alvarado María Isabel⁽¹⁾, Vásquez Murrieta Maria Soledad⁽²⁾,
Cira Chávez Luis Alberto⁽¹⁾.

⁽¹⁾ Instituto Tecnológico de Sonora, 6 de abril, 818. Ciudad Obregón, Mexico. ² Instituto Politécnico
Nacional, Manuel Carpio, 471. Ciudad de Mexico, Mexico. C.P. 85000

*Corresponding author: luis.cira@itson.edu.mx



Rapid urbanization and industrialization has resulted in large quantities of contaminants such as toxic metals to be released into the environment and represent a hazard for all forms of life. Chromium is widely used in industrial activities whose effluents often contain its most toxic and mobile form (VI). Traditionally, these effluents are treated by physical-chemical methods that are often expensive, inefficient and result in the generation of other toxic waste. Because of this, there is great interest in the development of environmentally friendly technologies such as bioremediation. Extremophiles, particularly halophiles, are of great interest for this purpose due to their ability to thrive in harsh conditions such as high salinity, pH, presence of organic contaminants, and toxic metals, among others. The present work consisted in the screening of halobacteria for chromate tolerance and removal, as well as the identification of mechanisms involved in the detoxification process such as production of exopolysaccharides (EPS) and siderophores. 10 strains were found to grow in presence of chromium ranging from 15 to 40 mM. 6 Strains (M2P, M1Q, BLSS1AM2035, N31, 30042 and YRAM72) showed a decrease of over 95% of Cr(VI) in liquid media after 120 h. There was no production of EPS by any of the strains. All strains showed production of siderophores in presence of Cr(VI), which could indicate the involvement of these molecules in the mobilization of the metal for the detoxification process. These results indicate that at least 6 of the strains assayed in this work could be interesting candidates for their use in hexavalent chromium remediation processes.

Keywords: Bioremediation, chromium, extremophile, halophile.



Removal efficiency of organic matter in a sequential batch activated sludge reactor for treatment of municipal wastewater

A. N. Salinas-Pacheco⁽¹⁾, C. Estrada-Vázquez*⁽¹⁾, E. Peralta-Reyes⁽¹⁾, A. R. Galado-Méndez⁽¹⁾
⁽¹⁾ Universidad del Mar (UMar) campus Puerto Ángel, Ciudad Universitaria, Puerto Ángel, Distrito de San Pedro Pochutla, Oax., México
*Corresponding author: carlosesvz@yahoo.com



This pilot-scale study aimed to test the removal efficiency of organic matter in a sequential batch activated sludge reactor (LOA-SBR) for treatment of diluted municipal wastewater, from small population; this population includes cafeteria, habitable zone and library areas of the UMar campus. The bioreactor was operated in a 24 h cycle with an aeration time of 22.4 h at constant flow rate, for a period of 365 days. The environmental conditions that mentioned the reactor operated was established from a multiparameter, resulting in a pH of 7.96, a temperature of 26.9 °C and electrical conductivity of 1700 mS/cm. The average results obtained show that the reactor had a removal efficiency of organic matter expressed as COD of 68.1%, with a sludge concentration of 3100 mg/L and a volumetric sludge index of 120 ml/mg. In spite of the high variation of organic matter concentration in the tributary (with COD values ranging from 180 to 540 mg O₂/L), the LOA-SBR showed no impairment in its functioning. The color of the mud remained brown, there was no presence of odors in the effluent. The difference in color between the affluent and the effluent was visible. The accumulation of solids at the edges was minimal and there was no foam on the surface of the reactor. Finally, the sludge concentration was modified to improve the operation of the reactor in concentrations of 3000, 2500 and 2000 mg/L, with an organic load of 2500 mg O₂/L. It was observed that decreasing the sludge concentration at 2500 mg/L, the reactor efficiency increases up to 70% organic matter removal. In conclusion, the removal efficiency of organic matter in an LOA-SBR reactor tested is low compared with other reactor types described in literature, so it is necessary to improve the operating variables of the tested reactor to raise the efficiency and that this type of reactors will be useful in small populations where the generation of this type of wastewater is common.

Keywords: activated sludge, pilot-scale, SBR, wastewater.



Multivariable Robust Regulation of Alkalinities and VFA in Continuous AD Processes against both Uncertain Kinetics and Operational Perturbations

V. Alcaraz-González^{*(1)}, F. A. Fregoso-Sánchez⁽¹⁾, V. González-Álvarez⁽¹⁾, J.P. Steyer⁽²⁾

⁽¹⁾ University of Guadalajara, Blvd. Gral. M. García Barragán 1451, 44430, Guadalajara, Mexico

⁽²⁾ LBE, INRA, 102 Avenue des Étangs, 11100, Narbonne, France

*Corresponding author: victor.alcaraz@cucei.udg.mx



In this paper we propose a simple but powerful robust Multiple-Input, Multiple-Output (MIMO) approach for regulating alkalinities, volatile fatty acid concentration and the strong ions concentration in continuous anaerobic digestion processes used in wastewater treatment. The system exhibits a large lack of knowledge on kinetic functions and their parameters. Moreover, these functions exhibit a very high nonlinear behavior. In addition, key state variables, like biomass concentrations, which cannot be measured, are estimated by using a Luenberger observer. Furthermore, process inputs are not well known. Thus, even under this highly uncertain scenario, the proposed control law uses minimal and partial available information for regulating exponentially the control variables stated beforehand. The control objectives are firstly established upon physicochemical criteria based on normal operational conditions and then formally stated in terms of the concerned state variables. In order to deal with these important uncertainties, the proposed control law is based upon the basis of adaptive robust exponential approaches¹. Two control inputs are used: the dilution rate and a supplementary NaOH supply. The resulting control law is tested and validated experimentally in a 1 m³ pilot plant for the treatment of red wine vinasses under the several uncertain scenarios established above. Results show that the multivariable control law is able to recover the system stability around the pre-specified set points despite this large variation in the process inputs as well as the high uncertainty in the process inputs and kinetics, showing a very high performance and usefulness for this kind of processes.

Keywords: Alkalinity, Anaerobic Digestion, Robust Regulation, Wastewater Treatment.

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Biodegradation of benzotriazole under nitrifying conditions

R. Trejo Castillo⁽¹⁾, F.M. Cuervo López⁽¹⁾, A.-C. Texier^{*(1)}

⁽¹⁾ Universidad Autónoma Metropolitana-Iztapalapa, Div. CBS, Departamento de Biotecnología, Av. San Rafael Atlixco, N° 186, Col. Vicentina, C.P. 09340, Ciudad de México, México.

*Corresponding author: actx@xanum.uam.mx



The presence of emerging organic contaminants (EOCs) as benzotriazole (Bt) in the water environment has been recognized as one of the emerging issues in wastewater treatment due to their potential harmful ecological and health effects¹. The ability of nitrifying sludge to simultaneously oxidize ammonium (NH₄⁺) and recalcitrant aromatic molecules, including EOCs, has been previously reported^{1,2}; however, information on the role of nitrification in Bt biodegradation is still limited. The purpose of this study is to evaluate the effect of the addition of increasing NH₄⁺ concentrations on Bt biodegradation. Both nitrification and Bt (5 mg/L) biodegradation processes were evaluated in batch cultures inoculated with a sludge produced in a continuous reactor under steady-state nitrification. Bt provoked a decrease on both the NH₄⁺ consumption efficiency from 99.8 to 17.0% and the nitrate (NO₃⁻) yield from 0.99 to 0.62. Specific rates of NH₄⁺ consumption and NO₃⁻ formation decreased by 50 and 40%, respectively, showing the inhibitory effect of Bt on nitrification. At 100 mg N-NH₄⁺/L, there was no Bt biodegradation even after 144 h, confirming its high recalcitrant character. According to recent studies, the increase in the concentration of NH₄⁺ as primary substrate could favor the cometabolic degradation of EOCs^{1,2}. Therefore, higher NH₄⁺ concentrations will be added in the cultures to favor Bt biodegradation.

Keywords: benzotriazole, biodegradation, emerging organic contaminants, nitrifying conditions.

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Inhibitory effects of benzotriazole in the nitrification ammonium and nitrite oxidizing processes

D. Maturano⁽¹⁾, D.I. Bejarano⁽²⁾, F.M. Cuervo López⁽¹⁾, A.C. Texier⁽¹⁾

⁽¹⁾ UAM-Iztapalapa, Av. San Rafael Atlixco, N° 186, Col. Vicentina, Ciudad de México, México

⁽²⁾ CINVESTAV-IPN, Av. IPN, N° 2508, San Pedro Zacatenco, Ciudad de México, México

*Corresponding author: actx@xanum.uam.mx



Benzotriazole (BT) is one of the major emerging organic contaminants (EOCs) detected in effluents from wastewater treatment plants and can present a high-risk factor for living beings^{1,2}. Its effect on nitrification process commonly used in the biological treatment of wastewater to remove ammonium (NH_4^+), is unknown. The aim of this work was to evaluate the physiologic and kinetic effects of BT on the ammonium and nitrite oxidizing processes, both in microbial respiratory pathways for nitrifying bacteria. Control batch nitrifying cultures were performed in the absence of BT and others with different initial concentrations of BT (0.1-10 mg/L). At 0.1-0.3 mg BT/L, the efficiency of NH_4^+ consumption ($E_{\text{NH}_4^+}$) remained high, but the nitrate yield ($Y_{\text{NO}_3^-}$) decreased by 27% and nitrite (NO_2^-) was accumulated. Specific rate of nitrate production decreased by 53%, showing that BT caused an inhibition in the nitrite oxidizing process. On the contrary, at 2.5-10 mg BT/L, $E_{\text{NH}_4^+}$ decreased by 24%, but $Y_{\text{NO}_3^-}$ remained high without NO_2^- accumulation. Specific rate of ammonium consumption decreased up to 45%, evidencing that BT provoked an inhibitory effect on the ammonium oxidizing process. These results indicate that the presence of BT in waters can favor the accumulation of NH_4^+ or NO_2^- by causing different inhibitory effects on the nitrification oxidizing processes depending on the BT concentrations.

Keywords: Benzotriazole; Emerging organic contaminants; Nitrification.

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Ampicillin biotransformation by a nitrifying consortium

J.J. Ramírez Muñoz⁽¹⁾, D.I. Bejarano Ortiz⁽²⁾, F.M. Cuervo López⁽¹⁾, A.C. Texier⁽¹⁾

⁽¹⁾ UAM-Iztapalapa, Department of Biotechnology, Av. San Rafael Atlixco, N° 186, CDMX, México

⁽²⁾ CINVESTAV-IPN, Av. IPN, N° 2508, San Pedro Zacatenco, CDMX, México

*Corresponding author: actx@xanum.uam.mx



More than half of the antibiotics produced worldwide are β -lactams and many of them have been detected in effluents from sewage treatment plants, including ampicillin (amp)¹. The pollution of water by nitrogen is mainly due to the presence of ammonium (NH_4^+) in wastewater, and its elimination in treatment plants is mainly carried out through biological processes such as nitrification and denitrification. Studies on the biotransformation of amp by nitrifying sludge and the effect of this antibiotic on nitrification are scarce. The physiological and kinetic behavior of a nitrifying consortium in the presence of amp (10, 25, and 50 mg/L) was evaluated in batch cultures. Under the experimental conditions (320 ± 8 mg bacterial protein/L, C/N = 2.4, 24 h), the sludge behavior was very similar in the controls without amp and the assays with antibiotic and there was no effect on efficiency ($E_{\text{NH}_4^+} = 99.7 \pm 4.2\%$) and yields ($Y_{\text{NO}_2^-} = 0$, $Y_{\text{NO}_3^-} = 1.0 \pm 0.1$ mg N/mg N- NH_4^+ consumed) of nitrification. Specific rates of both NH_4^+ oxidation and NO_3^- formation were similar between controls and cultures with amp, showing that amp did not cause inhibition on nitrification. The insensitivity to amp of the nitrifying bacteria may be explained through the bacteria's slow growth and other survival strategies². The elimination of amp was mainly attributed to physico-chemical processes (16.0-16.5%), biosorption (23.2-47.0%), and biotransformation (10.0-29.8%). With the increase in the initial amp concentration, there was a greater participation of the biotransformation process, associated with an increase in the specific amp consumption rate. The sludge was able to oxidize NH_4^+ to NO_3^- by nitrification and eliminate amp biologically, but without reaching full mineralization.

Keywords: Ampicillin, Biotransformation, Biosorption, Nitrification.

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Oxidation of ammonium and benzotriazole in a sequencing batch reactor

M.L. Aceves Zamora, F.M. Cuervo López, A.-C. Texier*

Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco, N° 186, Col. Vicentina, C.P. 09340, Ciudad de México, México.

*Corresponding author: actx@xanum.uam.mx

Nitrification is a viable alternative for the simultaneous removal of ammonium and recalcitrant aromatic compounds from wastewater¹. Benzotriazole (BTA) is one of the emerging organic compounds (EOCs) that has been commonly detected in effluents from wastewater treatment plants². Very little is known about the role of nitrifying sludge in the biodegradation of BTA and the effect of this compound on nitrification. In the present work, the nitrifying respiratory activity and biodegradation capacity of a sludge exposed to BTA were evaluated along the operation cycles of a sequencing batch reactor (SBR). A 2 L SBR was inoculated with 350 mg/L of microbial protein and fed with nitrifying lithoautotrophic medium (C/N of 2.5±0.2). It was operated at 24 h cycles: feeding (5 min), biological aerobic reaction (23 h), settle (45 min), and draw (10 min). Firstly (cycles 1-39), a high nitrifying respiratory activity of the sludge was stabilized in the SBR fed with NH₄⁺. In the first cycle (cycle 40) of BTA (5 mg C/L) addition, inhibitory effects on nitrification were observed with decreases of 58 and 62% in specific rates of NH₄⁺ consumption (qNH₄⁺) and NO₃⁻ generation (qNO₃⁻), respectively. Along the cycles, the inhibitory effect decreased more rapidly in the qNH₄⁺ than in the qNO₃⁻. Efficiency (ENH₄⁺) and nitrate yield (YNO₃⁻) were also diminished by the addition of BTA, however, they increased again through the cycles, obtaining in cycle 81, high values of 99.3% and 0.79, respectively. BTA remained totally recalcitrant in the first cycle of its addition, however 7 cycles later the consumption of BTA was of 100% in only 5 h. The qBTA improved as the cycles passed, obtaining a maximum value of 4.79 mg BTA-C/g SSV·h, showing metabolic adaptation of the sludge for oxidizing this compound. The results show that the use of SBR can be a good alternative to reduce the inhibitory effect of BTA on nitrification throughout the operation cycles and obtain the simultaneous oxidation of ammonium and BTA.

Keywords: biodegradation, benzotriazole, nitrification, sequencing batch reactor.

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TiO₂ and N-TiO₂-photocatalytic degradation of salicylic acid in water:

characterization of transformation products by HPLC-MSⁿ



Bracco, Estefanía⁽¹⁾, Butler, Matías⁽¹⁾, Carnelli, Patricio⁽¹⁾, Alfano, Orlando⁽²⁾, **Candal, Roberto^{(1)*}**
⁽¹⁾ *Instituto de Investigación e Ingeniería Ambiental (3iA), CONICET, Universidad Nacional de San Martín. Av 25 de Mayo y Francia, San Martín, Buenos Aires, Argentina.*
⁽²⁾ *Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), CONICET-UNL, Predio CONICET "Dr. Alberto Cassano" Paraje El Pozo, (3000) Santa Fe, Argentina.*

*Corresponding author: rjandal@gmail.com

TiO₂ is one of the most employed compounds for the treatment of recalcitrant pollutants by heterogeneous photocatalysis. However, TiO₂ is only activated by UVA light (around 5% of the solar light), which represents a limitation on its use as photocatalyst. Therefore, a potential alternative to improve its photocatalytic activity is through nonmetals doping, although byproducts and reaction rate may be affected by light wavelength and doping. In this work, sol-gel process coupled with urea coprecipitation was used to prepare nanoparticulated powders of nitrogen doped TiO₂ (N-TiO₂), since it has been shown as a nontoxic dopant contributing to the activity under visible light¹. A control sample of pure TiO₂ was synthesized in a similar way, except for the addition of urea. Powders were characterized by means of scanning electron microscopy (SEM) and X-ray diffraction (XRD). Photocatalysis experiments were performed using salicylic acid as a model contaminant at different initial concentrations (10⁻⁴ M and 2·10⁻⁵ M). A homemade photoreactor equipped with two different light sources, UVA (372 nm) and blue led (462 nm), was used for the experiments with each synthesized photocatalyst besides commercial P-25 TiO₂. The evolution of the experiments was followed via the initial and final measurement of temperature, pH and TOC, as well as through the analysis of samples taken at regular times by UV-Vis spectrophotometry and high performance liquid chromatography coupled to mass spectrometry (HPLC-MSⁿ). The latter was applied to detect the presence of transformation products in the samples via a linear ion trap with electrospray ionization in negative ion mode. The analysis of the results showed a faster decrease in the concentration of salicylic acid when using P-25 TiO₂, followed by N-TiO₂ and pure TiO₂ catalysts under both light sources. Accordingly, the calculated quantum yields obeyed the same trend, although N-TiO₂ displayed the highest photon absorption efficiencies and photonic efficiencies similar to the quantum yields with both lights. The major differences found in the amount and identity of the transformation products were owed to the different light sources and to a lower extent to the photocatalyst. Further structural characterization of the degradation products was made by mass spectrometry through fragmentation (MSⁿ) and analysis of derivatives.

Keywords: Degradation; Photocatalysis; Salicylic acid; Transformation products.

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Chloramphenicol elimination in aquaculture wastewater using

electrooxidation



Romero-Soto I.C.⁽¹⁾, O. Dia⁽³⁾, L.A. Leyva-Soto⁽¹⁾, P. Droguí², G. Buelna⁽²⁾, L. M. Díaz-Tenorio⁽¹⁾, R. G. Ulloa-Mercado⁽¹⁾, P. Gortáres-Moroyoqui⁽¹⁾

⁽¹⁾ Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora. 5 de Febrero 818 Sur, 85000 Ciudad Obregón, Sonora, México.

⁽²⁾ Institut national de la recherche scientifique (Centre Eau, Terre et Environnement), Université du Québec, 490 rue de la Couronne, Québec, Qc, Canada, G1K 9A9.

⁽³⁾ Centre de recherche industrielle du Québec (CRIQ), 333 Franquet, Québec, Qc, Canada, G1P 4C7.

*Corresponding Author: pablo.gortares@itson.edu.mx

Nowadays, the use of antibiotics have increased and with it, the bacterial resistance towards them. Chloramphenicol (CAP) is a compound forbidden in Mexico since 2012¹. However, it is still used in animal farming and aquaculture industries. In this study, electrooxidation (EO) process using Ti/PbO₂ anode and Ti cathode was applied to investigate the CAP degradation in real aquaculture wastewater. The experimentation was conducted in a batch reactor agitated by a magnetic stirrer. The CAP measurement was performed by liquid chromatography tandem mass spectrometry (Thermo TSQ Quantum Access). The liquid chromatography separation was achieved in a HyperSil Gold C18 column (Thermo Scientific) heated to 35°C. To obtain and apply the optimal conditions in real wastewater, the CAP removal was evaluated in synthetic solution obtaining the next conditions: current intensity (I) of 0.65 A, treatment time (t) of 34 minutes, and CAP initial concentration of 0.5 mg/L. In real wastewater the antibiotic was not present and, for this reason, 0.5 mg/L of CAP were added and treated by EO using the optimal conditions. Under these conditions, a complete removal of CAP was obtained with an energy consumption of 4.65 kW/h/m³. Also, the presence of NO⁻², NO⁻³, PO₄⁻³, SO₄⁻² and NH₄⁺ were evaluated using standard methods² before and after the process, finding a slight increase of 7.39% in SO₄⁻² and of 41.4% in PO₄⁻³, which may be due to oxidation of the initial organic compounds, coming from different residual nutrients added during feeding in the aquaculture activity and transformed into inorganic forms by the treatment, that were not detected at the initial stage of the process. The NO⁻², NO⁻³ and NH₄⁺ were eliminated by 34.21%, 10.71% and 33.75%, respectively. With these results, EO could be considered as an interesting approach to remove antibiotics and other organic compounds.

Keywords: Aquaculture, chloramphenicol, electrooxidation, wastewater.

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Oxytetracycline biosorption and degradation by *Chlorella sp*

Burboa Charis, V. A.⁽¹⁾, Ulloa Mercado, R.G.^{*(1)}, Hernández Chávez, J. F.⁽³⁾, Leyva Soto, L. A.⁽¹⁾, Gortáres Moroyoqui, P.⁽¹⁾, Serrano Palacios, D.⁽²⁾, Meza Escalante, E.R.⁽²⁾

⁽¹⁾ Dpto. Biotecnología y Ciencias Alimentarias,

⁽²⁾ Dpto. Ciencias del Agua y Medio Ambiente,

⁽³⁾ Dpto. Ciencias Agronómicas y Veterinarias. Instituto Tecnológico de Sonora, Av. Antonio Caso 2266, Cd Obregón, Sonora, México.

*Corresponding author: ruth.ulloa@itson.edu.mx



The increase in intensive animal production for obtaining food for human consumption [1] has led to an indiscriminate use of antibiotics, especially Oxytetracycline (OTC). The use of integrated systems with microalgae presents important advantages, since they can directly biodegrade the contaminants through heterotrophic metabolism [2], they can also improve biodegradation indirectly through symbiosis with bacteria [3]. The objective of this work was to evaluate the biodegradation, photodegradation, adsorption and desorption of OTC by *Chlorella sp.* Bioassays were carried out in Bold medium with *Chlorella sp.* at concentrations of 0.4, 0.8 and 1.6 mg/L of OTC, in 800 mL reactors, with an initial density of 2×10^6 cell/mL, for 10 days; a daily cell count was performed in the Neubauer Chamber to determine the growth rate (μ) and the consumption rate (mg OTC/mg of dry biomass). An abiotic test was performed to determine the photodegradation of the antibiotic in three different conditions, with a concentration of 1 mg/L of OTC; 10 mL of sample was taken, centrifuged and filtered with 0.45 μm membrane for both tests; the quantification of OTC was by capillary electrophoresis. The biomass of the bioassays was collected to evaluate the adsorption and absorption by the solid phase extraction method [4]. Kinetics with inactive biomass was performed at a concentration of 2 mg/L for 72 hours to determine adsorption. The samples were filtered through 0.22 μm pore size and analyzed on HPLC. The bioassays in bold basal medium with *Chlorella sp.*, added with OTC, the microalga under study showed inhibition in the presence of different concentrations of OTC, decreasing its growth rate by increasing the concentration of the antibiotic compared with the control. In 72 hours, the presence of the antibiotic in the aqueous phase was not detected, which can not be attributed to degradation by algal activity, since it can be adsorbed and absorbed by the microorganism. However, the light showed a significant effect on degradation of the pollutant; We can establish that there is a photodegradation of the antibiotic.

Keywords: Antibiotics, Microalgae, waste-water treatment, micropollutant

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Avilés Saucedo Ana Isabel, **Tabares Escamilla Luis**, Estrada Alvarado María Isabel; Cira Chávez Luis Alberto*

⁽¹⁾ Instituto Tecnológico de Sonora, Departamento de Biotecnología Ciudad Obregón Sonora C.P. 85000

*Corresponding author: authorluis.cira@itson.edu.mx



The effluents from industries textile contribute with 80,000 tons of dyes a year, these pollutants contain aromatics amines, that are a largest group of organic compounds and represent an environmental hazard. Between 1 and 20% of the dyes are lost in the production during these processes and are released to the environment ¹. Biotechnology is in search of new technologies for the treatment of effluents. One of them is the application of microorganisms or enzymes with the ability to degrade dyes. Halophiles are microorganisms that grow between 1 to 30% of NaCl and have the capacity to produce enzymes for the degradation of dyes ². The objective of the present work is to select bacteria capable of degrading the direct red dye and determine the enzymatic activity oxidoreductase as a possible mechanism of action. For the above, 132 halophilic bacteria isolated from saline soils were evaluated. Once the degrading strains of the direct red dye were selected, decolorization kinetics were carried out in saline medium supplemented with the dye up to 300 ppm. During the kinetics of discoloration tests were performed to determine oxidoreductase activity: lignin peroxidase, azobenzene reductase and laccase. Two strains of 132 analyzed presented the ability to degrade 80% of the direct red dye concentration of 300 and 200 ppm were obtained and were positive in azobenzene reductase and laccase.

Keywords: Halophile, Red direct dye, degradation, isolation

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Biosorption and degradation of chlortetracycline by microalgae

Cortés Astengo, C.⁽¹⁾, Burboa Charis, V. A.⁽¹⁾, Ulloa Mercado, R. G.*⁽¹⁾, Gortáres Moroyoqui, P.⁽¹⁾, Rentería Mexía, A.M.⁽¹⁾, Leyva Soto, L. A.^(1,2), Hernández Chávez, J. F.⁽³⁾,

⁽¹⁾ Dpto. Biotecnología y Ciencias Alimentarias

⁽²⁾ Dpto. Ciencias Agronómicas y Veterinarias: Instituto Tecnológico de Sonora, Av. Antonio Caso 2266, Cd Obregón, Sonora, México

*Corresponding author: ruth.ulloa@itson.edu.mx



Antibiotics and hormones are frequently used to guarantee productivity in pig farming, ending up in wastewater¹ in concentrations between ngL^{-1} to μgL^{-1} as micro-contaminants². Conventional wastewater treatments are not designed for the elimination of these residues, making them present in superficial and underground water causes². They trigger important negative effects in human health and the environment³ such as antibiotic resistance to bacteria¹ and causes adverse effects to different types of organisms including hormonal, reproductive, growth, fertility, chronic toxicity, bioaccumulation, etc.². The objective of this work was to evaluate the biosorption and degradation of chlortetracycline (CTC) by microalgae, to determine the effects of CTC in the growth of microorganisms. The bioassays were made in bold basal medium added with 0.5, 1 and 2 mg/L of CTC; the growth was evaluated for 12 days at 25 ± 2 °C with cycles of 12 hours light and 12 hours dark by cell counting with chamber Neubauer method for determinate the growth rate, the biomass was recollected by centrifugation, then be analyze to establish the bioabsorption and bioadsorption with a solid phase extraction methodology. Also performed an abiotic test with three different conditions to determine the photodegradation of the antibiotic. In 96 hours, the presence of CTC in the aqueous phase was not detected, which can not be attributed to degradation by algal activity, since it can be adsorbed and absorbed by the microorganism. The light was significant effects in the photodegradation of the antibiotic.

Keywords: Antibiotic, Chlortetracycline, microalgae, micro-contaminant

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Removal efficiency of organic matter in a sequential batch activated sludge reactor for treatment of municipal wastewater

A. N. Salinas-Pacheco⁽¹⁾, C. Estrada-Vázquez*⁽¹⁾, E. Peralta-Reyes⁽¹⁾, A. Regalado-Méndez⁽¹⁾
⁽¹⁾ *Universidad del Mar campus Puerto Ángel, Ciudad Universitaria, Puerto Ángel, Distrito de San Pedro Pochutla, Oax., México*

*Corresponding author: carlosesvz@yahoo.com



This pilot-scale study aimed to test the removal efficiency of organic matter in a sequential batch activated sludge reactor (LOA-SBR) for treatment of diluted municipal wastewater, from small population; this population includes cafeteria, habitable zone and library areas of the UMar campus. The bioreactor was operated in a 24 h cycle with an aeration time of 22.4 h at constant flow rate, for a period of 365 days. The environmental conditions that mentioned the reactor operated was established from a multiparameter, resulting in a pH of 7.96, a temperature of 26.9 ° and electrical conductivity of 1700 mS/cm. The average results obtained show that the reactor had a removal efficiency of organic matter expressed as COD of 68.1%, with a sludge concentration of 3100 mg/L and a volumetric sludge index of 120 ml/mg. In spite of the high variation of organic matter concentration in the tributary (with COD values ranging from 180 to 540 mg O₂/L), the LOA-SBR showed no impairment in its functioning; the color of the mud remained brown, there was no presence of odors in the effluent; the difference in color between the affluent and the effluent was visible; the accumulation of solids at the edges was minimal and there was no foam on the surface of the reactor. Finally, the sludge concentration was modified to improve the operation of the reactor in concentrations of 3000, 2500 and 2000 mg/L, with an organic load of 2500 mg O₂/L. It was observed that decreasing the sludge concentration at 2500 mg/L, the reactor efficiency increases up to 70% organic matter removal. In conclusion, the removal efficiency of organic matter in an LOA-SBR reactor tested is low compared with other treatment systems used in literature, so it is necessary to improve the operating variables of the tested reactor to raise the efficiency and that this type of reactors will be useful in small populations where the generation of this type of water is common.

Keywords: activated sludge, pilot-scale, SBR, wastewater.



Microbial diversity and contamination in Reconquista River sediments: detection of bacteria with potential biotechnological applications

Natalia Porzionato⁽¹⁾, Agustina Ziliani⁽¹⁾, Celeste Grimolizzi, Ana E. Tufo⁽¹⁾, Susana Vázquez⁽²⁾, Angela Cabezas Da Rosa⁽³⁾, Gustavo Curutchet^{(1)*}

⁽¹⁾ Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, Bs. As., Argentina.

⁽²⁾ NANBIOTEC UBA-CONICET, Cátedra de Biotecnología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, CABA, Argentina

⁽³⁾ Department of Environmental Sciences, Technological University (UTEC), Francisco Antonio Maciel s/n, CP: 97000, Durazno, Uruguay.

*Corresponding author: gcurut@gmail.com



Sediments from contaminated water courses accumulate large amounts of organic matter and toxic persistent pollutants like heavy metals and pesticides. The stress caused by severe contamination leads to the selection of microorganisms which, in addition to being involved in *in situ* self-purification processes, have a clear biotechnological potential. In this work, we studied the microbial communities from fluvial sediments in areas with different degree and type of contamination at the Reconquista River basin, one of the most polluted watercourses in the metropolitan area of Buenos Aires, Argentina. Our aim was not only to get insight on the composition of bacterial communities in these habitats but also to recover in culture bacteria capable of growing at the expense of the pollutants. The sediment samples were analyzed to determine their physicochemical parameters and respirometry experiments were performed. Bacterial community structure in sediment samples was analyzed through 16S rRNA gene amplicon sequencing (V4 variable region, Illumina MiSeq platform, and data were processed using Mothur and R packages). Cultures were performed to enrich in sulphate and iron reducers and sulfur and iron oxidizers using selective media, and isolates were obtained in solid media. Taxonomic identification of isolates was achieved by partial sequencing of the 16S rRNA gene after DNA extraction using the Wizard® Genomic DNA Purification Kit (Promega) and PCR amplification using the universal primers 27F/1492R for bacteria and ITS5/ITS4 for yeasts. Growth kinetics and metabolites production were studied for the enrichment cultures and isolates obtained. Bacterial communities from areas with different contamination status differ in their structure (and therefore, in their functional potential and performance). Aerobic and anaerobic isolates, affiliated to *Acidithiobacillus*, *Clostridium*, *Desulfovibrio*, and *Shewanella* among others, were obtained. These microorganisms possess a great potential for applications in environmental biotechnological processes.

Keywords: Bacterial communities. Persistent pollutants. Reconquista River.



Oxytetracycline removal in porcine wastewater through an electrooxidation system using a photovoltaic panel as power supply

Osuna-Lizárraga S.D.E⁽¹⁾, Romero-Soto I.C., L.A. Leyva-Soto, L. M. Díaz-Tenorio, R. G. Ulloa-Mercado P. Gortáres-Moroyoqui*

⁽¹⁾ *Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora. 5 de Febrero 818 Sur, 85000 Ciudad Obregón, Sonora, México.*

*Corresponding Author. Pablo.gortares@itson.edu.mx



Currently, the study of emerging pollutants has caused great impact due to the environmental deterioration that these generate, both the aquatic and terrestrial ecosystems, as well as the resistance that it causes in microorganisms¹. Likewise, the use of alternative energies (from renewable sources) has been popularized to reduce damage to the environment. In the present work, the removal of oxytetracycline (OXI) in the general effluent of a swine farm by electrooxidation was evaluated. This investigation was carried out with real residual water (with a dilution 1:25) enriched with OXI using a factorial design (2²) and a central composite design, where the effect of 2 experimental variables, over the removal of OXI (analyzed by HPLC), was evaluated: experimental time (10 minutes to 30 minutes) and the concentration of antibiotic (0.5 mg/L to 1 mg/L) at a fixed current intensity (2.8 A). In the process, a Ti cathode, a Ti/PbO₂ anode, and 1 g/L Na₂SO₄ electrolyte were used. In addition, a photovoltaic cell was used to provide the necessary energy to the system in order to reduce the costs of electrochemical treatment. The highest removal obtained was 63.46% at a concentration of 0.5 mg/L of OXI and an operating time of 30 minutes. The variable of greater relevance in the process was the experimental time with a contribution of 53.34% followed by the concentration of antibiotic with 44.86%. The optimization of the system was established by maximizing the removal rates and minimizing the experimental times at a high concentration of OXI, whose conditions were 40 minutes and 0.8 OXI mg/L, to obtain 64% removal. Therefore, it is concluded that electrochemical systems are highly efficient to removal organic compounds, also in conjunction with the use of renewable energy the environmental impact is reduced favorably.

Keywords: Antibiotics, central composite, electrooxidation, renewable source.

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Evaluation of physiological development of *Salicornia bigelovii* exposed

to antibiotics during the phytoremediation of shrimp aquaculture wastewater



Molina-Avila B.⁽¹⁾, López-Heraldez V.⁽¹⁾, Escoboza- Barceló D.⁽¹⁾, Leyva-Soto L.⁽¹⁾, Gortáres-Moroyoqui P.*⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 5 de Febrero, 818, Ciudad Obregón, México.

*Corresponding author: Pablo.gortares@itson.edu.mx



Intensive aquaculture employs antibiotics to prevent and counteract infectious diseases. In Northwest Mexico, this is a primary activity of great importance, which applies 95% enrofloxacin (ERO) and oxytetracycline (OXY) alone or combined. Phytoremediation consists of a set of technologies that exploit the ability of some plants to absorb, accumulate or metabolize contaminants. Halophytes such as *Salicornia bigelovii* are viable plants to treat these micropollutants in saline wastewater since use these mechanisms to cope with saline stress¹. The objective of this work was to evaluate the effect on the physiological development of *S. bigelovii* juveniles in the presence of stress induced by different levels of ERO and OXY alone (1, 5, 10, 20, 50, 100, 1000 µg/L). Height, root length, number of nodes and branches are used to monitor impact of exposure to antibiotics, during the treatment of shrimp residual effluent. Ten plants were installed in a batch hydroponic system, with 60 mL of working volume containing seawater, 20% Hoagland solution, and corresponding antibiotic concentration under laboratory conditions during 30 days of exposure. Results obtained for enrofloxacin and oxytetracycline show that increase in nodes and branches is not significant, being of 1.5 ± 0.49 , 3.8 ± 2.25 and 2.5 ± 0.84 , 1.9 ± 2.18 cm respectively. In contrast, the root length and the aerial part showed an increase of 100% in the levels of 100 and 1000 µg/L of enrofoxacin, reaching 4.6 ± 0.8 cm and 25.1 ± 4.3 cm for aerial part and root length respectively. A similar case was observed with oxytetracycline in the root length in all concentrations studied, reaching until 26.4 ± 4.10 cm. This phenomenon could occur because of highest concentrations are favorable for the plant, since active the mechanism of phytohormones production such as auxins and cytokinins that promote plant development². Therefore, we can conclude that *S. bigelovii* has no apparent physiological effects due to antibiotics present in shrimp aquaculture wastewater, highlighting the phytoremediation of this plant.

Keywords: *Salicornia bigelovii*, enrofloxacin, oxytetracycline, Wastewater treatment.

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cell inoculated with *Shewanella oneidensis*



Mendoza-Chávez E.⁽¹⁾, Khosravanipour-Mostafazadeh A.⁽²⁾, Drogui P.⁽²⁾, Buelna G.⁽³⁾, Gortáres-Moroyoqui P.*⁽¹⁾

⁽¹⁾ Instituto tecnológico de Sonora, 5 de febrero, 818 sur, Ciudad Obregón, Sonora, México

⁽²⁾ Institut National de la Recherche scientifique, 490 rue de la Couronne, Québec, QC, Canada

⁽³⁾ Centre de recherche industrielle du Québec (CRIQ), 333 Franquet, Québec, Qc, Canada,

*Corresponding author: pablo.gortares@itson.edu.mx

In order to reduce the extensive use of fossil fuels and the environmental problems they create, non-pollutant energy sources such as hydrogen have been proposed to meet the world energy demand. Microbial electrolysis cell (MEC), is a system that allows hydrogen generation from wastewater through the metabolism of electrochemically active bacteria¹. The aim of the present research work was to evaluate the hydrogen production efficiency obtained by a MEC inoculated with a pure culture of *Shewanella oneidensis*. Four preliminary tests were carried out in a MEC at two different voltages (0.7 and 0.9 V), both in aerobic and anaerobic conditions, with and without growing medium. The highest hydrogen production was obtained in anaerobic conditions using a single chamber with a voltage applied of 0.7 V and without growing medium; with a total volume produced of 145 ml of hydrogen. The effect of voltage applied on hydrogen production efficiency was also determined with experiments in the same operational conditions at two different voltages (0.7 and 0.9). In these experiments, although more biogas was produced at 0.9 V the percentage of hydrogen in the biogas was higher at lower voltage of 0.7V; with a total volume of hydrogen produced of 42.98 and 32.92 ml produced with 0.7 and 0.9V, respectively. Although there was not significant biogas production when the MEC was operated in oxic conditions, hydrogen concentrations were detected on the headspace of the reactor, showing the capacity of *S. oneidensis* to produce hydrogen at this conditions; contrary to other exoelectrogenic species strictly anaerobic. Based on this information, a MEC with a pure culture of *S. oneidensis* can be considered as an effective alternative to produce hydrogen from wastewater treatment.

Keywords: Microbial electrolysis cell, hydrogen production, *Shewanella oneidensis*.

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Evaluation of atrazine effect on *Salicornia bigelovii* growth indicators during agricultural wastewater remediation

López Heraldez, V.⁽¹⁾, Escoboza Barceló, D. O.⁽¹⁾, Molina Ávila, B. G.⁽¹⁾, Leyva Soto, L. A.^(1,2), Gortáres Moroyoqui, P.*⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 5 de Febrero, 818, Ciudad Obregón, México;

⁽²⁾ ICC1, Departamento de Cátedras, CONACYT;

*Corresponding author: pablo.gortares@itson.edu.mx



Atrazine is an herbicide that is associated with effects on human health and impacts on wildlife, so it is a priority to remove this pollutant from environmental matrices. 1. Phytoremediation has received special attention because it is an innovative biological alternative over conventional techniques used for this purpose. 2. *Salicornia bigelovii* phytoremediation capacity has been proven before, and due halophytes are adapted to environmental stress and can tolerate xenobiotics presence³, this plant is potential candidate to remove atrazine from wastewaters. Phytoremediation plants must show sufficient pollutant tolerance to maintain proper physiological conditions while decreasing their concentration. 4. This work's objective was to evaluate *S. bigelovii* atrazine tolerance and its impact on plant growth indicators during agricultural wastewater remediation. *S. bigelovii* seedlings were put in contact with atrazine in hydroponic systems at different concentrations in two contact experiments. Attributes aerial part length, root length, number of nodes and lateral branches, were measured initially and at the end of contact time to know increments. Contact time for treatments with 0.1, 0.4 and 0.7 mg of atrazine per L of agricultural wastewater, was 3 weeks, and 2 weeks for 1.0, 5.0 and 9.0 mg/L treatments. All treatments had influence in root length, nodes and lateral branches increment, in relation to control without contaminant. Roots showed a decrease due to herbicide action causing a root fragmentation by tissue deterioration, and nodes and lateral branches didn't show growth in all concentrations probed. Aerial part growth was also affected in all concentrations tested, except in 0.1 mg/L concentration where increase was obtained, although statistically different and smaller than control. Affected plants presented dehydration and necrosis, due atrazine action on susceptible plants's metabolism produces imbalance in vital functions which leads growth decrease and even death. Only in 0.1 mg/L concentration there was herbicide tolerance with 70% surviving plants, and 100% mortality in the rest of the treatments by phytotoxic effect. As conclusion, atrazine exposition had a significant effect on evaluated growth indicators in tested concentrations, it is proposed to carry out experiments with lower concentrations that allow a long-term contact and find the appropriate to allow growth and development, as required in phytoremediation plants.

Keywords: pesticides, phytoremediation, phytotoxic effect, tolerance

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Slaughterhouse wastewater treatment in SBR

Vianka Celina Hernández-Fydrych ^{*(1)}, Patricia Castilla-Hernández⁽²⁾, Ricardo Beristain-Cardoso⁽³⁾, María del Carmen Fajardo-Ortiz⁽¹⁾

⁽¹⁾ Department of Biotechnology, Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco 186, Col. Vicentina, 09340, Mexico City, Mexico.

⁽²⁾ Department. of the Man and his Environment. Universidad Autónoma Metropolitana-Xochimilco. Calz. del Hueso 1100 W309, Col. Villa Quietud, Coyoacán, 04960 Mexico City.

⁽³⁾ Department Land Resources, Universidad Autónoma Metropolitana-Lerma. Av. De las Garzas No. 10, Col. El Panteón Lerma de Villada, 52005, Mexico State.

*Corresponding author: vhdezfydrychj@xanum.uam.mx

In Mexico, municipal slaughterhouses are generating wastewater contains high amounts of pollutants. Researchers suggesting that an anaerobic treatment helps to diminish the COD, but release $\text{NH}_4^+\text{-N}$ from proteins (Jia et al. 2012), therefore a pos-treatment is requiring; for this propose Sequencing Batch Reactor (SBR) is preferred for its small space requirement, low engineering investment and operational cost. In the present study the effect to pre-fermented slaughterhouse wastewater for simultaneous removal of COD, protein and $\text{NH}_4^+\text{-N}$ was evaluated. The slaughterhouse wastewater was pre-fermented anaerobically for 7 days, attaining 53% of COD Soluble reduction, 78% of protein hydrolysis and a production of 139 mg $\text{NH}_4^+\text{-N/L}$. Subsequently the pre-fermented wastewater (PW) was occupied to biodegradability assays in SBR operated under oxic/anoxic (O/A) and anoxic/oxic (A/O) conditions; raw slaughterhouse wastewater (RW) was used as control assay. Low values of protein hydrolysis and COD reduction were obtained with PW: (O/A) 44% and 46%, and (A/O) 34% and 54%, respectively; while COD reduction and protein hydrolysis were better con RW (above 60%). Contrary to expected, the pre-fermentation was not able to improve the biodegradability; however, the treatment of RW under A/O conditions produced an effluent with quality to be discharged in according to Mexican Normative.

Keywords: Anoxic-Oxic, Slaughterhouse wastewater, Oxic-Anoxic.

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Formation of aerobic granules in Sequential Batch Reactors (SBR)

operated at different airflow velocity



Oucilane I. M. Alves ^{*(1)}, Julliana M. P. Araújo ⁽²⁾, Poliana M. J. Silva ⁽¹⁾, Sávia Gavazza ⁽¹⁾, Mário T. Kato ⁽¹⁾, Lourdinha Florêncio ⁽¹⁾

⁽¹⁾ Federal University of Pernambuco, Architecture Avenue, s/n, University City, Recife, Brazil

⁽²⁾ Federal Institute of Education, Science and Technology of the Sertão Pernambucano, Road Tamboril, s/n, Ouricuri, Brazil

*Corresponding author: oucilane@hotmail.com



During the aerobic granulation process, the shear force is considered as determinant in the physical process for the formation of the granules, being controlled by the airflow velocity applied in the system. Studies have shown that the application of airflow velocity higher than $1.2\text{cm}\cdot\text{s}^{-1}$ is recommended to obtain more regular and compact granules¹. On the other hand, Devlin et al. (2017) using three laboratory scale reactors, low organic loads and airflow velocity of $0.41\text{cm}\cdot\text{min}^{-1}$ was successful with granulation². Thus, the aim of this study was to evaluate the formation of aerobic granules in RBS feeding with diluted wastewater under different airflow velocity. Three pilot Sequential Batch Reactors (SBR) with effective volume of 115.5 L, height of 2.45 m, internal diameter of 0.245 m (ratio of height to diameter 10) and volumetric changes of 59% each were used as a methodological strategy. The feeding of reactors was supplied by a Wastewater Treatment Plant (WWTP) that treats domestic wastewater and the airflow velocity applied were $1.3\text{ cm}\cdot\text{s}^{-1}$, $1.1\text{ cm}\cdot\text{s}^{-1}$ e $0.88\text{ cm}\cdot\text{s}^{-1}$ for SBR I II and III respectively. The SBRs were operated in 3-hour cycles divided into four phases: (i) down feed (1 min), (ii) aeration (165 min), (iii) settling (10 min) and (iv) discard (4 min). No seed sludge was used. Granules were obtained at 71, 51 and 53 days of operation with average sizes of 2.0 mm 2.1 mm and 3.1 mm in RBS I II and III, respectively. The retention of granules was higher in RBS III, with lowest airflow velocity ($0.88\text{ cm}\cdot\text{s}^{-1}$). The COD removal was $89,5 \pm 3,8\%$ $81.2 \pm 7.8\%$ and $78.3 \pm 9.8\%$, in RBS I II and III, respectively. The presence of nitrite in the effluent (RBS I - $8,82 \pm 7,09\text{ mg}\cdot\text{L}^{-1}$ RBS II $3.0 \pm 4.9\text{ mg}\cdot\text{L}^{-1}$ and RBS III - $9.3 \pm 7.1\text{ mg}\cdot\text{L}^{-1}$) indicated the nitrification process didn't occur until its last stage of oxidation. Due to no maturation of the granules and the consequent lack of anaerobic microzones, significative phosphorus removal was not observed in any reactor (RBS I - $18,2 \pm 17,5\%$ RBS II - $21.0 \pm 8.9\%$ and RBS III - $17.6 \pm 7.8\%$). To sum up, the configuration applied in this study allowed to obtain granules treating dilute domestic wastewater and these were more resistant using the lower airflow velocity. This condition promoted greater aggregation of the particles selecting the granules of better settling proprieties.

Keywords: airflow velocity; domestic wastewater; pilot scale; SRB.

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case study



Luz Dehni Acosta-Moyado⁽¹⁾, Alejandro Regalado-Méndez⁽²⁾, Héctor M. Poggi-Varaldo⁽³⁾,
Carlos Estrada-Vázquez^{*(1)}.

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

(2) *Universidad del Mar, Instituto de Industrias, Puerto Ángel, Oaxaca, México*

(3) *Environmental Biotechnology and Renewable Energies Group, CINVESTAV-IPN, México*

*Corresponding author: carloesvz@yahoo.com



Puerto Angel is located close to the Mexican South Pacific shore, latitude 15°40'18"N and longitude 96°29'45"W. It belongs to the San Pedro Pochutla county, in the State of Oaxaca, Mexico. It has 18 neighborhoods and 2,645 inhabitants devoted to activities such as fishing and touristic services. Only 35% of the households are connected to a sewerage net that feed two inactive wastewater treatment plants (based on activated sludge process). Therefore, on site sanitation based on Imhoff-like tanks seem to be the alternative for sanitation. These tanks can be envisioned as anaerobic reactors operating at ambient temperature. Domestic effluents from showers, kitchen (grey wastewater) as well as those from the WCs are discharged to Imhoff tanks without segregation. The goals of this work were (i) to determine the status of a selected sample of existing Imhoff tanks; (ii) to survey the population regarding several issues related with on site sanitation by interviews and questionnaires, and (iii) to develop a proposal for a sound on site sanitation of Puerto Angel. We encompassed several examined 5 tanks and performed 100 interviews. The latter questions related to qualitative and quantitative knowledge and personal attitudes related to wastewater pollution, health, and disposal. We found that (i) the tanks were small to medium size, 4 m³ to 12 m³; (ii) the operation of all the tanks was deficient due to flaws in design and construction; (iii) there were negative impacts on the quality of drinking water wells, whose water showed total and fecal coliforms concentrations exceeding by far the maximum permissible levels of the Mexican regulation NOM-127-SSA-1994; (iv)

in general population is unaware of the environmental and health impacts that could be generated by deficient management of wastewaters. Finally, a proposal was issued with the purposes of upgrading design and construction of existing tanks, correct design of new tanks, treated effluent proper fate and disposal, environmental and health education of the population, recommendations for sound management of the sanitation system including financial resources for construction and maintenance, and fostering the participation of the Universidad del Mar in the process of sanitation improvement in Puerto Angel.

Keywords: anaerobic treatment, Imhoff tank, septic tank, strategic planning, wastewater treatment



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Chapter 8 Risk Assessment and Environmental Impact



CO₂ generated in the construction of a social housing by using regional materials in the city of Obregón Sonora, México

H. Aceves Gutierrez ⁽¹⁾, O. López Chávez ⁽¹⁾, J.L. Arévalo Razo ⁽¹⁾, L. A. Cervantes García ⁽¹⁾, G. Ayón Murrieta ⁽¹⁾, J. M. Campoy Salguero ⁽¹⁾, J. R. Osuna Corona ⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 5 de febrero, 818 sur, Ciudad Obregón, Sonora, México

*Corresponding author: haceves_itson@hotmail.com

Demographic growth leads to the expansion of towns and cities, causing the need for living places, urban infrastructure and services, which means that 50% of world resources are currently being absorbed. Construction is the least sustainable activity on the planet, because its form of production is characterized by the consumption of raw material and by generating waste and, along with the construction material producing industry, it is the biggest consumer of natural resources such as wood, minerals, water and energy. Since 1970 the emission of greenhouse gases has tripled in the construction sector and it employs nearly one third of energy consumed worldwide, it also generates one third of the direct and indirect emissions of CO₂ related to energy. This sector contributes a lot to pollution due to its nature which generates waste and degrades the ground, it is estimated that at a worldwide level, around three million tons of raw matter are being used to create construction products, additionally, only a few of the elements that are wasted in this industry get re-used, for that reason there is a need to try out new technologies that allow the recycling of rubbish. Because of what was already mentioned, it is of our best interest to consider the environmental impact of the constructive activities in Mexico and, for that issue, the “Análisis del Ciclo de Vida” or “Lifecycle Analysis” (ACV) tool could be used, it allows us to know the potential environmental impact of the products by taking into account the stages from the extraction of materials, to its use and consumption, as well as its final disposition. The ACV tool is standardized by the ISO4040 and ISO 14044 norms of the International Organization for Standardization (ISO). The objective of this work is the analysis of environmental impact related to CO₂ emissions derived from the construction process of a social housing of 48 m² by applying construction materials that are generally used in Sonora, Mexico. The ACV methodology was utilized up to the construction phase, using for that the amount of materials that were needed in the living place, an inventory of CO₂ emissions was used for each of the materials by reaching different sources of information and doing the necessary conversions for each type of work package and these results were obtained: a social housing of 48 m² in its construction process produces 6725 Kg of CO₂ which is the equivalent of 138.07 Kg of CO₂/M². As a conclusion and considering that the trends of CO₂ emissions are towards zero we will have to consider that it is necessary to use other types of materials in the construction process that are more friendly and sustainable with the environment, such as ecological concrete, paints with natural pigments, straw panels and many more existing alternatives, to reduce emissions gradually as much as possible.



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Keywords: ACV. CO2, Construction, Pollution



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Comparison of the environmental impacts of wheat production using chemical versus biofertilizers using a Life Cycle Assessment



Ismael Fragoso-Sosa⁽¹⁾, Robert Handler⁽²⁾, Sergio de los Santos-Villalobos⁽¹⁾, María Fernanda Lares-Orozco⁽¹⁾, Agustín Robles-Morúa⁽¹⁾

⁽¹⁾ Department of Water and Environmental Sciences, Technological Institute of Sonora, 5 de Febrero 818 Sur, Cd. Obregón, Sonora, 85130, México.

⁽²⁾ Sustainable Futures Institute, Michigan Technological University, 1400 Townsend Dr., Houghton, MI, 49931, USA.



Plant growth promoting rhizobacteria (PGPR) have been studied due to their beneficial mechanisms to increase the availability and uptake of mineral nutrients for plant growth. The use of PGPR as a biofertilizer (BF) in agriculture has also increased at a rapid pace, as an alternative to limit the excessive use of chemical fertilizers (QF) in crop production and reduce environmental impacts. Uncertainty remains about the ultimate environmental benefits of using BFs, compared to typical chemical fertilizer use. Life cycle assessment (LCA) has become an increasingly useful approach for identifying, quantifying, and evaluating the total potential environmental impacts of products and services. Using a standardized cradle to field (LCA) methodology, this study aims to: 1) Develop an LCA module of biofertilizer production, 2) Compare wheat production using different fertilization rates through local intensive agriculture in the Yaqui Valley in northwest Mexico. Using Ecoinvent life cycle inventory data with the SimaPro LCA model, this study evaluates the global warming potential, depletion of natural resources, terrestrial ecotoxicity and eutrophication potential of several wheat production scenarios using different fertilizer types and quantities. Various scenarios that maintain similar yields with mixtures of BF and QF were evaluated. Results from the study will allow for the quantification of the environmental impacts associated with specific components in the process to produce BFs and of the environmental benefits in the production of wheat when using BFs and less QF. The LCA study identified the optimal fertilization approach for wheat cultivation, and was used to suggest improvements in the BF production in an effort to further reduce environmental impacts for this novel product.

Keywords: biofertilizer, LCA, sustainability, wheat production.



**Tentative baseline values of less frequently regulated elements in urban
park soils of Alcalá de Henares, Spain**



Antonio Peña-Fernández^{*(1)}, Gevorg Tepanosyan⁽²⁾, M. Carmen Lobo-Bedmar⁽³⁾

⁽¹⁾ Faculty of Health and Life Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK.

⁽²⁾ The Center for Ecological-Noosphere Studies, National Academy of Sciences, Abovian-68, Yerevan 0025, Armenia.

⁽³⁾ Departamento de Investigación Agroambiental. IMIDRA. Finca "El Encín", A-2, Km, 38.2, 28800 Alcalá de Henares, Madrid, Spain.

*Corresponding author: antonio.pena-fernandez@dmu.ac.uk



Background or baseline concentrations of metals in urban soils are critical to determine risks and establish clean-up decontamination thresholds to protect the public, but these concentrations remain unknown for less frequently regulated and monitored metals in most of the European cities. Iterative 2σ -technique¹ and boxplot methods² were used to establish tentative background values for a variety of metals in soils from urban parks in Alcalá de Henares (Spain), which resulted in the same median values for the elements studied (mg/kg): Ag (0.003), Co (0.8), Mo (0.24), Pt (0.26), Rh (0.15), Sb (0.15) and Y (5.1). The percent differences when comparing these values with the average continental crust³ values were as follows: 47.1, 84.2, 78.8, 83, 34.5, 158, 52.8 and 75.4 for Ag, Co, Mo, Pt, Rh, Sb and Y, respectively. These differences could be explained by the geochemical peculiarities of Alcalá's soils. Our results are a preliminary step for developing background values following implementation of a further monitoring programme in the urban soils studied.

Keywords: baseline, park soil, trace elements

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PM_{2.5} concentration in the urban area of Aguascalientes, México, during winter and summer

J. A. González-Torres⁽¹⁾, E. M. Ramírez-López^{*(1)}, A. J. Meraz-Jimenez⁽¹⁾, H. A. Balzen⁽²⁾, D. Ramírez⁽²⁾, P. Rangel-Jiménez⁽¹⁾

⁽¹⁾ Universidad Autónoma de Aguascalientes, Av. Universidad 940, Ciudad Universitaria, Aguascalientes, México.

⁽²⁾ Texas A&M University - Kingsville, 700 University Blvd., Kingsville, TX, USA.

*Corresponding author.: emramir@correo.uaa.mx



The state of Aguascalientes is located at the centre of the Mexican Republic. The municipality of Aguascalientes has four industrial parks, approximately 458 small brickworks businesses for the artisan production of brick and a vehicular load of approximately 342 thousand automobiles. These activities are emission sources of particles smaller than 2.5 μm in size (PM_{2.5}). The PM_{2.5} can cause serious health problems¹. The goal of this study was to determine the air quality at the east and north of the municipality of Aguascalientes by measuring the ambient concentration of PM_{2.5} at these locations and the PM_{2.5} dispersion during the winter and summer of 2017. The monitoring of the respirable PM_{2.5} at the eastern location was carried out following the procedures as per NOM-035-SEMARNAT-1993, with a TAS® minivol equipment, in winter and summer of 2017. The dispersion study at the east of Aguascalientes was carried out using the weather information of the station El Cedazo-INIFAP, and RUOA-UNAM-UAA to the north. The air quality at the east of the municipality of Aguascalientes presented a chronic population exposure to PM_{2.5}. Concentrations of PM_{2.5} were up to 80 μgm^{-3} , exceeding the 45 μgm^{-3} established by the NOM-025-SSA1-2014. A similar behaviour occurred in the north of the city. The air quality index (AQI) went from good to unhealthy for both seasons of 2017. The dispersion was predominant from the NE to the SW, and from the S to the N. The PM_{2.5} emissions probably came from the vehicular combustion, industrial activities and brickworks and transported from other nearby areas.

Keywords: Aguascalientes, air quality, dispersion, minivol, particulate matter.

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Germination toxicity tests in fireworks contaminated soils using seeds of radish (*Raphanus sativus*) and corn (*Zea mays*)

IV Robles-González^{(1)*}, VS Robles-González⁽²⁾, J Zafra-Soto⁽³⁾ and A Ordaz-Cortés⁽³⁾

⁽¹⁾ Instituto de Investigaciones en Materiales, UNAM. Circuito Exterior, Ciudad Universitaria, Coyoacán, 04510, Ciudad de México, México.

⁽²⁾ Universidad Tecnológica de la Mixteca, Carretera a Acatlima Km 2.5 C.P. 6900. Huajuapán de León Oaxaca, México

⁽³⁾ Universidad Mexiquense del Bicentenario. Av. Ex-Hacienda de Portales S/N, Col. Villa Esmeralda, C.P. 54910. Tultitlán, Estado de México, México.

*Corresponding author: irerirobles@iim.unam.mx



The use of fireworks in religious, cultural, sports, national and family celebrations is an important cultural activity, that takes place in different parts of the world. Unfortunately, there are not enough reports about the levels of environmental pollution (in air, soil, water and sediments) and the health damage in the population exposed to the manufacture and use of fireworks. In Mexico, the main production of handcraft fireworks is produced in the Estado de Mexico, specifically in the location of Tultepec, where is characterized for concentrating around of 40% the elaboration and commercialization of the fireworks. The aim of this work was to determine the effect of the used and production of the fireworks on the soil by performing of acute ecotoxicity testing (AET), using seeds of agricultural importance. Samples of contaminated soils (SCS) was collected during three seasons of increased production, sale and burning fireworks. The SCS were physicochemical characterized. Aqueous extracts soils (AES) were obtained and evaluated in the AET using seeds of radish (*Raphanus sativus*) and corn (*Zea mays*). Inhibition in the radicle elongation of corn seed was observed in all sites evaluated, mainly in the Santiago Teyahualco site, whose length of the radicle was three times less than the control. Not significant effect of inhibition was observed on the growth of the radicle elongation of seeds radish. In the present work was observed that soils contaminated by the use and manufacture of fireworks could have a negative effect on the germination of a basic crop such as corn.

Keywords: acute ecotoxicity, soil, pollution, fireworks



Levels and spatial distribution of pollutants in an aquatic ecosystem in western Mexico

Celia De La Mora-Orozco⁽¹⁾, Hugo Ernesto Flores-López*⁽¹⁾, Álvaro Agustín Chávez-Durán⁽¹⁾, Irma Julieta González-Acuña⁽²⁾, Juan Napoles-Armenta⁽³⁾, Edgardo Martínez-Orozco⁽³⁾

(1) *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Av. Biodiversidad 2470, Tepatitlán de Morelos, Jalisco.*

(2) *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Carretera Internacional México - Nogales Kilómetro 6, Centro, 63300 Santiago Ixcuintla, Nay.*

(3) *Instituto Tecnológico José Mario Molina Pasquel y Henríquez, Avenida José Guadalupe Tejeda Vázquez, 557, Arandas, México*

*Corresponding author: flores.hugo@inifap.gob.mx

Water quality evaluation is an important parameter to classify the use of water. The La Vega dam is located at 9 km south of Teuchitlán town and 5 km south of the Ameca River in Jalisco México. The main dam water sources are fountains and the Teuchitlán river (to the north) the Salado River (to the south-east), and seasonal agricultural runoff. This water body is supplying approximately 90% of the water for irrigation for about 5,314 ha of sugar cane farming and others relatively minor agricultural products. Besides irrigation, fishing is an important activity to support families from the surrounding areas. The objective of this study was to evaluate the level of pollution in La Vega dam in Jalisco Mexico. Physical-chemical parameters like electrical conductivity, As, Cd, Hg, Co, Cr, Fe, Mg, Na, Pb, Zn, Ni, B, Ca, Mn and K were analyzed to determine the water quality available in the dam. Seven water samples were collected along the dam and five from the main water inflows. Samples were collected after the rain season of 2014. Results showed that Cd, Co, Cr, Hg, Pb, Zn and Ni, were under the range detection limit. Meanwhile Fe (0.129 ppm) and Mg (0.078 ppm) were under the Mexican Standards for irrigation and aquaculture. Elements such as Na, Ca, K and Mg were identified in high concentrations (22.73, 20.12 and 9.212 ppm). Special attention has to be taken to B and As, the concentration were above the Mexican Standards for irrigation and aquaculture, the mean B concentration was 4.5 ppm, meanwhile the As average concentration was 0.15 ppm. The results clearly demonstrated severe restriction for crop irrigation due to the high levels of boron, arsenic, and sodium (SAR). The Salado River at the south was identified as the main pollution source, where high sodium, calcium, arsenic and magnesium concentrations were detected.

Keywords: pollutants, spatial distribution, water.



Chapala, Michoacán. An Environmental Risk Assessment



H. Andrade Prado ⁽¹⁾, M. A. Velázquez Machuca* ⁽¹⁾, J. L. Pimentel Equihua ⁽²⁾

⁽¹⁾ Instituto Politécnico Nacional-CIIDIR Michoacán. Justo Sierra 28, Centro, Jiquilpan, Mich. México.

⁽²⁾ Colegio de Postgraduados-Campus Montecillo. Carr. México- Texcoco km 36.5, Montecillo, Estado de Mex.

México.

*Corresponding author: mvelazquezm@ipn.mx



The contamination by heavy metals and boron is an environmental problem that is increasing continuously due to anthropic activities, especially in agricultural soils where urban wastewater is mixed with irrigation water. On the other hand, it is considered that sediments in water bodies are the final deposit of heavy metals because solid substances accumulate over time and chemical forms soluble in water can precipitate, flocculate, agglomerate and form complexes through the adsorption to inorganic substances 1. The objective of this paper was to determine the concentration of heavy metals and boron in irrigation water, sediments and agricultural soils of the "Ciénega de Chapala", Michoacán, District of Irrigation 024 and analyze their potential environmental risk. 20 samples of irrigation water, sediments (channels and/or main drains) and agricultural soils were collected in places with intense agricultural activity. In water, boron (B) was determined by the method of azomethine-H, dissolved heavy metals (Fe, Mn, Cu, Zn, Pb, Cd, Sr, Ni, direct reading in Atomic Absorption Spectroscopy) and totals (acid digestion, Atomic Absorption Spectroscopy). In sediments and soils, B and total metals (Pb, Cd, Sr, Ni) and available metals (Fe, Mn, Cu, Zn, DTPA) were analyzed. The multivariate statistical analysis was used to group the variables. In water, the content of B was of nd to 1.99 mg/L and no heavy metals were detected. The principal components analysis (PCA) indicates that two factors explain 82% of the variance and were associated with the dissolution of ions from natural sources and wastewater. In sediments, the concentration of B was 2.33 to 16.8 mg/kg and for total metals Fe, Zn, Mn and Pb was 2889 to 15328, 6.95 to 52.6, 103.9 to 338.2 and 1.95 to 23.8 mg/kg, respectively. 25% of the samples exceeded the limit of 16 mg/kg (low risk) for Pb₂, so it is inferred that this metal will be causing a moderate impact on biota in those sites. The sites with the highest concentrations of Pb were located in the irrigation zone on the left bank of Lerma River. In soils, the concentration of B was 0.03 to 6.92 mg/kg and the total Pb was 6.6 to 22.3 mg/kg. For boron, 55% of the samples exceed the limit of 1.0 mg/L for sensitive crops (citrus, blackberry, strawberry, bean, wheat). The Pb enrichment factor was 2.9. Six factors were found that explain 86% of the variance and were associated with: geothermal influence, rock weathering, salinity of irrigation waters, municipal wastewater and agricultural drainages.

Keywords: heavy metals, boron, environmental risk, pollution.

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Environmental impact on the river water quality using multivariate statistical techniques

Elizabeth Ríos-Ramos ⁽¹⁾, José A. de Diego ⁽²⁾, Luz Breton-Deval ^{*(1)}

⁽¹⁾ CONACYT-Instituto de Biotecnología de la Universidad Nacional Autónoma de México, Avenida Universidad 2001, Chamilpa, 62210 Cuernavaca, Mor.

⁽²⁾ Instituto de Astronomía, Universidad Nacional Autónoma de México, Av Universidad 3000, Cd. Universitaria, Coyoacán, 04510 Ciudad de México, CDMX

*Corresponding author e-mail: lbreton@ibt.unam.mx, bretondeval@gmail.com



The measurements of the water quality parameters along the rivers allows to determine hot spots where the level of contamination increases due to the quantity of discharges or due to some topographic change of level which make the waters stagnate or go slower. Elucidate these changes are important for designing an integrated management of the resources of each particular hydrological basin (Muchitsch et al., 2011). Moreover, the regular evaluation of river quality water through time allow observe seasonal variations or anthropogenic changes. Currently, the use of multivariate statistics, such as principal components and cluster analysis becomes a useful methodology for the reduction of a large number of physicochemical parameters and for grouping areas of similar water quality. The aims of this study are to obtain a longitudinal profile of the water quality in the Apatlaco River and identify the principal variables that have an influence on the water quality of the river. The Apatlaco River is in México at Morelos state, it is 63 km long and runs throughout 10 districts of Morelos. Water samples were collected at 17 sampling points along the Apatlaco River. The pH, dissolved oxygen, turbidity and temperature were measure in situ using a HANNA multi-parametric sonde HI9828. While, parameters for instance ammonia, nitrate, nitrite, biological oxygen demand, chemical oxygen demand, total phosphorus, Kjeldahl nitrogen, orthophosphorus, total carbon, turbidity, and total coliform bacteria were analyzed using the Standard Methods techniques. Currently, the water quality is not the same along the river due to the different kinds of discharges. There are 321 discharges, 158 are from the industrial sector and 137 and 26 are from domestic and agricultural sector, respectively. The general tendencies of water quality for year showed that total coliforms increased by 59% in the last year. On the other hand, total carbon, biological oxygen demand, nitrate, turbidity, Kjeldahl nitrogen and chemical oxygen demand parameters decreased in the last year. There are some parameters such as pH, total phosphorus, orthophosphorus, and nitrite that their tendency is the same along these years.

Keywords: water quality, multivariate statistics, River, principal component



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Water quality assessment in an aquatic ecosystem in Jalisco, Mexico

Edgardo Martínez-Orozco⁽¹⁾, Luz Stephanie Valadez-Romo⁽¹⁾, Irma Julieta González-Acuña⁽²⁾,
Juan Napoles-Armenta⁽¹⁾, Celia De La Mora-Orozco^{*(3)}

⁽¹⁾ *Instituto Tecnológico José Mario Molina Pasquel y Henríquez, Avenida José Guadalupe Tejeda Vázquez, 557, Arandas, México*

⁽²⁾ *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Carretera Internacional México - Nogales Kilómetro 6, Centro, 63300 Santiago Ixcuintla, Nay.*

⁽³⁾ *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Av. Biodiversidad 2470, Tepatlán de Morelos, Jalisco.*

*Corresponding author: delamora.celia@inifap.gob.mx

Human activities may cause degradation in surface and ground water affecting its potential use for domestic, industry, recreational and animal consumption among others. Pollution problems associated with water quality is affecting most ecosystems around the planet. Water quality evaluation is an important parameter to classify the use of water. The El Tule dam is located at south of Arandas town, the main dam water sources are the Lagunillas and Coyotes creek (to the north) and the Recibidora creek (to the east), moreover the municipal wastewater from a small town Santa María del Valle (to the east), besides the seasonal agricultural runoff. The water body is used for irrigation, recreational activities, fishing and the last year intensive blueberry production using greenhouses is an increasing activity around the dam. The objective of this study was to evaluate the water quality conditions of El Tule dam. Ten water samples were collected along the dam to evaluate the water quality, physical-chemical parameters like electrical conductivity, pH, PO₄, NO₃-N, NH₄-N, Na, Mg, Ca, K and B were analyzed. The Sodium Adsorption Ratio (SAR) was calculated, this includes the concentration of sodium, calcium and magnesium. The SAR values results demonstrated a low salinity risk (0.68) the SAR values were similar along the dam, with slightly difference at the east (0.72) where the main inflow is the domestic wastewater. Moreover, the results demonstrated also low boron concentration (0.394 mg/L). The average pH was 7.5, and the electrical conductivity of 0.165 μS/cm, the NO₃-N, and NH₄-N concentration were under the Mexican Standards for irrigation and aquiculture. As a conclusion, the water quality from El Tule dam is acceptable and meet the Mexican Standards for irrigation, aquiculture, industry and recreational activities. However, research continues to determine the seasonal water quality variation.

Keywords: Sodium Adsorption Ratio (SAR), irrigation water, Jalisco, México.



Distribution and fractionation of heavy metals in surface sediments of the Bacanuchi river, Sonora, Mexico

León-García, G.J.⁽¹⁾, Meza-Figueroa, D.M.⁽²⁾, Valenzuela-García, J.L.⁽¹⁾, Encinas-Romero, M.A., Villalba-Atondo, A.I.⁽³⁾, Encinas-Soto, K.K.⁽¹⁾, Gómez-Álvarez, A.^{*(1)}

⁽¹⁾ Department of Chemical Engineering and Metallurgy, University of Sonora, Blvd. Luís Encinas and Rosales, Hermosillo, Sonora 83000, México.

⁽²⁾ Department of Geology, University of Sonora, Luis Encinas and Rosales, Hermosillo, Sonora 83000, México.

⁽³⁾ Department of Scientific and Technological Research, University of Sonora, Luis Encinas and Rosales, Hermosillo, Sonora 83000, México.

*Corresponding author: agustin.gomez@unison.mx

The objective of this study was to evaluate levels of heavy metals in sediments of the Bacanuchi river, impacted by the mining activity of the region. A sequential extraction analysis was performed using the BCR method. The evaluation of heavy metal contamination was performed using Enrichment Factor (EF), Geoaccumulation index (Igeo), Low Effect Level (LEL), and the Severe Effect Level (SEL). Total metal concentration (mg/kg) fluctuated in the following ranges: Cd (<BDL), Cu (8-716), Cr (8-90), Fe (7,300-52,400), Mn (80-938), Ni (6-48), Pb (14-210) and Zn (41-470). A sequential extraction analysis indicated the following order of geochemical fractions: residual>Fe/Mn oxides> exchangeable>organic matter. A significant percentage of metals was observed in the non-residual fraction, indicating anthropogenic contributions. In this fraction, the order of mobility and/or bioavailability of metals was: Mn>Cu>Ni>Pb>Zn>Fe>Cr. EF showed an anthropogenic enrichment in both rivers for Cu, Cr, Mn, Ni, Pb and Zn, mainly derived from the mining activity. Values of Igeo were classified as non-contaminated to moderately contaminated. The Bacanuchi station (before confluence), showed moderate to strong contamination of Cu and Pb. The LEL criterion was exceeded for Cr, Cu, Fe, Mn, Ni, Pb and Zn. The SEL criterion was only exceeded for Cu and Fe. This may represent a danger to biota of the river, due to the high mobility and bioavailability of metals.

Keywords: Metals, Bioavailability, Sediments, Bacanuchi river.



Exposure to Glyphosate and Aminomethylphosphonic acid in Communities of Northwestern Mexico

Balderrama-Carmona A.P.*⁽¹⁾, Flores-Gil J.A.⁽¹⁾, Leyva-Soto L.A.⁽²⁾, Gortáres-Moroyoqui P.⁽²⁾

⁽¹⁾ Universidad de Sonora, Lázaro Cárdenas, N°100, Navojoa, Sonora, México.

⁽²⁾ Instituto Tecnológico de Sonora, 5 de febrero, N° 818, Cd. Obregón, Sonora, México.

*Corresponding author: pabal@navojoa.uson.mx



Glyphosate is the most sold herbicide worldwide because of its high solubility, whose main metabolic intermediary is aminomethylphosphonic acid (AMPA). Both products are harmful for human health⁽¹⁾. This research intends to provide relevant information on environmental health by assessing exposure to glyphosate and AMPA in rural communities of scarce resources where deficiencies to obtain water and sanitation exist. The area of study is located in El Valle del Mayo with a total of 307,390 inhabitants. The description of the population was performed by means of socioeconomic, consumption and symptomatology surveys. With respect to the environmental description, water samplings were taken from irrigation, soil (sediment and drains) and private well water to determine the concentrations of glyphosate and AMPA. The samples were analyzed by high resolution liquid chromatography. Exposure to potentially contaminated water was higher for housewives and children as they spend more time in the address, which makes them the main groups in risk; a significant relationship between socioeconomic status an exposure to potentially contaminated water and consumption of private well water and frequency of diseases were calculated ($p \leq 0.05$). As to the concentrations of glyphosate in all the samples, they were under the detection limits ($N = 24$); nevertheless, as to AMPA concentrations, 75% of soil samples were positive in concentration oscillating from < 15 to $342.75 \mu\text{g/g}$, which evidences exposure. regulations. In addition, we determinate that water consumption from the private well near to irrigation canals correlated statistically with diabetes ($p \leq 0.03$) and hypertension ($p \leq 0.004$). It is necessary to develop more toxicity studies that indicate an accurate value to establish that the herbicide and its metabolite do not cause harm to the human beings in a short and long-term.

Keywords: contamination, soil, water, herbicide

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Bioindicators of As, Pb and Hg in irrigation water, soil and chile (*Capsicum Annuum*) in an agricultural area near to mine tailings

D.M. Orozco Corona ⁽¹⁾, C. Letechipía de León ⁽¹⁾, H. R. Vega Carrillo ⁽¹⁾, I. Gavilán García ⁽²⁾, R. Basurto Gaytán ⁽¹⁾

⁽¹⁾ Universidad Autónoma de Zacatecas, Jardín Juárez 57, Zacatecas, México

⁽²⁾ Universidad Autónoma de México, Circuito Exterior S/N, Ciudad de México, México.

*Corresponding author: ing.dulceorozco87@gmail.com



The food harmlessness is a means to protect the economic and sustainable development of a country and, at the same time to promote and take care of public health which represents a global challenge. The contamination by potentially toxic elements is a serious environmental and human health problem. In such a way that it is significant to determine bioindicators of the presence of this. The objective of the research was to evaluate the concentration of As, Pb and Hg in irrigation water, soil and chile (*Capsicum Annuum*) in agricultural plots at the municipality of Morelos, Zacatecas, for an agricultural area with a mining history. Nine agricultural plots were taken (120 samples) with reference to the established norms. For the soil and chile matrices, they were digested by the wet acidic microwave digestion method, prepared for the determination of the analyte concentration and measured by EAA-F and HG. The average values of the concentrations for As, Pb and Hg in irrigation water were 0.0130 mg / L, 0.0107 mg / L and 0.0005 mg / L, respectively. For the soil matrix the average values for the same analytes were: 83.373 mg / kg, 78.179 mg / kg and 7.877 mg / kg and finally for the chile matrix these were: 8.557 mg / kg, 6.115 mg / kg and 5.253 mg / kg. The concentrations of the bioindicators As, Pb and Hg found in irrigation water, soil and chile were significantly relevant when compared with the concentrations reported in the different regulations, Hence, we can estimate some potential risk of the presence of these contaminants for the environment and human consumption. Resulting in complications of public health. Furthermore, it is necessary to carry out activities of prevention and remediation of the study site.

Keywords: Heavy Metals, atomic absorption, mine tailing



Health risk assessment for drinking water consumption in communities near from Cuitzeo Lake, Michoacán, México

L.N. Rodríguez- Cantú^(1,2), M.M. Meza- Montenegro⁽²⁾, M.A. Martínez-Cinco⁽³⁾, M.M. Navarro-Farfán⁽³⁾, R. Alfaro Cuevas-Villanueva⁽⁴⁾.

⁽¹⁾ Programa de Doctorado en Ciencias en Biotecnología, Instituto Tecnológico de Sonora, Cd. Obregón, Sonora, México.

⁽²⁾ Departamento de Recursos Naturales, Instituto Tecnológico de Sonora, Cd. Obregón, Sonora, México.

⁽³⁾ División de Estudios de Posgrado de la Facultad de Ingeniería Química, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Múgica S/N, Morelia, Mich., México.

⁽⁴⁾ Instituto de Investigaciones en Ciencias de la Tierra, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Múgica S/N, Morelia, Mich., México.

*Corresponding author: lauranrc@gmail.com

Cuitzeo Lake is located in the volcanic region of the Tarascan Plateau in northern Michoacán, México. It was formed within a tectonic basin and is the second largest lake in México. Some studies show the presence of heavy metals and arsenic in the Cuitzeo Lake basin and communities around it¹. Arsenic is a toxic and carcinogenic metalloid that represents a global health problem, and the infant population represents greater risk because it is more susceptible than adults to the toxic effects of the metalloid². The purpose of this study is to assess the health risk from exposure to arsenic (As) through drinking water consumption of children living in the communities of Zinapécuaro and Araró, nearby to Cuitzeo lake, Michoacán, México. Sampling was carried out in well water, spring and household intakes of the study locations. The arsenic concentrations were determined by Atomic Absorption Spectrophotometry coupled to Hydride Generator (EAA-GH)³. In the Uranus well, the lowest concentration detected was 0.0114 mg As/L and the Taimeo spring had the highest level with a value of 0.0645 mg As/L. 100% of the samples exceeded the arsenic levels established by the World Health Organization (WHO) of 0.01 mg/L⁴ and 71% exceeded the limit established by NOM-127-SSA1 of 0.025 mg/L⁵. Risk quotients (HQ), values were obtained in a range of HQ = 0.80 for the Uranus well and HQ = 4.52 for the Taimeo spring, which means that children in some communities have risk of developing chronic diseases associated with the exposure of arsenic, for the consumption of drinking water.

Key words: Arsenic, Children, Drinking water, Risk analysis.

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Hydrogeochemical and bacteriological assessment of potable water in

Taxco de Alarcón, Guerrero, Mexico, a mining site



G. Sánchez Montoya⁽¹⁾, O. Talavera Mendoza*⁽¹⁾, **G. Hernández-Flores****⁽²⁾, E. Díaz Villaseñor⁽¹⁾,
A.H. Ramírez Guzmán⁽¹⁾, Z. Galarza Brito⁽¹⁾

⁽¹⁾ *Escuela Superior de Ciencias de la Tierra, Universidad Autónoma de Guerrero. Ex hacienda San Juan Bautista s/n, Taxco el Viejo, Guerrero, CP 40323, México.*

⁽²⁾ *CONACYT - Escuela Superior de Ciencias de la Tierra, Universidad Autónoma de Guerrero. Ex hacienda San Juan Bautista s/n, Taxco el Viejo, Guerrero, CP 40323, México.*

*Corresponding author: otalaver@email.arizona.edu, otalavera.uagro@gmail.com

** Corresponding author: gioheflo_10@hotmail.com, ghernandez@conacyt.mx

Taxco de Alarcón is an important mining site in Mexico. The town is a well known tourist destination where the potable water quality is important for guaranteeing the health of their users. Due to the altitude of the city, the surface and rain water are the main sources of water. Thus, the aims of this study were i) to analyze the hydrogeochemistry and bacteriology of the potable water of Taxco de Alarcón, Guerrero, Mexico, regarding the NOM-127-SSA1-1994 and ii) to characterize the composition and chemical speciation of particulate matter suspended in water to determine the content of potentially toxic elements. The representative samples were taken and analyzed from the municipal water distribution network, the water treatment plant, the Chacualco spring and a mixture of the waters of the Chontalcuatlán River, the Tenería spring and the San Marcos and El Sombrerito dams. At each sampling site, the temperature, pH, Eh and electrical conductivity were determined. On the other hand, bacteriological analyzes, anions, cations, alkalinity, sulfate, nitrate, fluorine and turbidity contents were analyzed at laboratory. Finally, to assess the presence, concentration and bioavailability of toxic metals in the water, the extractable metal content was determined. It was determined that 94% of the analyzed samples did not comply with the standard NOM-127-SSA1-1994. Moreover, the distribution of the analyzed elements in the different chemical fractions of the particulate showed that the concentration of Cd (4.17 mg/kg) is associated with the carbonate fraction. This means Cd is moderately bioavailable. On the other hand, concentrations of Pb, Zn, Mn and Cu, 62.2, 42.3, 243 and 1026 mg/kg, respectively, were detected in Fe-Mn oxyhydroxide fraction, also moderately bioavailable, whereas the higher concentrations of As, Fe and V, 24.0, 7583 and 31.3 mg/kg, respectively, were associated with the residual fraction, considered as non-bioavailable. Overall, particulate matter is characterized by high concentrations of total metals (Al, Cu, Fe, Pb, As, Cr, V, Zn, Sr, Ni) and to improve potable water quality, the results indicate that modifications must be incorporated along the water treatment processes, in the distribution network and management.

Keywords: Chemical speciation; Hydrogeochemistry; Particulate; Potable water.



Concentration of lead (pb) in muscle and hepatic tissues in estuarine

fishes from the south of sinaloa (gulf of california)



Brigitte Gil-Manrique^{(1)*}, Omar Nateras-Ramírez⁽¹⁾, Ana I. Martínez-Salcido⁽¹⁾, Jorge Ruelas-Inzunza⁽²⁾, Felipe Amezcua⁽³⁾

⁽¹⁾ Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Circuito Exterior s/n Ciudad Universitaria, 04510 México, DF, México.

⁽²⁾ Instituto Tecnológico de Mazatlán, Corsario 1 No. 203, Colonia Urías, 82070 Mazatlán, SIN, México.

⁽³⁾ Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Joel Montes Camarena s/n, 82040 Mazatlán, SIN, México.

* Corresponding author: biomarbrigitte@gmail.com

Pollution of aquatic environments with harmful trace metals, and its subsequent impact on organisms, is more dramatic within estuaries, lagoons, and semi-closed coastal zones. The studied lagoon systems: Urías, Huizache-Caimanero and Teacapán, are located in the SE of the Gulf of California; they are economically important for small scale fisheries. The three lagoons are impacted by diverse activities (agriculture, aquaculture, mining, and dams) that may increase metal loads and favor biota accumulation, which affect the organisms including commercial fish. Since polluted fish from this area may become a public health concern, information on elemental concentration in fish species in these lagoon systems becomes of great importance. Lead (Pb) was determined in liver and muscle of twelve demersal fishes, with the purpose of comparing concentrations of Pb in the three ecosystems and to assess the potential human health risk based on the levels established for Mexican government and FAO-WHO. Fish were collected bimonthly during two sampling campaigns (2011–2012 and 2013–2014); in the rainy season (june to october, n=235) and dry season (rest of the months, n = 265). Concentrations of Pb in liver and muscle samples were measured by graphite furnace atomic absorption spectrophotometry (GF-AAS, Varian SpectrAA 220). The quality control of the analytical method was assessed by using certified reference materials of fish muscle (DORM-3, NRC-Canada), recovery percentage 103.7 and relative standard deviation 10% and liver (DOLT-4, NRC-Canada), recovery percentage 103.9 and relative standard deviation 18%). Results are expressed as micrograms per gram on a dry weight basis; the limit of detection was 0.040 µg g⁻¹. The levels of Pb in liver were higher than in muscle; with respect to the sites, mean levels of Pb were higher in Huizache > Urías > Teacapán. Regarding individual values, the highest Pb concentrations were measured during the rainy season in the muscle (1.01 µg kg⁻¹ dw) and liver (6.01 µg kg⁻¹ dw) of *D. peruvianus* from Urías and Huizache, respectively. Cluster and nMDS analysis of Pb concentrations formed clear cut groups according to the locality (Teacapán Huizache, Urias), tissues (liver, muscle) and sizes (small, medium, large); but not with season (dry, rainy), residence to estuary (resident, intermediate, migrant), trophic habits (carnivorous, herbivorous, omnivorous), and trophic level (2, 3, 4). Results were also corroborated by ANOSIM, and determined statistically differences from SIMPER. The Pb concentrations in muscle tissue (wet weight) did not exceed the Mexican norm (1 µg/g–1 ww), but four fishes (0.8%) exceeded the level established by FAO-WHO (0.3 µg/g–1 ww), *Diapterus peruvianus* (0.72 µg/g–1 ww), *Cynoscion xanthulus* (0.48 µg/g–1 ww), *Mugil curema* (0.36 µg/g–1 ww) and *Pomadasys macracanthus* (0.34 µg/g–1 ww) from Teacapán.

Keywords: lead, tissue distribution, lagoon systems, Gulf of California.



An ethical and environmental perspective of technology that incorporates genetically modified organisms into food

Yáñez Vergara A.G.*⁽¹⁾, Sanabria García, D. A.⁽¹⁾, Carrasco González, J. A.⁽¹⁾, García Moreno, T. N.⁽¹⁾, Poggi Varaldo, H. M.*⁽²⁾.

⁽¹⁾ CINVESTAV-IPN, DCTS, P. O. Box 14-740, CDMX (D.F.) 07000, Mexico.

⁽²⁾ CINVESTAV-IPN, Environmental Biotechnology and Renewable Energies Group, P. O. Box 14-740, CDMX (D.F.) 07000, Mexico.

* Corresponding author: alejandra.yanez@cinvestav.mx ; r4cepe@yahoo.com



Today more food than enough is produced for everyone, however, about 815 million people suffer from chronic hunger, and malnutrition affects one out of three people on the planet. The development of GM crops has also brought a great controversy among academia, environmental associations, GM seed companies, the agricultural sector, government institutions and consumers. The use of GMOs still has no ruling for or against, however, as it is an incredible source of income through patents, different companies continue to promote the use of GMOs despite not knowing all possible side effects these GMOs could generate in humans or in the environment. In the present work, the current situation in the GMO issue is analyzed from a socioeconomic and environmental perspective. This work is approached from the following points: (i) Current situation on nutrition in the World; (ii) socio-economic aspects; (iii) ethical aspects; (iv) environmental aspects. So far, the lack of information regarding the risks that GMOs can cause to health and the environment is predominant. Among the most important benefits obtained from the production of foods based on GMOs, there is a better nutritional composition or of substances with therapeutic effects and the improvement in food processing through the alteration of the enzymes present in them, it is the increase of more than 370 million tons of crop yield with the consequent positive environmental impact by more efficient use of agricultural land. The latter can have a negative environmental impact because it implies intensifying intensive agriculture. On the other hand, it is debatable to put the accent on the amount of food because the bottlenecks seem to be of another type. Studies on environmental and economic effects of the application of GM technology in agriculture have been published that show some positive environmental effects. With the application of GM crops, the use of pesticides (1996-2008) has been reduced by 352 million kg (-8.4%) of active ingredient. To measure the effects of incorporating GMOs in food, it is necessary to carry out case-by-case studies, with complex systems analysis tools due to the existing variety of crops and GM foods, and the large number of variables that act. Although the scientific development of new GM technologies will continue to evolve, it is necessary to carry out an environmental evaluation of GMOs, as well as an ethical analysis that takes into account the social and cultural context of the country or region where these new technologies are implemented. Also, evaluate that they adhere to a regulatory framework that provides clear and accurate information to consumers, including benefits and risks to health and the environment. In this way the population will have sufficient arguments to decide whether to consume these foods or not.

Keywords: ethics, environmental, technology, genetic modification.



services



Julliana M. P. Araújo*⁽¹⁾, Maria A. P. Cecílio⁽¹⁾, Maria V. R. Ramos⁽¹⁾

⁽¹⁾ Federal Institute of Education, Science and Technology of the Sertão Pernambucano, Road Tamboril, s/n, Ouricuri, Brazil

* Corresponding author: jullianameloaraujo@gmail.com



Under Brazilian law number 11.445¹, among the fundamental principles of basic sanitation are universal access, comprehensiveness, availability, efficiency and sustainability. The sanitation system should operate so that the water supply is performed in appropriate quality and quantity; sewage and solid waste is collected, treated and disposed properly, and management of rainwater is carried out to prevent flooding. However, in general, in Brazil, these systems are not designed in a fair, economical, efficient and / or environmentally viable. This scenario brings up the need for new positions in policy management, which value popular participation and social control. Sanitation needs to be seen by the community as a set of actions that directly affect the health, well-being and the relationships of people, as the community uses the same space, and all have the same rights and duties. The starting point for understanding the environmental policies is precisely this duality between the rights and duties of the population in the community. All have the right to enjoy a healthy environment, as guaranteed in the Constitution. On the other hand, everyone should protect and guarantee the rights of others. That is, for the proper functioning of the systems, and the installation and periodic maintenance on the premises by the government, the community served should use them consciously, avoiding waste and damage. Therefore, this study aimed to know the perception of communities in the city of Ouricuri-PE on the scope and deficits of the existing sanitation infrastructure and empower them through targeted environmental education enhancement and use of sanitation services, stressing its importance to the environment, quality of life and health. As a methodological strategy, we were initially applied questionnaires in three communities of the municipality: Santo Antônio Neighborhood; Nossa Senhora Maria de Fátima Neighborhood; and Center District. The tabulation of the data collected offered subsidies to carry out the next step, holding events in the community, being one Gymkhana in a public-school present in a community and two short courses for analysis of water quality consumed facing the residents of community. This work stimulated the citizens to perceive themselves as actors of community transformation and enabled the construction of together knowledge (summarized in a booklet) and encouraged the exchange of experiences between the community and the Federal Institute of Education, Science and Technology of the Sertão Pernambucano of Ouricuri *Campus*.

Keywords: Empowerment. Basic sanitation. Environmental education.

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Chapter 9 Control and Modeling of Environmental Processes



Multivariable Robust Regulation of Alkalinities and VFA in Continuous AD Processes against both Uncertain Kinetics and Operational Perturbations

V. Alcaraz-González^{*(1)}, F. A. Fregoso-Sánchez⁽¹⁾, V. González-Álvarez⁽¹⁾, J.P. Steyer⁽²⁾

⁽¹⁾ University of Guadalajara, Blvd. Gral. M. García Barragán 1451, 44430, Guadalajara, Mexico

⁽²⁾ LBE, INRA, 102 Avenue des Étangs, 11100, Narbonne, France

*victor.alcaraz@cupei.udg.mx.



In this paper we propose a simple but powerful robust Multiple-Input, Multiple-Output (MIMO) approach for regulating alkalinities, volatile fatty acid concentration and the strong ions concentration in continuous anaerobic digestion processes used in wastewater treatment. The system exhibits a large lack of knowledge on kinetic functions and their parameters. Moreover, these functions exhibit a very high nonlinear behavior. In addition, key state variables, like biomass concentrations, which cannot be measured, are estimated by using a Luenberger observer. Furthermore, process inputs are not well known. Thus, even under this highly uncertain scenario, the proposed control law uses minimal and partial available information for regulating exponentially the control variables stated beforehand. The control objectives are firstly established upon physicochemical criteria based on normal operational conditions and then formally stated in terms of the concerned state variables. In order to deal with these important uncertainties, the proposed control law is based upon the basis of adaptive robust exponential approaches¹. Two control inputs are used: the dilution rate and a supplementary NaOH supply. The resulting control law is tested and validated experimentally in a 1 m³ pilot plant for the treatment of red wine vinasses under the several uncertain scenarios established above. Results show that the multivariable control law is able to recover the system stability around the pre-specified set points despite this large variation in the process inputs as well as the high uncertainty in the process inputs and kinetics, showing a very high performance and usefulness for this kind of processes.

Keywords: Alkalinity, Anaerobic Digestion, Robust Regulation, Wastewater Treatment.

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Removal of Hexavalent chromium by chromate tolerant halobacteria isolated from saline soils in Sonora, Mexico

Yépiz Gonzalez Armando ⁽¹⁾, Estrada Alvarado María Isabel ⁽¹⁾, Vásquez Murrieta Maria Soledad ⁽²⁾, **Cira Chávez Luis Alberto** ⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 6 de abril, 818. Ciudad Obregón, Mexico.

⁽²⁾ Instituto Politécnico Nacional, Manuel Carpio, 471. Ciudad de Mexico, Mexico. C.P. 85000.

*Corresponding author: luis.cira@itson.edu.mx



Rapid urbanization and industrialization has resulted in large quantities of contaminants such as toxic metals to be released into the environment and represent a hazard for all forms of life. Chromium is widely used in industrial activities whose effluents often contain it's most toxic and mobile form (VI). Traditionally, these effluents are treated by physical-chemical methods that are often expensive, inefficient and result in the generation other toxic waste. Because of this, there is great interest in the development of environmentally friendly technologies such as bioremediation. Extremophiles, particularly halophiles, are of great interest for this purpose due to their ability to thrive in harsh conditions such as high salinity, pH, presence of organic contaminants, and toxic metals, among others. The present work consisted in the screening of halobacteria for chromate tolerance and removal, as well as the identification of mechanisms involved in the detoxification process such as production of exopolysaccharides (EPS) and siderophores. 10 strains were found to grow in presence of chromium ranging from 15 to 40 mM. 6 Strains (M2P, M1Q, BLSS1AM2035, N31, 30042 and YRAM72) showed a decrease of over 95% of Cr(VI) in liquid media after 120 h. There was no production of EPS by any of the strains. All strains showed production of siderophores in presence of Cr(VI), which could indicate the involvement of these molecules in the mobilization of the metal for the detoxification process. These results indicate that at least 6 of the strains assayed in this work could be interesting candidates for their use in hexavalent chromium remediation processes.

Keywords: Bioremediation, chromium, extremophile, halophile.



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Chapter 10 Environmental Biotechnology



Influence of assay conditions on the recombinant POXA 1B laccase activity detection.

L.D. Ardila-Leal^(1,2), M.C. Ferrucho-Calle⁽¹⁾, C.M. Rivera-Hoyos⁽¹⁾, A.M. Pedroza-Rodriguez⁽¹⁾, B.E. Quevedo-Hidalgo⁽¹⁾, R.A. Poutou-Piñales⁽¹⁾, **D.C Niño-Gómez⁽¹⁾**

⁽¹⁾ Pontificia Universidad Javeriana, Carrera 7 No 40-62, Bogotá, D.C., Colombia.

⁽²⁾ Universidad Francisco de Paula Santander, Avenida Gran Colombia No. 12E-96, Cúcuta, Norte de Santander, Colombia.

*Corresponding author: e-mail: rpoutou@javeriana.edu.co



Laccases (E.C. 1.10.3.2) are glycoproteins widely distributed in nature. Their structural conformation includes three copper sites in their catalytic center, which is responsible for substrates oxidizing; reaction that generates H₂O instead of H₂O₂^{1,2}. Measurement of laccase activity (UL⁻¹) is the way to demonstrate its production and functionality; however, results may vary depending on the type of laccase, buffer, redox mediators and substrates used. The aim was to select best conditions for detection of rPOXA 1B laccase activity produced in *P. pastoris*. Two "D-Optimal" Experimental Designs were developed (one for acetate buffer (AB) and another for citrate (CB)); in each design included 3 factors that were evaluated (reaction pH, substrate concentration (2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid, ABTS) and wavelength (λ) for measurement (using the corresponding molar extinction coefficient) so: AB (pH 4.0 and 4.5 ± 0.2, ABTS 0.5 and 2 mM, $\epsilon_{420\text{ nm}} = 36000\text{ M}^{-1}\text{ cm}^{-1}$ and $\epsilon_{436\text{ nm}} = 29300\text{ M}^{-1}\text{ cm}^{-1}$), CB (pH 3.0, 4.0 and 4.5 ± 0.2, ABTS 0.5 and 2 mM, $\epsilon_{420\text{ nm}} = 36000\text{ M}^{-1}\text{ cm}^{-1}$ and $\epsilon_{436\text{ nm}} = 29300\text{ M}^{-1}\text{ cm}^{-1}$). Best treatment conditions were used for the enzyme kinetics of concentrated rPOXA 1B. Optimum condition determined for laccase measurement was CB T2 (pH 3.0 ± 0.2; $\epsilon_{420\text{ nm}}$, 2 mM ABTS) with 5,291.665 ± 83.795 UL⁻¹, these assay conditions increased the affinity of the enzyme for the substrate which was corroborated with the decrease in K_m (0.0372 mM), compared to the same kinetic parameters previously obtained in acetate buffer K_m (1.716 mM). In addition, several of the combinations tested for acetate buffer surpassed the results obtained with the classic Tinoco *et al.*, (2001) technique.

Keywords: ABTS, acetate buffer, citrate buffer, laccase.

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Effect of fishmeal replacement with a vegetable protein mixture on the

generation of residual compounds in shrimp culture *Litopenaeus vannamei* in low salinity



J.C. Gil Núñez⁽¹⁾, R. Casillas Hernández⁽¹⁾, R. Servín Villegas⁽²⁾, F.J. Magallon Barajas⁽²⁾, R.A. Bórquez López⁽¹⁾, J.R. González Galaviz⁽¹⁾, M.B. Flores Pérez⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 5 de febrero, 818 sur, Ciudad Obregón, Sonora, México.

⁽²⁾ Centro de Investigaciones Biológicas del Noroeste, La Paz, Baja California Sur, México.

Corresponding author: jcgil0612@gmail.com



Shrimp nutrition is very important in the aquaculture and it need for quality ingredients and better formulations for food efficient and sustainable¹. Shrimp farming have problems of environmental impact in aquatic ecosystems due to nitrogen released. In low salinity culture the risk of contaminating surrounding bodies of water used for human activities and primary production activities is major². Therefore, the aim of this study was to determine whether the protein sources and levels used in balanced diets for *L. vannamei* in low salinity cause variation in the concentrations of soluble nitrogen inorganic in the water culture. Six diets were prepared according to Calderon (2007). Two protein sources were used at low, medium and high percentage: (APD_(L); APD_(M); APD_(H)) with FM and (AVD_(L), AVD_(M), AVD_(H)) with SM/SPC. 18 tanks (100 L) were used. 10 organisms per tank were used with a weight of 1.41±0.3g. Conditions: 28±0.5°C; O₂, ≥4mg/L; 3UPS.

Was used a Spectroquant 300 Merk spectrophotometer for the measurement of NH₄⁺, NO₂⁻, NO₃⁻. A one-way ANOVA test was performed, followed by a Tukey test for multiple comparisons. Polynomial regression of second order was used for analysis of residuals with STATGRAPHICS (P <0.05). The values show an increase in the concentration of NH₄⁺ as the percentage of protein and size of the shrimp increased regardless of the protein source. Diets APDs released a higher concentration of ammonium in both sizes. It is considered that to minimize the impact to the surrounding lands, the low salinity cultivation needs a stricter control of effluents³. Avoiding destruction of mangroves, contamination of water sources, eutrophication.

Keywords: *Low salinity, Replacement, Shrimp, Soybean*



Heterologous expression of *Aspergillus niger* Phytase A in *Pichia pastoris*

D. Niño-Gómez⁽¹⁾, V. González-Méndez⁽¹⁾, R. Poutou-Piñales⁽¹⁾, C. Rivera-Hoyos*⁽¹⁾

⁽¹⁾ Pontificia Universidad Javeriana, Carrera 7 No 40-62, Bogotá, D.C., Colombia.

*Corresponding author: claudia-rivera@javeriana.edu.co



Phytases (E.C. 3.1.3.8), are phosphocatalytic enzymes that perform partial or total hydrolysis of orthophosphates present in phytic acid. Phosphorus and inositol are stored mainly as phytic acid in cereals and legumes that are used commercially as feed for monogastric animals and in oilseeds, constituting between 60-90% of the total content of phosphorus in plants¹. Phytases hydrolyze phytic acid to one molecule of inositol and six molecules of inorganic phosphate. However, these phytates are not hydrolyzed in the intestine of monogastric animals, and the associated phosphates remain unabsorbed, requiring the addition of phosphates in the diet to avoid phosphorus deficiencies, and decrease their presence in the excreta of these animals. The aim, was to express the recombinant 3-Phytase A, obtained from the optimized coding sequence of *Aspergillus niger* in *Pichia pastoris*. The synthetic gene *PhyA* was cloned under the control of the constitutive promoter of glyceraldehyde-3-phosphate dehydrogenase (*GAP*) of *P. pastoris*. Two recombinant clones were selected to evaluate the growth and enzyme production parameters at a 100 mL scale in a total incubation time of 204 hours. The maximum phytase activity for each clone was obtained after 10 hours with 15.86 mUmL⁻¹ and a specific activity of 0.48 mUm⁻¹ for clone 1, and 26.87 mUmL⁻¹ with a specific activity of 0.81 mUm⁻¹ for clone 2. The results suggest that previously carried out design to clone and express the *PhyA* of *A. niger* in *P. pastoris* under a constitutive promoter^{2,3} is feasible, and it is proposed to scale the production process, as well as the improvement of the culture medium to obtain greater phytase activity, in order to generate an economic alternative to the production of phytases for use in the livestock industry.

Keywords: 3-PhyA, *Aspergillus niger*, GAP promoter, Phytases, *Pichia pastoris*.

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Mezcal vinasses as inducer of laccase enzyme from *Trametes versicolor*

in batch submerged bioprocess



VS Robles-González⁽¹⁾, IV Robles-González^{(2)*} and S Castillejos-Márquez⁽¹⁾

⁽¹⁾ Instituto de Hidrología, Universidad Tecnológica de la Mixteca. Carretera a Acatlima Km 2.5
Huajuapán de León, P.O. Box 69000, Oaxaca, México.

⁽²⁾ Instituto de Investigaciones en Materiales, UNAM. Circuito Exterior, Ciudad Universitaria,
Coyoacán, 04510, Ciudad de México, México.

*Corresponding author: ireriobles@iim.unam.mx



Laccase (EC 1.10.3.2 p-benzenediol/oxygen oxidoreductase), are multi-copper oxidases that have been considered a versatile enzyme since they catalyze redox reactions from which all their applications arise. Laccase is an enzyme that is being increasingly studied as a biocatalyst for industrially and environmental biotechnology relevant processes and considering themselves as green tools for sustainable ecological processes. White-rot basidiomycetes produce laccases in the presence of several inducers as, e.g., copper, manganese, some organic solvents and phenolic compounds. The objective of the present study was to evaluate the mezcal vinasses (MV), as a possible inducer of laccase from *Trametes versicolor* (*Tv*). In the MV, two concentrations of total phenols expressed as equivalents of gallic acid (EGA) 63 and 84 mg EGA / L were tested, and pellets formed with *Tv* and bagasse powder from *Agave* leaves were used, and their effect on the enzymatic activity of laccase (*EALac*), was evaluated. The fermentation was carried out in a batch submerged bioprocess at 24 ° C. The *EALac* obtained with vinasse at 84 mg EAG/L was 2 times higher than the maximum *EALac* obtained using vinasse with a concentration of 63 mg EGA/L and 60 times higher than that obtained with the control. The results showed that MV can be used to produce laccase, besides that the concentration of phenols present in the MV has a positive effect on the enzymatic activity.

Keywords: Fermentation, laccase, mezcal vinasses, *Trametes*.



Removal of copper from simulated wastewater using cross-linked chitosan beads with *Aspergillus australensis* biomass, alginate and TPP

Ana G. Contreras-Cortés^(1*), Francisco J. Almendáriz-Tapia⁽²⁾, Armando Burgos-Hernández⁽¹⁾, Francisco Rodríguez-Félix⁽¹⁾, Manuel A. Quevedo-López⁽³⁾, Agustín Gómez-Álvarez⁽²⁾, Maribel Plascencia-Jatomea⁽¹⁾

⁽¹⁾Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Universidad de Sonora, Blvd. Luis Encinas y Rosales S/N, Col. Centro, Hermosillo, Sonora, México, C.P. 83000

⁽²⁾Departamento de Ingeniería Química y Metalurgia, Universidad de Sonora, Blvd. Luis Encinas y Rosales S/N, Col. Centro, Hermosillo, Sonora, México, C.P. 83000.

⁽³⁾Department of Material Science and Engineering, University of Texas at Dallas, Texas, EUA.

*Corresponding author: rayne2129@hotmail.com

The presence of heavy metals in the environment has generated great concern worldwide due to an increase in the discharge of metals and their toxic nature; also due to adverse effects that are caused in the receiving waters⁽¹⁾. The toxicity of heavy metals is related to bioaccumulation process and increased exposure may result in damage to the central nervous system, blood composition, lungs, liver and other vital organs⁽²⁾. Biosorption or sorption of heavy metals using biological materials is recognized as an emerging technique for the treatment of wastewaters⁽¹⁾. Chitosan is a biopolymer of interest in the adsorption of metal ions, since it contains amine and hydroxyl groups in the chitosan chain that act as good chelating sites for metal ions and it is considered a very versatile polymer that can be combined with other biomaterials. The purpose of this research was to cross-link chitosan with other biopolymers like fungi biomass, alginate and TPP in order to increase chitosan functionality for heavy metal removal. It was possible to obtain composites of chitosan-alginate-fungi biomass and TPP with a size of 0.3 ± 0.06 cm. Batch biosorption experiments were carried out at 35°C and different pH (5.5, 5.0 and 4.5 respectively) using fresh and lyophilized beads. The results showed that for the experiments using fresh beads statistical differences were found for every pH used compared to control (21.8 ppm of Cu^{2+}), being the highest removal at pH 4.5 with a residual concentration of 10.48 ± 0.5 ppm of Cu^{2+} , this represents 52% of copper removal from the solution. Experiments with lyophilized beads also presented statistical differences in every pH, being pH 5 and 5.5 where the most copper was removed with a percentage of removal of 79%. The residual copper concentration for pH 5 and 5.5 was an average of 4.56 ± 0.13 ppm of Cu^{2+} . These results indicate that lyophilized beads performed better compared to fresh beads. Cross-linked chitosan beads showed high capacity for heavy metal removal like Cu^{2+} in different pH conditions, which makes this material a good candidate for heavy metal removal.

Keywords: Chitosan, composites, biosorption, copper.

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Alkaline protease produced by the moderate halophilic strain LT14: Characterization and potential application in commercial detergents

Juan Carlos Coronado Corral ⁽¹⁾, María Isabel Estrada Alvarado ⁽¹⁾, Lilia Arely Prado Barragan ⁽²⁾,
Luis Alberto Cira Chavez ⁽¹⁾

⁽¹⁾ *Departamento de Biotecnología y Ciencias Alimentarias. Instituto Tecnológico de Sonora.
Avenida 5 de Febrero 818 sur. Cd. Obregón, Sonora, 85000 México.*

⁽²⁾ *Ciencias Biológicas y de la Salud (Departamento de Biotecnología y Fermentación en Estado
Sólido) de la Universidad Autónoma Metropolitana*

*Corresponding author: luis.cira@itson.edu.mx



Enzymes are commercially exploited in the detergent, food, pharmaceutical, diagnostic and chemical industries. The alkaline proteases (or subtilisins, E.C. 3.4.21.14) are physiologically and commercially important group of enzymes. Mesophilic organisms; they work mainly in a narrow range of pH, temperature and ionic strength. The microorganisms of extreme environments, mainly halophiles; they are considered an important source of enzymes, and their specific properties are expected to result in new applications in biotechnological processes. The objective of this research was to characterize the enzymatic extract of strain LT14., As well as to determine its potential as a detergent additive. To verify if the enzymatic extract can be exploited commercially in the detergent industry, its stability and compatibility with seven liquid and powder detergents at 50°C was investigated. The protease was stable and compatible for 1 hour at that temperature with Persil powder as in liquid 123% and 134% respectively, Liquid Ace also had a compatibility of 120%. Viva, Tide and Great Value stand out with compatibility values to the extract. The preparation of the enzyme extract Persil was able to completely eliminate the blood stain of the white cotton fabric, having a whiteness index higher than the other treatments. The properties of the enzymatic extract of the strain LT14 and the compatibility with commercial detergents, it removes completely blood stain on cotton cloth. These properties have the potential to be exploited commercially as an ideal candidate for detergent ingredients.

Keywords: biocatalyst, detergent, halophile, protease



Antiviral activity of Brazilian propoli extract

Silva-Beltrán N.P.⁽¹⁾, Encinas-Valenzuela K.L.⁽¹⁾, Balderrama- Carmona A.P.⁽²⁾, Umsza Guez M.A.⁽³⁾, Souza Machado B.A.⁽⁴⁾, Barreto G.A.⁽⁴⁾

⁽¹⁾ *Universidad de Sonora, Departamento de Ciencias de la Salud, Campus Cajeme, Ejido Providencia, C.P. 85010, Cajeme, Sonora, México.*

⁽²⁾ *Universidad de Sonora, Departamento de Ciencias Químico Biológicas y Agropecuarias, Blvd. Lázaro Cárdenas 100, Colonia Francisco Villa C.P. 85880, Navojoa, Sonora*

⁽³⁾ *Universidade Federal Bahia, Departamento de Biointeração do Instituto de Ciências da Saúde, Avenida Reitor Miguel Calmon, s/n, Vale do Canela, CEP 40-110-100, Salvador, Bahia. Brasil*

⁽⁴⁾ *Centro Universitário SENAI CIMATEC – Serviço Nacional de Prendizagem Industrial, 41650-010, Salvador - BA, Brasil.*

*Corresponding author: norma.silva@unison.mx

The propolis is a resinous substance collected by honeybees, their pharmacology properties have been reported previously. Brazil have a great biodiversity of propolis, with different composition and biological activity, and several distinct propolis types have been described in this country¹, however, there are few studies related to its effect in enterovirus. Recently, different extracts were reported to have antiviral activities against viral surrogates and human enteric viruses². The objective of the present study was to evaluate the antiviral effects of extracts of green propolis on the inhibition of AD-08 bacteriophage, which was used as human enteric viral surrogates. Green propolis was collected from Paraná Brazil and was dried, pulverized and macerated with ethanol solution. The extract was confronted with the virus at different contact times and concentrations. To determine the reduction of virus decimal dilutions they were performed. The results were expressed in log₁₀ PFU (Plaque Forming Units). The replication of AD-08 bacteriophage was significantly suppressed in the presence of 100, 500, and 1000 µg/mL of green propolis. We found that propolis began to inhibit AD-08 replication after 30 min of incubation showed viral reductions of 4 to 5 log₁₀ PFU, and showed a dose-dependent manner. The activity of propolis against both AD-08 was confirmed by a significant decrease in the number of viral copies.

Keywords: Bacteriophage, Propolis.

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Identification of candidate genes for Arsenic tolerance using Genome-Wide Association Mapping in *Arabidopsis*

A.C. Delgado Chávez ⁽¹⁾, C.E. López Ortiz ⁽¹⁾, T. Saminathan ⁽²⁾, P. Nimmakayala ⁽²⁾, L.E. Montañez Hernández ⁽¹⁾, U. Reddy ^{*(2)}, N. Balagurusamy ^{*(1)}

⁽¹⁾ Universidad Autónoma de Coahuila, Carretera Torreón-Matamoros Km. 7.5, Torreón, Coahuila

⁽²⁾ West Virginia State University, Hamblin Hall, H, Institute, West Virginia

*Corresponding author: bnagamani@uadec.edu.mx; ureddy@wvstateu.edu



Arsenic (As) is considered one of the most toxic elements worldwide, its source comes from either natural or anthropogenic activities. High concentrations of arsenic have been reported to affect crops and causing losses of about 20 – 30%¹. Further, consumption of these plants or plant products can result in multiple health problems. Techniques such as bioremediation and phytoremediation have been suggested for remediation of contaminated soils and water and to counteract the effects of heavy metals. In this study, Genome-Wide Association Studies (GWAS) and reverse genetic approach was applied to 167 ecotypes from 1001 *Arabidopsis* genome project. The plants were grown in a growth chamber and were exposed to 100 µm of arsenic concentration. A cloud-based platform easyGWAS and different phenotypic root traits were used to associate SNP variation to morphological root traits under arsenic stress conditions. In our GWAS study, around 1 million 700 hundred SNP were found and were present across all 5 chromosomes of *Arabidopsis*. The observed SNP were found to affect diverse genes, among them, mainly genes involved in basic function for plant development, and as well as candidate genes for arsenic tolerance. Genes involved with oxide-reduction processes found to help in reducing or counteracting effects of the high concentrations of reactive oxygen species (ROS), metal transporters detoxifying the metal uptake, DNA repair genes, specific genes for arsenic tolerance such as HAC1, among other genes with unknown function. This study provided a selection of candidate genes that could be related to arsenic tolerance and the results of this study will be presented. It can be concluded that these results would aid in understanding the molecular mechanisms used by plants and to counter the effects due to heavy metal uptake by plants and as well as to develop arsenic tolerant plants.

Keywords: Arsenic tolerance, GWAS, plant engineering.

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CO₂ biomitigation from flue gas by *Scenedesmus obtusiusculus* using a hybrid photobioreactor

Adrián Estrada⁽¹⁾, Sergio Hernández⁽²⁾, Marcia Morales^{(2)*}

⁽¹⁾ Licenciatura en Ingeniería Biológica, UAM-Cuajimalpa, Vasco de Quiroga, 4871, Ciudad de México, México

⁽²⁾ Departamento de Procesos y Tecnología, UAM-Cuajimalpa, Vasco de Quiroga, 4871, Ciudad de México, México

*Corresponding author: mmorales@correo.cua.uam.mx



Microalgae based systems have gained importance over the past decades due to the capacity of these microorganisms to capture carbon dioxide from different sources. Microalgae and cyanobacteria are responsible of 50 % of the global oxygen production¹, through the process of oxygenic photosynthesis. These systems are also important for the generation of a broad range of products such as: biomass, pharmaceuticals, biofuels and fertilizers². The accumulation of greenhouse gases (GHG) from anthropogenic activities is the main cause for climate change which causes a rise in the temperature contributing to the global warming effect. Among the different GHG, carbon dioxide is the main contributor to this phenomenon and is produced mainly through the burning of fossil fuels. The development of environmental friendly technologies for the mitigation of this gas is essential to reduce the harmful effect of climate change on the ecosystems. Therefore, the aim of this work was to develop a CO₂ capture system using the microalgae *Scenedesmus obtusiusculus* for the mitigation of a flue gas stream produced through a combustion process of a portable gas LP powered generator using a 100 L-hybrid photobioreactor (HPR). The HPR was located in a greenhouse and consisted of a raceway-pond coupled with a bubble column. Initially the system was fed with 100 mL/min of synthetic flue gas (15% CO₂, 100 ppm NO, 100 ppm SO₂, nitrogen balance) and afterwards a real flue gas was used. The biomass concentration and biochemical composition of *Scenedesmus obtusiusculus* were determined, additionally a portable flue gas analyzer was used to measure the composition of the exhaust gas and environmental variables were continuously recorded on-line by a data acquisition module. The values of the specific growth rate and volumetric productivity were 0.1382 d⁻¹, 58.2 mgL⁻¹d⁻¹ respectively, and the protein, carbohydrate, lipid and chlorophyll contents were 50, 30, 15, 2%, respectively. The HPR presented a removal efficiency above 99% for CO₂, NO and SO₂ in the exponential phase of the culture. The microalgal-HPR offered an adequate alternative for removal of flue gases and cell growth.

Keywords: CO₂ capture, flue gas, microalgae, photobioreactor.

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Rhamnolipid and pyocyanin metabolites produced by *Pseudomonas aeruginosa* in a stirred tank reactor

Carolina Guatemala Hernández⁽¹⁾, Abdi Escalante Sánchez⁽²⁾, Maricruz Hernández Cruz⁽³⁾,
Josefina Barrera-Cortés⁽⁴⁾

^(1,2,4) Dept. Biotechnology and Bioengineering, CINVESTAV – IPN. Av. IPN 2508. Col. San Pedro
Zacatenco. Ciudad de México, C.P. 07360

⁽³⁾ Instituto Tecnológico Superior de Álamo Temapache (ITSAT). Xoyotitla, Álamo Temapache, Ver.
México.

*Corresponding author:: jbarrera@cinvestav.mx



Biosurfactants and phenazines produced by different species of *Pseudomonas aeruginosa* are respectively, environmentally friendly alternatives for removal of hydrophobic pollutants contained in polluted soils, and antimicrobials in the biological control of pest in the agricultural sector. Despite its potential application in these two sectors, the metabolites application at field level presents the drawbacks of high production costs, low yields and the requirements of a solid knowledge about the biological processes involved. The aim of this study was the implementation of a batch culture of *Pseudomonas aeruginosa* ATCC 9027 focused to the simultaneous production of the rhamnolipids and pyocyanin metabolites. *P. aeruginosa* was propagated in a stirred tank reactor (STR) of 7L at the following conditions: 37 ± 0.2 °C, pH= 7 (NaOH 4N), 350 rpm, air flow rate of 1-4 vvm and 4L of PPGAS medium supplemented with glycerol at 3%. *P. aeruginosa* was cultivated for 90 h, during which culture broth samples (15 mL) were drained periodically for the analysis of biomass and metabolites production. Biomass was analyzed by dried weight (45°C) from the biomass separated by centrifugation (10,000 rpm at 4 °C for 30 min). The rhamnolipid production was quantified by the anthrone assay using a UV/VIS spectrophotometer (625nm). The pyocyanin was also analyzed by spectrometry of UV/VIS (693 nm) to pH 7.

Production, yield and productivity of the rhamnolipid metabolite was of 12.1g/L, 0.32g/g and 0.168 g/L/h, respectively. Regarding the pyocyanin, the corresponding values were of: 0.151 g/L, 3.9 mg/g and 2.09 mg/L/h. These data were congruent with those reported in the literature. The maximal production of these metabolites was observed in the stationary phase of the studied bacterium. The preliminary results suggest that *P. aeruginosa* ATCC 9027 is suitable for the production in batch culture of both metabolites. It is assumed that further studies would allow the scale up of the rhamnolipid and pyocyanin production process.

Keywords: batch culture, metabolites, production, *Pseudomonas aeruginosa*



Biotechnological potential of native strains of *Trichoderma* based on

enzyme activities and biocontrol against *Fusarium*



D.J. Hernández-Melchor^{*(1)}, R. Ferrera-Cerrato⁽¹⁾, A. Alarcon⁽¹⁾

⁽¹⁾ Colegio de Postgraduados, Carretera México-Texcoco Km. 36.5, Estado de México, México

*Corresponding author: dulcejazz@hotmail.com

Trichoderma is a cosmopolitan fungus whose importance lies in its ability to adapt and produce metabolites with biotechnological and environmental purposes¹. This fungus has industrial relevance for its ability to synthesize and release enzymes such as cellulases, chitinases, proteases, among others. In addition, this genus is a worldwide agent for biocontrol against phytopathogenic fungi such as *Fusarium oxysporum* due to its multiple mechanisms of action². Highlighting the importance of this fungus and the interest in the search for autochthonous organisms with biotechnological potential, this work evaluated the enzymatic capacity of native strains of *Trichoderma* and its antagonistic potential against *Fusarium oxysporum*. Fourteen fungal strains isolated from the maize rhizosphere of the State of Mexico were propagated. Growth kinetics of each fungal strain was performed; thus, qualitative tests were performed for determining enzymatic activities such as cellulases, chitinases and pectinases (pectatoliase and polygalacturonase). The best results in enzymatic activity and growth were obtained for strains EMV6SIC2, EMV6SIC4, EMV6SIC5, EMV6SIC6, EMV6SIC7, EMV6SIC8, EMMVrSIC4, and EMMVSIC2 with specific growth rates equal to 0.144, 0.137, 0.143, 0.149, 0.142, 0.147, 0.148 and 0.147 h⁻¹, respectively. During the qualitative enzymatic tests, these fungal strains showed higher hydrolytic halos than *Trichoderma reesei* strain (CDBB-H-356) obtained from the National Collection of Microbial Strains and Cell Cultures of CINVESTAV, which was used as a positive control. Afterwards, biocontrol tests were carried out, in which the selected fungal strains inhibited the growth (25%) of *Fusarium oxysporum* 17191.

Keywords: biocontrol, enzymes, *Fusarium oxysporum*, *Trichoderma*

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Development and evaluation of hybrids membranes to inactivate *E.coli* in

a photocatalytic process



López Vega, D. V*⁽¹⁾, Pérez Rodríguez F.⁽¹⁾, Arriaga García, S.L.⁽¹⁾, Acosta González, G.E.⁽²⁾
⁽¹⁾ Instituto Potosino de Investigación Científica y Tecnológica, Camino a la Presa San José 2055
Lomas 4ª Sección, C.P. 78216, San Luis Potosí, S.L.P, México
⁽²⁾ NANOMATERIALES, Ave. Milimex 215, Parque Industrial Milmex, C. P. 66637, Apodaca Nuevo
León.

*Corresponding author: valeria.lopez@ipicyt.edu.mx



In recent years the use of nanoparticles has increased in different areas such as physics, chemistry, biology, electronics or materials thanks to the improvement of its properties due to its great surface-volume ratio¹. However, due to its small sizes (1-100 nm) the recovery of these particles for reuse has been affected, therefore they are considered secondary pollutants when they are released into the environment. Among the alternatives to this problem have been used hybrid nanocomposites (glass, silica or incorporation in polymers)². The TiO₂ y ZnO nanoparticles have been used as catalysts in photocatalysis processes since they have a specific interaction with some contaminants and microorganisms present in water³. In this research, hybrid membranes based on polymethyl methacrylate (PMMA) and TiO₂ y ZnO nanoparticles were elaborated with the aim of minimizing fouling generated by microorganisms in microfiltration membranes that can cause decrease its efficiency in the process. The TiO₂ y ZnO nanoparticles were functionalized at 10% and 50% with stearic acid to anchor them to the polymer matrix, decrease the particle-particle interaction and increase the particle-matrix interaction. It generates a better dispersion of the nanoparticles, so that the photocatalytic process can be carried out uniformly throughout the membrane and inactivate the microorganisms present in water. The characterization of nanoparticles was carried out by analytical techniques such as FT-IR, TEM, TGA, XRD, DDL and antibacterial tests of CMI and CMB, getting a value of 1.25 mg/ml for individual nanoparticles and 0.312 mg/ml when combined, obtaining a synergy of their proprieties. While for the characterization of membranes was carried out by porosity, contact angle tests, and SEM. Based on the functionalization of the nanoparticles and under photocatalytic conditions, 100% of inactivation of *E.coli* were obtained with the combination functionalized of 10% Zn and 50% TiO₂ and with the individual nanoparticles to 10% functionalized. Thereby the superficial modification increases the inactivation of microorganism in a photocatalytic process, added to this the nanoparticles concentration utilized is less that other materials like carbon nanotubes.

Keywords: Inactivation, nanoparticles, photocatalysis

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Quantification of 17- β Estradiol in Biological Matrices

Morales JM⁽¹⁾, Díaz CA⁽¹⁾, Ulloa-Mercado RG⁽¹⁾, Burboa-Charis VA⁽¹⁾, Hernandez-Chavez JF*⁽¹⁾

⁽¹⁾ Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, Ciudad Obregón, Sonora, México.

*Corresponding author: juan.hernandez@itson.edu.mx



Intensive use of estrogens poses a risk for the environment and human health at concentration levels as low as ng-L⁻¹ (1). The monitoring of these chemicals demands the method development for its quantification in biological and environmental matrices. The aim of the present study was to validate an analytical method for the extraction and quantification of 17- β estradiol in animal serum using capillary electrophoresis. The scope covers concentrations from 1 to 10 mg/L and has a resolution of 1 mg/L; using the solid phase extraction significantly improves initial concentration folds. Serum samples were given by a regional farm and stored at 2°C prior to analysis. The spiked serum was homogenate and centrifugated at 7.2g in 50% v/v acetonitrile (ACN), and the supernatant analyzed by Micellar Electro kinetic Chromatography. The running buffer was composed of 120 mM SDS, 30 mM monosodium phosphate, 10 mM β -cyclodextrin, 20% v/v methanol adjusted to pH 2 with phosphoric acid 85% w/v, in a 50 μ m capillary, -22kV and 40°C separation conditions. Spiked samples (10 mL, 1 μ g L⁻¹) at 50% v/v ACN were concentrated in 60 mg Oasis-HLB cartridges and reconstituted in 20 μ L 50% ACN (500 folds). The method was validated in terms of linearity (R²), repeatability (%RSD), matrix effects (%ME) and recuperation (%R) (2). When the spiked samples were analyzed without pretreatment, the matrix effects were significant. Sample dilution with ACN reduces significantly the matrix effects, possibly due to sample electric field enhancement and protein precipitation (3). The analytical standard's linearity and repeatability were 0.998 and 3.97 respectively; whereas %ME and %R of the spiked serum were 2.98 and 72%. The method was validated successfully for the quantification and extraction of the hormone 17- β estradiol in biological matrices.

Keywords: Capillary Electrophoresis, Hormones, Quantification

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Wastewater as a substrate for the growth of thraustochytrid native strain,

VAL-B1.



C. R. Navarro Oyarzo⁽²⁾, D. Silva Rodríguez⁽²⁾, S.I. Sáez Elgueta⁽¹⁾, **B.H. Quilodrán Toloza⁽²⁾**
⁽¹⁾Environmental Engineering, Natural Resources and Environment Department, Universidad de Los Lagos, camino a Chiquihue km.6, Puerto Montt, CHILE.

⁽²⁾Natural Resources and Environment Department, Universidad de Los Lagos, camino a Chiquihue km.6, Puerto Montt, CHILE.

*Corresponding autor: bquilod@ulagos.cl

Domestic and industrial waste has been a focus of attention, because part of its components can be raw material to generate various products to be used as a source of carbon, nitrogen and energy by microorganisms, because one of the highest costs in fermentation is due to the value of the nutrients. Thraustochytrids are heterotrophic marine protists that are widely distributed throughout the marine world, and also produce and accumulate a high amount of lipids in their cells, especially long-chain polyunsaturated fatty acids (LC-PUFAs), such as docosahexaenoic acid (DHA, C22: 6, ω 3) and pigments of interest for human health and animal nutrition. An alternative to reduce the organic matter of wastewater is fermentative degradation, in this case it is proposed to use carbon sources of residual origin to obtain thraustochytrid biomass with lipid content and to reduce the COD in the medium. The native strain VAL-B1 isolated from coastal waters of Chile, identified morphologically and genetically as thraustochytrid, was cultivated using different culture media depending on the type of substrate (pure or in mixtures); glucose as a source of pure carbon (Glu, 10 gL⁻¹), edible fruit seed (RM, 10 gL⁻¹), grey water (RAG, 100%), and waste from the aquaculture industry (RHP, 100%), with or without yeast extract (EL, 2 gL⁻¹) and monosodium glutamate (GMS, 2 gL⁻¹), all media were adjusted to salinity 29 ‰, for the fermentative production of biomass. Strain VAL-B1 was cultured in triplicate with 100 mL of culture for 7 days at 180 rpm and 30 °C, then centrifuged at 5000 rpm, the biomass of each fermentation was lyophilized and quantified by gravimetry and the supernatant from the culture with the higher biomass production, the COD was determined by spectrophotometric method. VAL-B1 cultivated in Glu is obtained 2.09 ± 0.08 gL⁻¹ of biomass, when the RM is used in the medium, the biomass increases to 3.74 ± 0.12 gL⁻¹. When in the culture medium Glu is replaced by RAG maintaining RM, a higher biomass concentration is obtained, obtaining 4.04 ± 0.07 gL⁻¹. When 50% of RAG is replaced in this medium by RHP, the biomass production increases, obtaining 6.07 ± 0.06 gL⁻¹. In this medium the COD decreases by 27.41% in relation to the initial COD of the waste. The results suggest that the residues used are good substrates for the production of biomass using native thraustochytrid strain, VAL-B1. The changes in the time of the concentration of biomass, total lipids and LC-PUFA should be studied.

Keywords: biomass, grey water, industrial waste, thraustochytrid.



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Evaluation of different COD/SO₄²⁻ ratios on expression of *mcrA* and *dsrA* of a methanogenic and sulfidogenic consortia in lab scale reactors

G. Rafael Galindo⁽¹⁾, L.E. Montañez Hernández⁽¹⁾, I.O. Hernández De Lira⁽¹⁾, R. Oropeza Navarro⁽²⁾, T. Espinosa Solares⁽³⁾ and N. Balagurusamy^{*(1)}

⁽¹⁾ Laboratorio de Biorremediación, Facultad de Ciencias Biológicas, Universidad Autónoma de Coahuila, carretera Torreón-Matamoros km 7.5, Torreón, Coahuila, México.

⁽²⁾ Instituto de Biotecnología de la Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México.

⁽³⁾ Universidad Autónoma Chapingo, Chapingo, Estado de México, 56230, México.

*Corresponding author: bnagamani@uadec.edu.mx.

The competition between methanogens and sulfate-reducing bacteria (SRB) affect methane (CH₄) yield since hydrogen sulfide (H₂S) generated by SRB inhibit methanogens. Most of the cases, the quality of the biogas is affected, and it could lead to the failure of biogas plants¹. A real-time monitoring is essential to understand the functioning of the biodigesters and to keep control on the CH₄ yield and quality of biogas produced. In this regard, the present study was aimed to evaluate the different COD/SO₄²⁻ ratio (1:2, 1:1, 2:1, 3:1, 4:1, 5:1, and 6:1) on activity of methanogenic consortia on CH₄ and H₂S production. Physicochemical analyses were carried out to determine the COD removal, volatile fatty acid concentration, biogas produced, sulfate removal and H₂S production. It was found out that methanogenesis was not inhibited when the COD/SO₄²⁻ ratio was >3.0. However, when the ratio was <3.0, low COD removal, decrease in pH were observed along with an increase in dissolved sulfide concentrations. In the next phase, laboratory developed methanogenic and sulfidogenic consortia were evaluated in two different synthetic culture media in laboratory scale bioreactors (600 ml), out of which one favors methanogenic activity, while other favors sulfidogenic activity. While methanogenic consortia showed higher COD removal and increased the biogas production in medium favoring methanogenic activity, its activity was significantly affected in medium favoring sulfidogenic activity. Similarly, sulfidogenic activity was favored only in sulfidogenic medium. From these reactors, total genomic DNA of methanogenic and sulfidogenic consortia were extracted and amplified using universal 16s RNA primers for microbial diversity analyses. At the same time, total RNA was isolated from these reactors and genetic expression of key genes (*mcrA* and *dsrA*) is being studied². The results on the expression of these two genes at conditions favoring methanogenic and sulfidogenic activity will be presented. From the results, it can be concluded that medium composition along with COD/SO₄²⁻ ratio determined the gene expression of CH₄ formation and/or sulfate reduction.

Keywords: Anaerobic digestion, Competition, Genetic expression, Sulfate-reducing bacteria

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Scale-up and production of a photosynthetic, nitrogen-fixing microbial consortium

Vargas-Valtierra V⁽¹⁾, Reyna-Velarde R*⁽¹⁾, León-Tejera HP⁽²⁾, Ramírez-López C⁽¹⁾

⁽¹⁾ Universidad Mexiquense del Bicentenario, Unidad de Estudios Superiores Tultitlán, Av. Ex-hacienda de Portales S/N, Col. Villa Esmeralda, Tultitlán Estado de México, México

⁽²⁾ Laboratorio de Ficología Marina, Facultad de Ciencias UNAM, Av. Universidad 3000, Circuito Exterior S/N, Coyoacán 04510, Ciudad Universitaria, CDMX México.

*Corresponding author: r.reyna@umb.mx, fenixrv@yahoo.com.mx



After the water availability, nitrogen availability is the main factor that limits agricultural productivity, and the excess of chemical fertilization causes negative impacts to the environment. Microalgae Biotechnology offers us the possibility of generate biofertilizers, which provide a significant amount of nitrogen and do not harm the environment, taking advantage of the biological nitrogen fixing capacity of cyanobacteria. The objective of this work was to scale and produce a nitrogen-fixing photosynthetic microbial consortium, generated from native biomass from the Valley of Mexico. The consortium was obtained from a previous investigation of the working group. An airlift double-riser photobioreactor⁽¹⁾ 10 L working volume was inoculated and operated in semi-continuous regime with HTR = 14 d, artificial light, 12:12 h photoperiod, culture medium BG-11₀. The microbial culture was scaled to a volume of 30 L, under the (Ai / V) criteria. The microbial consortium maintained its morphological and performance characteristics at any time, detecting the presence of morphotypes like *Anabaena*, *Nostoc*, *Oscillatoria* and *Scenedesmus*, with a maximum biomass concentration of 1.51 g L⁻¹. This consortium will be utilized for biologic fertilization of Stevia plants.

Keywords: Microalgae, Nitrogen-fixing, Photobioreactor, Scale-up.

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Production of polyunsaturated fatty acids from crude glycerol, using strains of *thraustochytrium Kinney*

D. Silva Rodríguez*⁽¹⁾ and B. Quilodrán Toloza⁽¹⁾

⁽¹⁾ Universidad de Los Lagos, Departamento Recursos Naturales y Medio Ambiente, Camino a Chiquihue, km. 6 s/n, Puerto Montt, Chile

*Corresponding author: david.silva2@ulagos.cl

Thraustochytrids (TH) are unicellular marine and fungus-like heterotrophic protists within the clade Stramenopiles, several strains of which are currently recognized for their high potential to produce polyunsaturated fatty acids (PUFAs)¹, and some of them have also been adopted for commercial production of an omega-3 PUFA - docosahexaenoic acid (C22:6, DHA), eicosapentaenoic (C20:5, EPA) and docosapentaenoic (C22:5, DPA)-. In the last 10 years, crude glycerol (CG) is being used as an alternate source of carbon, because it is an abundant, lower cost compound that is generated as a by-product in a great variety of productive processes. The CG is obtained mainly from the production of biodiesel and can be transformed by TH to obtain primary metabolites (mainly PUFAs), thus giving an added value to the by-product obtained in the refining of biodiesel². In this study, two strains of TH (VAL-B1 and EMA-T5) of the same species (*Thraustochytrium Kinney*) but isolated from different coastal areas (central Chile and Chilean Antarctic) were used, and were grown in CG by comparing and analyzed if there is significant influence on the production of EPA, DHA and DPA. CG was obtained from the production of biodiesel from soybean oil and was used as substrate for cultures (pH = 6.4) at 20 gL⁻¹, which in a fermenter of 1L batch cultivation was carried at 25 °C, 180 rpm for 7 days. The results indicate that the PUFAs that obtain the highest percentage of production are DHA and EPA, with 44.84% and 8.75% respectively (as percentage of total fatty acids) for VAL-B1, overcoming the production values of EMA-T5 in more than 50%. With respect to the DPA for both strains, the values are below 1% (as percentage of total fatty acids). The maximum production of PUFAs based on the biomass generated and the volume of culture was also higher in VAL-B1, mainly in the DHA. The values were 82.68±4.76 mgg⁻¹ and 498.81±38.92 mgL⁻¹ and were obtained on the fifth day of culture. In conclusion, VAL-B1 produces higher amounts of PUFAs than EMA-T5, although they are strains belonging to the same species. It is clear that the place where they come from plays an important role, but still stands out in both cases, the biotransformation that make CG as a substrate, given that an industrial product is generating a product with high commercial value.

Keywords: crude glycerol, DHA, PUFAs, unicellular marine.

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Production of lovastatin using agriculture wastes as substrates



Amaury Ábrego-García ⁽²⁾, E. Rios-Leal ⁽¹⁾, Hector M. Poggi-Varaldo* ⁽¹⁾, T. Ponce-Noyola ⁽²⁾, G. Calva-Calva ⁽²⁾, D. Estrada-Bárceñas ⁽³⁾, V. Robles-Gonzales ⁽⁴⁾ and J. Tapia-Ramirez ⁽⁵⁾

⁽¹⁾ *Environmental Biotechnology and Renewable Energies Group, CINVESTAV-IPN, P.O. Box 14-740, Mexico City, 07000, Mexico*

⁽²⁾ *Depto. de Biotecnol. y Biong., CINVESTAV-IPN, Mexico City, Mexico*

⁽³⁾ *Colección Microbiana, CINVESTAV-IPN, Mexico City, México*

⁽⁴⁾ *Instituto de Agroindustrias, UTM. Huajuapán de León, Oaxaca, Mexico*

⁽⁵⁾ *Depto. de Genética y Biología Molecular, CINVESTAV-IPN, Mexico City, Mexico*

*Corresponding author: r4cepe@yahoo.com



Lovastatin (Lv) is a natural statin produced by filamentous fungi. It is an enzymatic inhibitor of methanogenic archaea and it could be used in strategies for reducing CH₄ emissions by ruminants that contribute to global greenhouse effect. Solid state fermentation (SSF) for Lv production is more advantageous than other processes: SSF uses agriculture wastes as substrates that are very economical and adequate as ruminant feedstocks. The objective of this study was to evaluate the effect of two strains of *Aspergillus terreus* and two types of agricultural wastes (wheat bran and oat straw) on Lv production by SSF. This experiment was carried out in a completely randomized experimental design arranged in a 2x2x4 factorial. The factors were the type of *Aspergillus terreus* (2 strains, ATCC10029 and A1156) and the type of substrates (oat straw and wheat bran), and sampling time (0,6,12 and 16 d). Lv was extracted from the fermented solids, concentrated in a rotavapor, and determined by HPLC. The strain ATCC10029 displayed the maximal production of Lv (23.83 mg Lv/gds,fed) with oat straw and 16 d of incubation. This result was significantly superior to the maximum yield of 19.95 mg Lv/gds,fed using soybean meal as substrate, reported in the open literature. With wheat bran as substrate, this strain exhibited a relative maximum 5.35 mg Lv/gds,fed at 6 days. On the other hand, the strain A1156 showed its highest Lv productions with both substrates at 16 d with 6.80 and 3.45 mg Lv/gds,fed for wheat bran and oat straw, respectively. Interestingly, dynamic curves of Lv vs time exhibited a sharp growing patten at the end of the incubation, particularly for the treatment ATCC10029/oat straw. This suggests that it would be likely to obtain Lv concentrations higher than 24 mg Lv/gds,fed.

Keywords: agricultural wastes; *Aspergillus terreus*; lovastatin; solid state fermentation.



Kinetics of carbendazim degradation by three axenic batch cultures

Alvarado Gutiérrez, M. L.^{*(1)}, Ruiz Ordaz, N.⁽¹⁾, Galíndez Mayer, C. J. J.⁽¹⁾

⁽¹⁾ Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu S/N, Ciudad de México, México.

*Corresponding author: missy_1802@hotmail.com



The fungicide carbendazim is an ecotoxic agent affecting zooplankton, amphibians, and macroinvertebrates living in fresh water 1, 2. Thus, it is essential to avoid the arrival of fungicides to water reservoirs, via agricultural or industrial wastewaters. The microbial degradation of xenobiotics is an alternative to diminish the water contamination by agrochemical compounds. In this work, bacteria of the genera: *Klebsiella*, *Flavobacterium*, and *Stenotrophomonas* were isolated and identified from the biofilm of the packed zones in a piston-flow reactor. The isolated bacteria were able to grow individually in selective medium with carbendazim. The degradation rates of the fungicide by *Klebsiella sp.*, *Stenotrophomonas sp.* and *Flavobacterium sp.* were 2.46 mg L⁻¹ h⁻¹, 2.46 mg L⁻¹ h⁻¹, and 2.40 mg L⁻¹ h⁻¹ respectively. The presence of the *mhl1* gene was found in *Klebsiella sp.*, *Stenotrophomonas sp.* and *Flavobacterium* which encodes the enzyme that catalyzes the first degradation reaction of carbendazim. *Klebsiella sp.* presented plasmids which were cured, the strain was able to continue degrading the fungicide at a similar rate, so it is deduced that this information is present in the chromosome.

Keywords: carbendazim, axenic cultures, degradation kinetics.

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Methanotrophs and microalgae co-culture for greenhouse gases Mitigation

P. Ruiz-Ruiz⁽¹⁾, T. L. Gómez-Borraz⁽¹⁾, M. Morales^{*(1)}, S. Revah^{*(1)}

⁽¹⁾ *Departamento de Procesos y Tecnología; Universidad Autónoma Metropolitana- Cuajimalpa, Vasco de Quiroga, 4871, Mexico City, Mexico*

**Corresponding author: mmorales@correo.cua.uam.mx ; srevah@correo.cua.uam.mx*



Co-culturing methanotrophic bacteria and microalgae is a promising approach for the global and simultaneous conversion of the greenhouse gases CH₄ and CO₂ into valuable biomass, through a robust metabolic coupling between methane oxidation and oxygenic photosynthesis. The aim of this work was to determine the maximum CH₄ specific biodegradation rates for different initial biomass ratios of a co-culture of a consortium of alkalophilic methanotrophic bacteria (AMB) and the green microalgae *Scenedesmus obtusiusculus* (GM) for further implementation in a stirred-tank reactor (STR). Experiments were carried out in batch closed reactors with NMS+BG11 medium (pH 9.15) using three different ratios of AMB:GM (w/w): 1:3, 1:1, 3:1 and CH₄ concentrations of 4% and 8% (v/v) in the headspace. Reactors were incubated in a shaker at 30±1°C and light exposed at 115 μmol m⁻² s⁻¹. A STR (2 L of working volume) was used to evaluate the co-culture under the selected biomass ratio and different N sources. CH₄, CO₂ and O₂ concentrations were measured by GC-TCD. The highest maximum specific CH₄ biodegradation rate, 384 mg CH₄ g biomass⁻¹d⁻¹, was obtained for the biomass ratio of 3:1 AMB-GM and 8% of CH₄; and 173 mg CH₄ g biomass⁻¹d⁻¹ was the lowest value achieved for a biomass ratio of 1:3 AMB-GM and 4% of CH₄. It was observed that CH₄ biodegradation ceased in closed systems by the pH increase due to the microalgal activity in the co-cultures. It suggests that microalgae was under CO₂ limitation and the need of pH control during continuous operation using AMB-GM co-cultures. Increased in O₂ concentration was observed for the three initial biomass ratios tested, with O₂ final values between 24 and 28%. Optimization of cell growth conditions for the methanotrophs-microalgae co-cultures leads to a promising strategy to mitigate greenhouse gases in one step with biomass and high added value compounds production.

Keywords: Greenhouse gases mitigation, co-culture, methanotrophs, microalgae.

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Effect of silver concentration and extracellular filtrates of *Alternaria*

alternata on AgNP production



Alamilla-Martinez Diana G⁽¹⁾, Rojas-Avelizapa Norma G.,^{(1)*}, Gómez-Ramírez Marlenne⁽¹⁾
⁽¹⁾ Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada, Instituto Politécnico
Nacional, Cerro Blanco 141, Colonia Colinas del Cimatario, 73090, Querétaro, México
*Corresponding author: nrojasa@ipn.mx.



The increase in market demand of silver nanoparticles (AgNPs) represents a business opportunity due to their potential applications. Biological synthesis of AgNPs include the use of plants, bacteria, yeast and fungi. The AgNPs biosynthesized present interesting characteristics, such as stability and solubility in aqueous solutions; moreover, AgNPs of fungi can be produced extracellularly. The aim of this study was to evaluate the effect of silver nitrate concentration and extracellular filtrates of *Alternaria alternata* MVSS-AH-5 on AgNP production. Extracellular filtrates were obtained from the biomass of *Alternaria alternata* produced in Sucrose and Czapeck media. Different concentrations of AgNO₃ (1, 1.5 and 2 mM) were evaluated. The AgNPs were evidenced by UV-visible absorption spectra from 200 to 800 nm and morphology by Transmission Electron Microscopy (TEM); images were analyzed by software SPIP 6.0.2. Results showed that higher 420 nm absorbance was obtained with the extracellular filtrate obtained from the biomass produced in Czapeck than Sucrose media. Absorbance of 2.2, 2.8 and 2.4 units corresponded to 1, 1.5 and 2 mM (AgNO₃), TEM analysis showed AgNPs with spherical morphology and sizes within 2 - 12 nm for all concentrations while size distribution corresponded to 68%, 84% and 76% respectively, that is, higher percentage of AgNPs within 2- 12 nm were found at 1.5 mM. Absorbance of extracellular filtrate from sucrose media was 0.44, 0.53 and 0.32 units at 1, 1.5 and 2 mM respectively, TEM analysis showed sizes within 2 - 12 nm with a size distribution of 73% for all concentrations of AgNO₃. In summary, silver nitrate concentrations and extracellular filtrates of *A. alternata* have effect on AgNP production; filtrate obtained with Czapeck media and silver nitrate concentration (1.5 mM) were the best conditions to produce AgNP, however none of both conditions had effect on morphology and size of AgNPs.

Keywords: fungi, culture media, nanoparticles, metals.



Hyper-rhizogenesis in vitro in *Typha domingensis*

Hernández-Piedra, G.*⁽¹⁾, Ruiz-Carrera, V.⁽¹⁾, J. Sánchez A.⁽¹⁾, Calva-Calva, G.⁽²⁾

⁽¹⁾ Universidad Juárez Autónoma de Tabasco, KM 0.5 Carr. Villahermosa-Cárdenas, Vhsa. México.

⁽²⁾ Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Col. San Pedro Zacatenco, D.F., México.

*Corresponding author: guadalupe.hernandezp@ujat.mx



The emergent aquatic monocotyledon *Typha domingensis* is used in the construction of artificial wetlands for the remediation of wastewater with limited efficiency. Nonetheless, their capacity phyto remediation may be potentiated through biotechnological strategies (1). Two trials were used to evaluate the factors that induce hyper-rhizogenesis. For the root transformation the strains of *Agrobacterium rhizogenes* (LBA9402, K599 and AR4), infection techniques (puncture, immersion and inoculation) and type of explant (somatic embryo, complex and simple) were evaluated. The trial non-transformed roots were performed with different concentrations of indolacetic acid (AIA) with kinetin (CIN) or benzylaminopurine (BAP) using control without phyto regulator and light and dark in culture environment. The experimental unit was rhizotron type. The length and number of roots were estimated by image analysis every 72 h or 7 d, according to the experimental set. Strain K599 and AR4 at 7 d induced hairs in somatic embryos. In simple explant foliar and radicular regeneration occurred, which was significant infected by the AR4 strain. In vitro roots growth with addition of AIA-CIN and AIA-BAP showed inhibition in the number and length of roots compared with the control. Treatments with low concentrations of AIA had the highest number of roots ($p < 0.05$). The best rhizogenic system will be used to generate the hyperproduction line protocol of *T. domingensis* for future phyto remediation studies.

Keywords: *Typha domingensis*, Agroinfection, Phyto remediation, Hyper-rhizogenesis

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Bioleaching of metals from mine tailings by *A. thiooxidans* DSM 26636

Rivas-Castillo A. M.⁽¹⁾, Gomez-Ramirez M.⁽²⁾, Rojas Avelizapa N. G.^{(2)*}

⁽¹⁾ Universidad Tecnológica de Zona Metropolitana del Valle de México, Blvd. Miguel Hidalgo y Costilla 5, Los Héroes de Tizayuca, Tizayuca, Hgo., 43816, México.

⁽²⁾ Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada del IPN Unidad Querétaro, Cerro Blanco 141, Col. Colinas del Cimatarío, Querétaro, Qro., 76090, México.

*Corresponding author: nrojasa@ipn.mx.



Among the diverse types of pollutants produced by anthropogenic activities, metals represent a serious threaten, due to their accumulation in ecosystems and their elevated toxicity¹. Mining activities generate considerable amounts of high metal content solid residues in the form of mine tailings², and bioleaching has been considered as one bioremediation solution for the treatment of these residues. Previous studies have reported the promising capability of the sulfur oxidizing bacteria *Acidithiobacillus thiooxidans* for the bioleaching of metals like As, Zn, Pb, and Cu contained in mine tailings³. Specifically, *A. thiooxidans* strain DSM 26636 has been able to leach Al, Ni, V, Fe, Mg, Si, and Ni contained in slags from coal combustion wastes⁴. The ability of *A. thiooxidans* DSM 26636 for the bioleaching of metals contained in two different mine tailing samples (MT1 and MT2) was assessed, and it was observed that Al, Fe, and Mn were removed from both mine tailings, in 36.3 ± 1.7 , 191.2 ± 1.6 , and 4.5 ± 0.2 mg/kg for MT1, and in 74.5 ± 0.3 , 208.3 ± 0.5 , and 20.9 ± 0.1 for MT2. Besides, < 1.5 mg/kg of Au and Ru were also biolixiviated from MT1, while it was also observed a 55.7 ± 1.3 mg/kg Zn bioleaching in MT2, besides < 1.5 mg/kg removal for As, Ir, Li, and 0.6 Os in this residue. These results show the potential of strain DSM 26636 for the bioleaching of different mine tailings.

Keywords: *A. thiooxidans*, bioleaching, mine tailings.

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Evaluation of physicochemical changes on activated carbon fibers

surface used as redox mediator in 4-nitrophenol biotransformation process

J. P. García-Rodríguez⁽¹⁾, H. J. Amezcuita-García^{*(1,2)}, C. Escamilla-Alvarado^(1,2), J. R. Rangel-Mendez⁽³⁾, P. Rivas-García^(1,2), J. Cano-Gómez⁽¹⁾, M.M. Alcalá-Rodríguez⁽¹⁾

⁽¹⁾ Universidad Autónoma de Nuevo León, Facultad de Ciencias Químicas, División de Estudios de Posgrado, Av. Vicente Guerrero s/n, Monterrey, México.

⁽²⁾ Universidad Autónoma de Nuevo León, Facultad de Ciencias Químicas, Centro de Investigación en Biotecnología y Nanotecnología, Parque de Investigación e Innovación Tecnológica, Autopista al Aeropuerto Internacional Mariano Escobedo Km. 10, Apodaca, México.

⁽³⁾ Instituto Potosino de Investigación Científica y Tecnológica, División de Ciencias Ambientales, Camino a la Presa San José 2055, San Luis Potosí, México.

*Corresponding author: hector.amezquitagr@uanl.edu.mx



Anaerobic biotechnology is a low-cost and environmental friendly alternative for treatment of toxic and recalcitrant xenobiotic compounds. Anaerobic sludge biotransformation of aqueous contaminants have been improved through the use of activated carbon as microbial support and redox mediator¹. However, there are no reports about changes on physicochemical characteristics of carbonaceous materials after their use as biofilm carriers and redox mediators. The objective of this work was to evaluate chemical changes on activated carbon fibers (ACFs) after the anaerobic biotransformation of 4-nitrophenol (4NP) to 4-aminophenol (4AP). For this purpose, commercial polyacrylonitrile ACFs (AW) were oxidized with HNO₃ (OX) and then functionalized with anthraquinone-2,6-disulfonate (AQ). The three materials were packed in up-flow anaerobic sludge blanket (UASB) reactors for continuous biotransformation of 4NP and applying a substrate-source restriction methodology². After 37 days of 4NP biotransformation, the ACFs were removed from reactors, washed and characterized. The ACFs were characterized (prior and after biotransformation process) by different titrations to quantify the acid and base surface functional groups (Boehm technique) and determining the point of zero charge (PZC). Finally, active biomass was estimated in sludge and ACFs biofilms by volatile solids (VS) technique. Results indicate that PZC of the ACFs were acid (AW: 5.00, OX: 3.38, AQ: 3.09) and neutral (AWEOXEAQ: 7.6 on average) prior and after 4NP biotransformation, respectively. Concentration of both acidic and basic functional groups also increased in all ACFs probably due to biofilm components (e.g. ions, exopolysaccharides) on ACFs surface. Such physicochemical similarities on ACF surface might have explained the observed results where all packed reactors biotransformed 4NP with a similar efficiency of 60%. The un-packed control reactor reduced only 30% of 4NP, which means ACF-packed reactors improved 2 times the biotransformation of 4NP. VS content in un-packed and ACF-packed reactors were (on average) 7.4 and 15.7%, respectively. Since the surface chemistry of ACFs were the similar after biofilm formation, the enhanced 4NP biotransformation could only be explained by the additional biofilm attached onto ACFs. Therefore, it was concluded that surface chemistry of individual ACFs had no significant effect over 4NP biotransformation process and the higher amount of active microorganisms promoted the increased 4NP biotransformation efficiency. This study offers an alternative explanation (i.e. biofilm quantity) to the surface chemistry of activated carbons as the main responsible of the observed increased biotransformation of recalcitrant pollutants.

Keywords: Activated carbon fibers, surface chemistry, biofilm, nitrophenol,

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Preliminary study of bisphenol-A removal in water samples by a

combined biocatalytic-ultrafiltration process at laboratory scale



Karen Avilez Cuahquentzi⁽¹⁾, Angélica Panohaya Rancho⁽¹⁾, Eduardo Torres Ramírez⁽²⁾,
Georgette Rebollar Pérez⁽¹⁾

⁽¹⁾ Facultad de Ingeniería Química, Benemérita Universidad Autónoma de Puebla, Av. 18 sur y San Claudio S/N, Ciudad Universitaria, Col. San Manuel, C.P. 72570, Puebla, Puebla, México.

⁽²⁾ Centro de Química-ICUAP, Edificio IC8, Ciudad Universitaria, Puebla, Puebla, México.

*Corresponding author: georgette.rebollar@correo.buap.mx



Emerging contaminants (EC) are harmful compounds due to their persistence and toxicity. They enter the environment through several anthropogenic sources. The conventional treatment processes hardly remove these recalcitrant contaminants and thus alternative processes have been developed to reduce their ecological impact. The use of biocatalysis as an advanced oxidation process, has been proved efficient to degrade different EC, such as pharmaceuticals¹. The objective of the present work is thus to present the preliminary results of bisphenol-A (BPA) removal through a combined biocatalytic-ultrafiltration (UF) treatment in a two-stage process. In the first stage, several samples of BPA were oxidized at room temperature by horseradish peroxidase (HRP, 2.8 μM) in a medium reaction (25 mL) consisting of phosphate buffer (60 mM), H_2O_2 (1 mM) and chitosan as an adsorbing agent. In the second stage, the samples were filtered using a pressurized cell (1 kg/cm^2) with commercial 1 kDa UF membranes. BPA oxidation/adsorption/retention was followed by UV-Vis analysis. A 2^k experimental design was chosen to optimize BPA removal. The effect of three parameters ($k=3$) on BPA removal was assessed: chitosan amount (250 and 500 mg), pH (6 and 7) and reaction time (15 and 24 h). All the experiments were done by duplicate. The results showed that the best BPA removal (90%) was obtained at pH 7, 15 h reaction time and 250 mg of chitosan.

Key words: bisfenol A, enzima HRP, ultrafiltración

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Removal of mercury by *Scenedesmus sp* as an alternative

bioremediation method



I.C. Guauque Díaz^(1,2), M. Peñuela Vásquez⁽¹⁾, A. Villegas Quiceno⁽²⁾

⁽¹⁾ Grupo de Bioprocesos, Universidad de Antioquia, Calle 67 # 53-108, Medellín, Colombia.

⁽²⁾ TERMOMECA, Universidad Cooperativa de Colombia, Calle 50 No 41-34. Medellín, Colombia.

*Corresponding author: ingrid.guauque@udea.edu.co

Free-living microorganism may become suitable models for recovery of heavy metals from wastewater as an alternative to conventional processes of environmental remediation¹. The dumping of highly harmful chemical compounds in waters has increased in parallel to industrial development since the regulatory laws for the adequate use of environmental resources are not complied. One of the sectors where this breach is most evident is in mining since some of the techniques used to obtain and purify minerals employ substances such as cyanide, strong acids, solvents, and heavy metals that seriously affect the ecosystems². The process of removing mercury by *Scenedesmus sp*, is discussed in this study. This microalgae has been found to be a very efficient biosorbent due to its ability to adsorb different metallic elements and chemical compounds. In addition, it has advantages over other species, such as higher growth rate, higher efficiency respect to metal removal, and its phytoremediation capacity³. The aim of the present study was to investigate the capability of *Scenedesmus sp* to remove Hg contained in a base medium contaminated with 0,005 g/l of mercury chloride ($HgCl_2$). The experimental methodology allowed to evaluate three initial concentrations of living microalgae (0,035,0,145,0,225 g/l). After 15 days the remaining mercury in the medium was measured by Atomic Absorption spectroscopy (AA). The generated dataset was analyzed by Analysis of variance (ANOVA) of biomass growth and metal uptake. The experimental results indicated high removal capability (greater than 90%); however growth inhibition was observed due to possible changes in the metabolism of the cells, since growth in presence of Hg^{2+} was significantly lower even at higher microalgae concentration. At 0,145 g/l initial concentration of microalgae it removed 98.6% of Hg from the solution, indicating this is promising and useful method for microalgae-mediated bioremediation.

Keywords: *Scenedesmus sp.*, mercury removal, bioremediation.

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Monitoring Ureolytic Biofilm formation from a microbial consortium on graphite surface



María C. Romero*⁽¹⁾, Guadalupe Ramos⁽²⁾, Ignacio González⁽²⁾, Florina. Ramírez⁽¹⁾
⁽¹⁾ Biotechnology Department, Metropolitan Autonomous University, Mexico City, Mexico
⁽²⁾ Chemistry Department, Metropolitan Autonomous University, Mexico City, Mexico

*Corresponding author: concepcionmacoro@gmail.com



The biological transformation of urea by bacteria to obtain ammonium (Ureolysis) is involved in engineering applications such as, contaminant remediation and, municipal wastewater treatment 1. To carry out the ureolysis the adsorption of bacteria on a graphite surface to form a biofilm may represent an advantage. To get this purpose, a microbial consortium was enriched from cow dung and the culturing conditions were established in order to increase biomass content, resulting in the complete hydrolysis of urea. The study of microbial adsorption on a graphite surface had been monitoring by the use of Electrochemical Impedance Spectroscopy (EIS) and confirmed by the employment of imagen techniques (CLSM and SEM)[1]. The study of the microbial population composition by simple sequencing of regions of 16s rRNA (Bacteria and Archaea), suggest that the biofilm has been enriched with a 99% of identity the species of *Amphibacillus indicireducens*, and *Amphibacillus iburiensis*. Also 98% of indetity is attributed to *Amphibacillus xilanus*, While 97% of *Amphibacillus sediminis*, *Aquibacillus halophilus* and *Paraliobacillus ryukyuensis*. The results could answer questions concerning the nature and role of microorganisms present in the biofilm to would be presented at the symposium.

Keywords: Ureolysis, Electrochemical Impedance Spectroscopy (EIS), Microbial Consortium.

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Chapter 11 Climate Change



Anaerobic methane oxidation linked to nitrous oxide reduction via humus mediated interspecies electron transfer in wetland sediment

Padilla Loma C. ^{*(1)}, Valenzuela-Reyes E.I. ⁽¹⁾, Cervantes Carrillo F.J. ^{*(1)}

⁽¹⁾ Instituto Potosino de Investigación Científica y tecnológica, Camino a la Presa San José, 2055, Lomas 4^a Sección, C.P. 78216 San Luis Potosí S.L.P, México

*Corresponding author: claudia.padilla@ipicyt.edu.mx; fcervantes@ipicyt.edu.mx

Wetlands are the main natural source of methane emission (CH₄), which results from the balance between methanogenesis and methanotrophy. Methanotrophy under anaerobic conditions has been documented by microbial processes linked to the reduction of sulphate, iron oxides and humic substances¹. Ecosystems, such as wetlands, possess high quantities of natural organic matter in their sediments (Humic substances). It has been documented that the activity of the quinone groups present in humic substances can act as redox mediators in biogeochemical cycles². On the other hand, nitrous oxide (N₂O) is the third most important gas within greenhouse gases. Biologically, the only N₂O sink known so far, is the reduction of N₂O to N₂ gas, which occurs in the last step of denitrification and is mediated by the nitrous oxide reductase enzyme, which is encoded by the NosZ gene³. The capacity of the microbial community, present in a wetland sediment, to carry out the anaerobic oxidation of methane (AOM) coupled to nitrous oxide reduction (NOR) mediated by humic substances was evaluated in this research. Fresh sediment (1 g) collected from the Sisal wetland (Yucatán, Mexico) was incubated with 15 mL mineral medium, under argon atmosphere in 25 mL serological bottles. Samples were spiked with 2 mL of ¹³CH₄ and 4 mL of ¹⁵N₂O. Besides this, 2 g/L Pahokee Peat (PP) was added as an external source of humic substances (Supplied by International Humic Substances Society, IHSS, USA). Production of ¹³CO₂ and ¹⁵N₂ was monitored daily by GC-MS for nine days. Experimental design included ten treatments; Sed-PP+¹³CH₄+¹⁵N₂O, Sed-PP+¹³CH₄, Sed+¹⁵N₂O, Sed-PP, Sed-PP+¹³CH₄+¹⁵N₂O+autoclaved, the remaining 5 treatments were placed with the same conditions described above, but without humic substances (PP). The results showed the significant impact of humic substances on the consumption rate of nitrous oxide in all treatments; however, in the complete treatment (Sed-PP + ¹³CH₄ + ¹⁵N₂O) at day nine ¹⁵N₂O was not detected in the gas phase, presenting a maximum consumption rate of 3.38 ± 0.07 mmol ¹⁵N₂O/g sed-d, which is ten times higher than the microcosm that did not contain humic substances. In addition, fifty percent of the reduced nitrous oxide was measured as ¹⁵N₂. On the other hand, 6.81% of methane reached the mineralization (¹³CO₂) in the complete treatment. From electron balance, a third of the ¹⁵N₂ production can be linked to methane oxidation, whereas other electron donors (reduced sulphur, ferrous Iron, originating from sediments) are needed to account for the nitrous oxide reduction observed. Finally, it could be said that under these conditions fifty percent of ¹⁵N₂O can be consumed on the site before being released into the atmosphere.

Keywords: Humic substances, methane, nitrous oxide, wetland

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Comparison of DNA extraction methods for molecular analyses of the ticks *Rhipicephalus sanguineus*, a warm-favored infection

C.E. Aragón-López⁽¹⁾, V. Ortiz-Encinas⁽¹⁾, J.R. Reyna-Granados⁽¹⁾, P. Luna-Nevárez⁽¹⁾, Y.L. Caraveo-Gutierrez⁽¹⁾

⁽¹⁾ Departamento de Ciencias Agronómicas y Veterinarias, Instituto Tecnológico de Sonora. Calle 5 de febrero 818 Sur, Colonia Centro, Cd. Obregón, Sonora, México. 85134.

Corresponding author: laloarg92@gmail.com



Diseases transmitted by ticks are of great importance in animal and public health, especially infection by the bacterium *Rickettsia rickettsii*, also called Stained Fever of the Rocky Mountains. This infection has caused dozens of deaths every year in the state of Sonora, and Cajeme is among the municipalities with a higher percentage of fatality, associating this percentage to adverse conditions such as lacking of a quick and effective diagnostic technique. In addition, warm climates predispose the disease allowing the replication of *Rickettsia rickettsia*. The objective of the present study was to compare different techniques of bacterial DNA extraction to analyze both integrity and quality of the DNA that allow a fast and precise diagnosis by PCR. The best DNA extraction procedure will help to promote the prevention and control of the disease, and also to increase the knowledge of diseases transmitted by the tick *Rhipicephalus sanguineus* in Cajeme, Sonora. Different DNA extraction techniques were performed to find the most suitable method. The best method for extracting DNA in ticks and tick eggs was the macerated with liquid nitrogen with the commercial kit DNeasy Blood and Tissue kit from Qiagen, using a modified protocol, later confirming the integrity with agarose gel electrophoresis and finally the quality and quantity of the genetic material in the biospect-nano quantifier SHIMADZU. The extracted DNA was able to be amplified in the conventional PCR technique, identifying the 16S ribosomal region for bacterial DNA. In conclusion, the macerated of ticks with liquid nitrogen was a suitable method to improve the timing of extraction, integrity and quality of DNA for subsequent molecular analysis in pathogenic bacteria related to the *Rickettsiae* group. We propose such technique as appropriate strategy to study a very dangerous disease which is exacerbated by the warm conditions that are common in semi-arid regions such as Sonora, and specifically, the Yaqui Valley.

Keywords: DNA Extraction, amplified, *Rickettsia rickettsii*, Yaqui Valley.



A SNP from the gene STAT5A associated to days open in lactating Holstein cows managed under the warm weather conditions of the Yaqui Valley

R. Zamorano-Algandar ⁽¹⁾, M.A. Sánchez-Castro ⁽²⁾, J.C. Leyva-Corona ⁽¹⁾, M.G. Thomas ⁽²⁾, R.M. Enns ⁽²⁾, S. E. Speidel ⁽²⁾, J. F. Medrano ⁽³⁾, G. Rincon ⁽³⁾, and P. Luna-Nevarez ⁽¹⁾

⁽¹⁾ Departamento de Ciencias Agronómicas y Veterinarias, Instituto Tecnológico de Sonora. Calle 5 de Febrero 818 Sur, Colonia Centro, Cd. Obregón, Sonora, México. 85134.

⁽²⁾ Department of Animal Sciences, Colorado State University, Fort Collins CO, USA. 80523. USA. ⁽³⁾

Department of Animal Sciences, University of California, Davis CA, USA. 95616. USA.

*Corresponding author: pluna@itson.edu.mx

Reproduction in Holstein cows is limited by the selection method, facilities, nutrition and weather, and the last one is potentiated by the climatic change. Marker assisted selection in cattle using single nucleotide polymorphisms (SNP) has been proposed as strategy to identify cattle with superior ability to tolerate heat stress. The signal transducer and activator of transcription (STAT6) is a complex gene system composed by seven proteins which participate as transcription factors and play a key role in cytokines signal pathways. The objective of this study was to associate SNP from the STAT5A gene with the reproductive trait days open (DO) in lactating Holstein cattle located and managed in a warm climate region. The study was performed in three commercial dairy herds from the Yaqui Valley and included 500 Holstein cows with reproductive records from 2011, 2012 and 2013. A blood sample was collected from each cow and spotted onto FTA cards which were used to genotype 17 SNP from the STAT5A gene. Such SNP were previously identified as associated to thermo-tolerance, milk production and fertility. Hardy-Weinberg equilibrium test and minor allele frequency estimation were conducted in PROC ALLELE. The associative analyses among SNP genotypes and the reproductive trait DO were performed using a mixed effects model for continuous traits. The model included DO as the response variable, genotype, number of lactations and herd as fixed terms, sire as a random term, and days in milk as a covariate. Allele substitution effects were calculated for each significant SNP ($P < 0.05$) through a mixed model that included the genotype term as covariate. The false discovery rate (FDR) analysis was performed using PROC MULTTEST. Only the SNP rs137182814 from the gene STAT5A was associated to DO ($P < 0.02$). Least square means among genotypes GG, CG and CC were 102 ± 3.84 , 110 ± 2.13 and 104 ± 2.72 days, respectively. The most favorable allele from the SNP was the G allele ($P < 0.001$) as it reduced DO in 6.4 ± 0.2 days. In conclusion, according to our results, we propose a SNP from the STAT5A gene as candidate marker to improve genetic selection in Holstein cattle managed under warm weather conditions. Further research should include denser SNP panels in order to discover a higher number of SNP that explain genetic variation associated to fertility traits in heat-stressed Holstein cattle as consequence of climatic changes.

Keywords: Days Open, Holstein, SNP, STAT5A.



Single nucleotide polymorphism associated with a behavioral trait sensitive to warm weather conditions in replacement gilt vaccinated against PRRS virus infection

C. M. Aguilar-Trejo⁽¹⁾, J. A. Romo-Rubio⁽¹⁾, G. Luna-Nevárez^{(1)*}, J. R. Reyna-Granados^{(2)*}, X. Zeng⁽³⁾, R. M. Enns⁽³⁾, S. E. Speidel⁽³⁾, M. G. Thomas⁽³⁾, and P. Luna-Nevárez^{*(2)}

⁽¹⁾ Faculty of Veterinary Medicine and Production, Universidad Autónoma de Sinaloa, Culiacán, Blvd. San Angel 3886, Mercado de Abastos, Fraccionamiento San Benito, 80260 Culiacán Rosales, Sin SIN 80260,

⁽²⁾ Departamento de Ciencias Agronómicas y Veterinarias, Instituto Tecnológico de Sonora. Calle 5 de Febrero 818 Sur, Colonia Centro, Cd. Obregón, Sonora, México. 85134.

⁽³⁾ Department of Animal Sciences, Colorado State University, Fort Collins CO, USA. 80523. USA.

*Corresponding author: pluna@itson.edu.mx

Porcine respiratory and reproductive syndrome (PRRS) is a disease of high negative impact on Mexican porcine production. Vaccination is the management strategy to protect replacement gilts from PRRS infection; however, their response is highly variable. Such variation is higher in sows managed in regions under increasing warm weather conditions as consequence of climatic changes. The objective of this study was to study genetic basis that underlies a behavior trait highly sensitive to climatic changes in replacement gilts vaccinated against de PRRS virus. This study included 6-mo-old 3/4-Landrace × 1/4-Yorkshire replacement gilts (n = 100). After a 7-d acclimation period, all gilts were vaccinated with a modified live PRRS virus (d 0) and they were kept under a quarantine program in a restricted area. Feed was provided twice daily and the gilts were observed during 15 min in order to measure posture, a categorical behavior trait (1=gilts eating, 2=gilts interested but not eating, and 3=gilts depressed/not interested/not eating). A blood sample was collected from each gilt approximately 40 d after vaccination and spotted onto FTA cards. All cards were processed for genomic analyses using a low-density chip to obtain genotypes from 8826 SNP (Infinium BeadChip, Illumina, San Diego, CA). A genomic multi-locus mixed model performed in SNP Variation Suite-7 identified five single nucleotide polymorphisms (SNP) associated with posture (P < 0.001), and these SNP were located within chromosomes 1, 3 and 15. In conclusion, there exist genetic components associated to posture, and this assumption is supported by the genomic analyses that suggests this behavioral train is gene-influenced. Therefore, this study provided evidence that a behavioral trait influenced by warm weather conditions created by the climatic change could be associated to a favorable response after vaccination against the PRRS virus.

Keywords: Gilts, PRRS, SNP, Vaccination.