



# ENVIS CENTER

on

# ENVIRONMENTAL BIOTECHNOLOGY



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## **ENVIS CENTRE**

**on**

## **ENVIRONMENTAL BIOTECHNOLOGY**

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## BACKGROUND

Environmental Information System (ENVIS) is established in the year 1984 as a network of Information Centres. It is planned by the Ministry of Environment and Forest. Aim of this centre is to provide descriptive and environmental subject related numerical data.

This ENVIS Centre is established in the focal theme area - Environmental Biotechnology at the Department of Environmental Science, University of Kalyani, Nadia-741235, West Bengal.

The objective of this centre is to collect data related to the above mentioned subject, from different major libraries mainly in West Bengal and also from other states in India, through consultation with different journals, Annual Reviews, Internet and to generate a database and create a website uploaded with these information. Besides, we publish biannually Abstract Volume on our thematic area Environmental Biotechnology under fifteen sub-heads. The volume contains abstracts of scientific articles from relevant national and international journals. Viewpoint of this abstract volume is to help the interested research workers, scientists, administrators and the general people.

This is the 21<sup>st</sup> publication of Abstract Volume of this ENVIS Centre. This contains the abstracts of research papers collected from the various areas of Environmental Biotechnology from different journals published in last six months upto December, 2012. In this issue, various topics like Bioenergy, Bioengineering, Bio-degradation, Bio-remediation, Bio-transformation etc. have been covered. We are grateful to the various libraries and their staff for their cooperation extended to us during the collection of the articles.

## Abstract Format

The format of the abstract is as follows:

- Abstract:** The abstracts are arranged in different subheads.
- Author:** Name of the authors are given in the order in which they appear in the original document. These names are given in succession.
- Address of Authors:** Address of the author is given in parenthesis at the end of the author's name. When the address of any other author is found, it is written after wards delimited by stop(.).
- Locus:** The name of the journal is followed by the volume number, issue number, year of publication and the page no.

## GENERAL INFORMATION

Abstract have been taken directly from source documents like research report, journals, internet, seminar proceedings, standards and patents. All the resources are published within last six months.

Abstract are broadly classified and arranged under the following 15 heads:

**Bioaccumulation:** Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things whenever they are taken up and stored at a rate faster than they are broken down (metabolized) or excreted. Understanding the dynamic process of bioaccumulation is very important in protecting human beings and other organisms from the adverse effects of chemical exposure, and it has become a critical consideration in the regulation of chemicals.

**Bioremediation:** It is a clean-up technology that uses naturally occurring microorganisms to degrade hazardous substances into less toxic or nontoxic compounds. The microorganisms may:

1. Ingest and degrade organic substances as their food and energy source,
2. Degrade organic substances, such as chlorinated solvents or petroleum products, that are hazardous to living organisms, including humans, and degrade the organic contaminants into inert products.

As the microorganisms occur naturally in the environment they are likely to pose little risks of contamination.

**Bio-Transformation:** This is a process of Biological changes of complex compounds to simpler one or toxic to non-toxic and vice-versa. Several microorganisms are capable of transforming a variety of compounds found in nature but generally in case of synthetic compounds they are unable to show any appropriate action. Biotransfer appears to be one of the major detoxication methods known so far.

**Biomarker:** It is a biological response to a chemical that gives a measurement of exposure and, sometimes, of toxic effect. It can be defined as any kind of molecule which indicate the existence (past or present) of living organisms. In particular, in the fields of geology and astrobiology biomarkers are also known as biosignatures. However, in environmental science a bio-markers can also be used to indicate exposure to various environmental substances in epidemiology and toxicology.

**Biofertilizer:** To reduce the impact of excess chemical fertilizers in the field of agriculture the biofertilizer is being considered as a potential tool; biologically fixed nitrogen

is such a source which can supply an adequate amount of Nitrogen to plants and other nutrients to some extent. Many free living and symbiotic bacteria, which fix atmospheric Nitrogen are used as biofertiliser material as a substitute for Nitrogen fertilizer. In general two types of biofertiliser are used

1. Bacterial Biofertilizer
2. Algal Biofertilizer

**Biocomposting:** It involves combining organic materials under conditions that enables them to decompose more quickly than they would in nature. Think about logs and leaves on the ground in a forest. The leaves will break down and disappear within a year. Logs of course will take much longer to crumble away. Composting is the process of converting all biodegradable wastes into organic manure. In composting process certain input should be made into waste to convert the process in a short time.

**Biopesticide:** Pest control by biological antagonism appears to be very useful tool in recent years. Bacterial pesticides are being developed. *Heliothis* complex, which lives in close association with plant roots, consists of two major crop pests budworm and ball worm. Biological insecticides against both these insects are being prepared by transfer of a gene from *Bacillus thuringiensis*

**Biodegradation:** It is nature's way of recycling wastes, breaking down organic matter into nutrients that can be used by other organisms. "Degradation" means decay, and the "bio-" prefix means that the decay is carried out by a huge assortment of bacteria, fungi, maggots, worms, and other organisms that eat dead material and recycle it into new forms.

In the nature, nothing is known as waste, because everything gets recycled. The waste products from one organism become the food for others, providing nutrients and energy while breaking down the waste organic matter. Some organic materials may break down much faster than others, but all will eventually decay.

By harnessing these natural forces of biodegradation, people can reduce wastes and clean up some types of environmental contaminants. Through **composting**, we accelerate natural biodegradation and convert organic wastes to a valuable resource.

**Biosensor:** Biosensor represents biophysical devices, which can detect the presence and measure the quantities of specific substances in a variety of environments. These specific substances may include sugars, proteins, or humas and variety of toxins in the industrial effluents. In designing a biosensor an enzyme or an antibody or even microbial cells are associated with microchip devices, which are used for quantitative estimate of a substance.

**Bioengineering:** It is a developing speciality featuring a multidisciplinary approach to the solution of problems in medicine and biology, based on the application of

advances in science, engineering and technology. It generally engineers the biological processes through biotechnological or genetic engineering interventions. It may also be a broad-based engineering discipline that involve product design, sustainability and analysis of biological systems.

**Pollen-Biotechnology:** This is a new field of science dealing with the pollen chemistry and allergenicity of aerospora. This subject also covers genetic manipulation of pollen development of haploid culture. Such haploid plants have immense values in genetic research.

**Biotechnology Policy Issue:** Biotechnology appears to be an emerging science in present decades. Genetic manipulation and development of genetically modified organism in human welfare is now showed a potential prospect and risk. Thus, researches and application of Biotechnology in diverse field is a major policy issue in the present decades.

**Agricultural Biotechnology:** Over the years, tremendous success has been made in diverse field of agriculture by applying Biotechnology. It includes development of genetically modified crops, genetic improvement in sericulture practices, improvement in Biofertilizer development and similar other aspects. Production of pest and disease resistant crop is also being considered to be an emerging area of Agricultural Biotechnology.

**Bioenergy:** In recent decades, efforts have been made for evolving were non-polluting bioenergy sources or energy generation from organic wastes and biomass. These are all ecofriendly solutions. Biomass energy supply-demand balances have become a component of energy sector analysis and planning and is propelled huge importance in the countries. Biomass, Biogas, Hydrogen are the example of Bioenergy.

**Nano Biotechnology:** Bionanotechnology, nanobiotechnology, and nanobiology are terms that refer to the intersection of nanotechnology and biology. Given that the subject is one that has only emerged very recently, bionanotechnology and nanobiotechnology serve as blanket terms for various related technologies.

This discipline helps to indicate the merger of biological research with various fields of nanotechnology. Concepts that are enhanced through nanobiology include: nanodevices, nanoparticles, and nanoscale phenomena that occurs within the discipline of nanotechnology. This technical approach to biology allows scientists to imagine and create systems that can be used for biological research

## ABBREVIATIONS USED IN ADDRESSES AND CITED JOURNALS

Acad	Academy	Chem	Chemistry
Adm	Administration	Cheml	Chemical
Admn	Administrative	Clinl	Clinical
Adv	Advance	Co	Company
Agri	Agriculture	Coll	College
Agricl	Agricultural	Comm	Committee
Amer	American	Commn	Commission
An	Annual	Comp	Comparative
Analyt	Analytical	Conf	Conference
Anat	Anatomy	Conv	Convention
Anim	Animal	Conserv	Conservation
Ann	Annals	Contl	Control
Appl	Applied	Contam	Contamination
Arch	Archives	Corp	Corporation
Archaeo	Archaeology	Coun	Council
Archaeol	Archaeological	Cult	Culture
Architect	Architecture	Cultl	Cultural
Assoc	Association	Curr	Current
Asst	Assistant	Dept	Department
Atom	Atomic	Dev	Development
Bacterio	Bacteriology	Develop	Developmental
Bacteriol	Bacteriological	Dig	Digest
Bd	Board	Div	Division
Bio	Biology	Divl	Divisional
Biochem	Biochemistry	Dte	Directorate
Biocheml	Biochemical	Dy	Deputy
Bioengg	Bioengineering	Eco	Ecology
Biol	Biological	Ecol	Ecological
Biometeo	Biometeorology	Econ	Economics
Biophys	Biophysics	Ecosys	Ecosystem
Biometeol	Biometeorological	Ecotoxico	Ecotoxicology
Biotech	Biotechnology(s)	Endocrinol	Endocrinological
Biotechno	Biotechnology	Engg	Engineering
Biotechnol	Biotechnological	Engrs	Engineers
Bldg	Building	Env	Environment
Bot	Botany	Environ	Environmental
Botl	Botanical	Epidemic	Epidemiology
Br	Branch	Epidemiol	Epidemiological
Bull	Bulletin	Estd	Establishment
Cent	Centre	Ethnopharmaco	Ethnopharmacology
Centl	Central	Expt	Experiment

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Exptl	Experimental	Microbiol	Microbiological
Fac	Faculty	Min	Ministry
Fd	Food	Monit	Monitoring
Fedn	Federation	Myco	Mycology
Fert	Fertiliser	Mycol	Mycological
Fmg	Farming	Nat	Natural
Gaz	Gazette	Natl	National
Genet	Genetics	N-E	North Eastern
Geo	Geology	Nut	Nutrition
Geogr	Geography	No	Number
Geogr1	Geographical	Occ	Occasional
Geol	Geological	Occupl	Occupational
Geosci	Geoscience	Oceanogr	Oceanography
Govt	Government	Org	Original
Hist	History	Orgc	Organic
Hlth	Health	Orgn	Organisation
Hort	Horticulture	Pharmaco	Pharmacology
Hosp	Hospital	Pharmacol	Pharmacological
Hydro	Hydrology	Phyl	Physical
Hydrol	Hydrological	Patho	Pathology
Immuno	Immunology	Pathol	Pathological
Immunol	Immunological	Petrochem	Petrochemical
Ind	Industry	Petro	Petrology
Inf	Information	PG	Post Graduate
Inst	Institute	Phys	Physics
Instn	Institution	Physio	Physiology
Int	International	Phytopath	Phytopathology
Irrig	Irrigation	Phytopathol	Phytopathological
J	Journal	Plang	Planning
Lab	Laboratory	Polln	Pollution
Lett	Letter(s)	Proc	Proceedings
Ltd	Limited	Prot	Protection
Malario	Malariology	Pub	Publication
Malariol	Malariological	Pvt	Private
Manag	Management	Qlty	Quality
Med	Medicine	Qr	Quarter
Medl	Medical	Rad	Radiation
Metab	Metabolism	Radio	Radiology
Metall	Metallurgy	Radiol	Radiological
Metallurg	Metallurgical	Rd	Road
Meteo	Meteorology	Recd	Received
Meteol	Meteorological	Reg	Region
Microbio	Microbiology	Regl	Regional

Rep	Report	Surv	Survey
Reptr	Reporter	Syst	System
Res	Research	Tax	Taxonomy
Rev	Review	Techl	Technical
Sch	School(s)	Techno	Technology
Sci	Sciences(s)	Technol	Technological
Scient	Scientific	Toxico	Toxicology
S-E	South East	Toxicol	Toxicological
Sec	Section	Transc	Transcations
Sect	Sector	Transpt	Transportation
Semin	Seminar	Trng	Training
Ser	Services	Trop	Tropical
Soc	Society	Univ	University
Socl	Social	Util	Utilisation
Stat	Statistics	Vet	Veterinary
Statl	Statistical	Zoo	Zoology
Stnd	Standard(s)	Zool	Zoological
Stud	Study/ (eis)		

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## Bioaccumulation

**Tímea Brázová<sup>1</sup>, Vladimíra Hanzelová<sup>1</sup>, Dana Miklisová<sup>1</sup>, Danka Šalgovičová<sup>2</sup>, Ľudmila Turčeková<sup>1</sup>.** (<sup>1</sup>Institute of Parasitology, Slovak Academy of Sciences, 04001 Košice, Slovakia. e-mail: barciova@saske.sk, <sup>2</sup>Centre for Evaluation of Food Contaminants, Food Research Institute, 82475 Bratislava, Slovakia). **Biomonitoring of polychlorinated biphenyls (PCBs) in heavily polluted aquatic environment in different fish species. Environmental Monitoring and Assessment, Volume 184 (11) (2012):6553-6561**

The distribution and concentrations of polychlorinated biphenyls (PCBs) were determined in fish species (European perch *Perca fluviatilis*, northern pike *Esox lucius*, pike perch *Sander lucioperca*, wels catfish *Silurus glanis*, common carp *Cyprinus carpio*, European eel *Anguilla anguilla*, freshwater bream *Abramis brama*, goldfish *Carassius auratus*, and roach *Rutilus rutilus*) in a heavily polluted water reservoir Zemplínska šírava (Slovakia). The study performed at two different time points 5 years apart (2004 and 2009) revealed serious PCB contamination of fish muscle tissue and significant interspecies as well as tissue-specific differences in PCB uptake by fish. Total PCBs broadly correlated with the trophic position of individual fish species within a food chain ( $P < 0.01$ ). The concentrations were particularly high in predatory fish species, perch, pike, and pike perch (108.0, 90.1, and 113.0 mg kg<sup>-1</sup> lipid wt, respectively), but comparable PCB values were also found in non-predatory detritivorous freshwater bream (128.0 mg kg<sup>-1</sup> lipid wt). The lowest PCB values were surprisingly assessed in European eel (17.1 mg kg<sup>-1</sup> lipid wt). Tissue analysis showed the highest storage capacity of the liver (hepatopancreas in cyprinids) with maximum concentrations recorded found in northern pike (214.0 mg kg<sup>-1</sup> lipid wt) and freshwater bream (163.0 mg kg<sup>-1</sup> lipid wt). Negative correlations, mostly not significant, between the total PCB concentrations and fish weight were observed ( $P > 0.05$ ). The study has shown that the kind of fish, its feeding habit, and specific conditions of the habitat are mutually interrelated factors that are responsible for significant variations in fish body burdens. A tendency to PCB biomagnification was also proved in some fish species of this water reservoir.

**Anuj Saxena<sup>1</sup>, Anjali Saxena<sup>2</sup>.** (<sup>1</sup>Department of Botany, Sacred Heart Degree College, Sitapur, 261001, Uttar Pradesh, India, anujsaxena2807@rediffmail.com. <sup>2</sup>Department of Chemistry, Bareilly College, Bareilly, Uttar Pradesh, 243005, India). **Bioaccumulation and glutathione-mediated detoxification of copper and cadmium in *Sphagnum squarrosum* Crome Samml. Environmental Monitoring and Assessment, Volume 184 (7) (2012): 4097-4103**

Physiological and biochemical responses, metal bioaccumulation and tolerance potential of *Sphagnum squarrosum* Crome Samml. to Cu and Cd were studied to determine its bioindication and bioremediation potential. Results suggest that glutathione treatment increases the metal accumulation potential and plays a definite role in heavy metal scavenging. High abundance of *Sphagnum* in metal-rich sites strongly suggests its high metal tolerance capabilities. This experiment demonstrates that *S. squarrosum* is able to accumulate and tolerate a high amount of metals and feasibility of its application as bioindicator and remediation test species of metal-contaminated environment.

**Mahesh Mohan, M. Deepa, E. V. Ramasamy, A. P. Thomas. (School of Environmental Sciences, Mahatma Gandhi University, 686 560 Kottayam, Kerala, India. e-mail: mahises@gmail.com). Accumulation of mercury and other heavy metals in edible fishes of Cochin backwaters, Southwest India. Environmental Monitoring and Assessment, Volume 184 (7) (2012): 4233-4245**

Mercury, a global pollutant, has become a real threat to the developing countries like India and China, where high usage of mercury is reported. Mercury and other heavy metals deposited in to the aquatic system can cause health risk to the biota. The common edible fishes such as *Mugil cephalus*, *Arius arius*, *Lutjanus ehrenbergii*, *Etroplus suratensis* were collected from Cochin backwaters, Southwest India and analysed for mercury and other heavy metals (zinc, cadmium, lead and copper) in various body parts. Kidney and liver showed highest concentration of metals in most fishes. The omnivore and bottom feeder (*E. suratensis*) showed high concentration of mercury (14.71 mg/kg dry weight) and other metals (1.74 mg/g—total metal concentration). The average mercury concentration obtained in muscle was 1.6 mg/kg dry weight (0.352 mg/kg wet weight), which is higher than the prescribed limits (0.3 mg/kg wet weight). The concentration of other heavy metals in the muscles of fishes were found in a decreasing order Zn>Cu>Cd>Pb and are well below WHO permissible limits that were safe for human consumption. Metal selectivity index (MSI) obtained for all the metals except mercury showed that both carnivores and omnivores have almost same kind of affinity towards the metals especially Zn and Cd, irrespective of their feeding habit. The MSI values also indicate that the fishes have the potential to accumulate metals. High tissue selectivity index (TSI) values were reported for kidney, muscle and brain for all metals suggests that the metal concentration in these tissues can serve as an indication of metal polluted environment. Even if the daily intakes of Zn, Cd, Pb and Cu from these fishes are within the provisional maximum daily intake recommended by WHO/FAO, the quality is questionable due to the high hazard index obtained for mercury (>1). Fishes like *E. suratensis* being a favourite food of people in this region, the high consumption of it can lead to chronic disorders as this fish has high concentration of metals.

**K. Kolaříková<sup>1</sup>, E. Stuchlík<sup>1</sup>, M. Liška<sup>2</sup>, J. Horecký<sup>1</sup>, J. Tátosová<sup>1</sup>, D. Hardekopf<sup>1</sup>, N. Lapšanská<sup>2</sup>, Z. Hořícká<sup>1</sup>, J. Hovorka<sup>1</sup>, M. Mihaljevič<sup>3</sup>, J. K. Fuksa<sup>4</sup>, W. von Tümpling<sup>5</sup>. (<sup>1</sup>Institute for Environmental Studies, Charles University in Prague, <sup>2</sup>Povodí Vltavy, State Public Enterprise, Prague, Czech Republic, <sup>3</sup>Institute of Geochemistry, Mineralogy and Mineral Resources, Charles University in Prague, Prague, Czech Republic, <sup>4</sup>T. G. Masaryk Water Research Institute, Prague, Czech Republic, <sup>5</sup>Helmholtz Centre for Environmental Research, Magdeburg, Germany Long-Term Changes in the Bioaccumulation of As, Cd, Pb, and Hg in Macroinvertebrates from the Elbe River (Czech Republic). Water, Air, & Soil Pollution, Volume 223 (6) (2012): 3511-3526**

The Elbe River was extremely loaded by heavy metals and arsenic during the second half of the 20th century as a consequence of intensive chemical industry activities. To assess long-term contamination trends in the Elbe after socio-economical changes in 1989, selected macroinvertebrate species were used to identify biotic accumulation levels in the period from 1993 to 2005. Concentrations of As, Cd, Pb, and Hg were determined in *Asellus aquaticus* (Crustacea), *Bythinia tentaculata* (Mollusca), *Erpobdella* spp. (Hirudinea), and larvae of the family Chironomidae (Diptera) over a 3-year period along the longitudinal profile of the Czech part of the Elbe River and the lower Vltava (the main Elbe tributary). In this study, new evaluative approaches were used to assess these multiparameter data sets. The results showed that the most contaminated Czech sites were located in the industry-heavy middle Elbe region

between Valy and Obříství. Surprisingly high concentrations of As, Cd, and Pb were observed in the upper stretch near the headwaters. Probably as a consequence of former mining activities and the metallurgic and glass industries, the lower Vltava was loaded with Cd and Pb. Despite substantial improvements in the water quality of the Elbe River within the studied time period, we found no general decreasing trend of As, Cd, and Pb in macrozoobenthos. A significant decrease of Hg was revealed, however, occurring already between 1993 and 1996. The results of interspecies comparisons showed that the investigated elements were most accumulated by chironomid larvae and *Erpobdella* spp.

## **Bioremediation**

**Chukwunonye Ojinnaka, Leo Osuji, Ozioma Achugasim. (University of Port Harcourt, Port Harcourt, Rivers, Nigeria). Remediation of hydrocarbons in crude oil-contaminated soils using Fenton's reagent. Environmental Monitoring and Assessment, Volume 184 (11) (2012): 6527-6540**

Sandy soil samples spiked with Bonny light crude oil were subsequently treated with Fenton's reagent at acidic, neutral, and basic pH ranges. Oil extracts from these samples including an untreated one were analyzed 1 week later with a gas chromatograph to provide evidence of hydrocarbon depletion by the oxidant. The reduction of three broad hydrocarbon groups—total petroleum hydrocarbon (TPH); benzene, toluene, ethylbenzene, and xylene (BTEX); and polycyclic aromatic hydrocarbon (PAH) were investigated at various pHs. Hydrocarbon removal was efficient, with treatment at the acidic pH giving the highest removal of about 96% for PAH, 99% for BTEX, and some TPH components experiencing complete disappearance. The four-ringed PAHs were depleted more than their three-ringed counterparts at the studied pH ranges.

**Soumya Chatterjee, Lokendra Singh, Buddhadeb Chattopadhyay, Siddhartha Datta, S. K. Mukhopadhyay. (Defence Research Laboratory, Post Bag No. 02, Tezpur, 784001 Assam, India. drlsoumya@gmail.com). A study on the waste metal remediation using floriculture at East Calcutta Wetlands, a Ramsar site in India. Environmental Monitoring and Assessment, Volume 184 (8) (2012): 5139-5150**

Use of specific plant species in remediation of heavy metal-contaminated soil and water was a promising eco-friendly technology. The present study indicated the possibilities of phytoremediation of metal-contaminated (namely Ca, Cr, Mn, Fe, Cu, Zn, and Pb) soil by using plant species important for floriculture of East Calcutta Wetlands, a Ramsar site at the eastern fringe of Calcutta city. Plant species like sunflower (*Helianthus annuus*), marigold (*Tagetes patula*), and cock's comb (*Celocia cristata*) grew on soil contaminated by industrial sludge and irrigated regularly with wastewater accumulated different metals in different plant parts in varied concentrations. Pot culture study in the laboratory setup was also done to ascertain the efficiency of these plants for ameliorating contaminated soil. It was found that general accumulation patterns of metals concerned in different plant parts were root > leaf > stem > flower. This work indicated the importance of cultivation of economically important, non-edible, ornamental plant

species as an alternative cost-effective practice to remediate heavily contaminated farmlands of East Calcutta Wetlands.

**K.A. Mackie<sup>a</sup>, T. Müller<sup>b</sup>, E. Kandeler<sup>a</sup>.** (<sup>a</sup> Institute of Soil Science and Land Evaluation, Soil Biology Section, University of Hohenheim, 27 Emil-Wolff-Straße, 70599 Stuttgart, Germany, <sup>b</sup> Institute of Crop Science, University of Hohenheim, Germany). **Remediation of copper in vineyards – A mini review. Environmental Pollution, Volume 167 (2012): 16–26**

Viticulturists use copper fungicide to combat Downy Mildew. Copper, a non-degradable heavy metal, can accumulate in soil or leach into water sources. Its accumulation in topsoil has impacted micro and macro organisms, spurring scientists to research *in situ* copper removal methods. Recent publications suggest that microorganism assisted phytoextraction, using plants and bacteria to actively extract copper, is most promising. As vineyards represent moderately polluted sites this technique has great potential. Active plant extraction and chelate assisted remediation extract too little copper or risk leaching, respectively. However, despite interesting pot experiment results using microorganism assisted phytoextraction, it remains a challenge to find plants that primarily accumulate copper in their shoots, a necessity in vineyards where whole plant removal would be time consuming and financially cumbersome. Vineyard remediation requires a holistic approach including sustainable soil management, proper plant selection, increasing biodiversity and microorganisms.

**Keywords:** Copper; Vineyard; Remediation; Phytoextraction; Microorganisms

**Yushuang Li, Xiaojun Hu, Xueying Song, Tiejing Sun.** (Key Laboratory of Regional Environment and Eco-Remediation (Ministry of Education), Shenyang University, Shenyang 110044, PR China). **Remediation of cadmium-contaminated soil by extraction with *para*-sulphonato-thiacalix[4]arene, a novel supramolecular receptor. Environmental Pollution, Volume 167(2012): 93–100**

Batch extractions were conducted to evaluate the performance of *para*-sulphonato-thiacalix[4]arene (STC[4]A), a novel supramolecular receptor, for removing cadmium (Cd) from soil. The extraction mechanism was investigated by determination of the conditional stability constants ( $\log K$ ) of the STC[4]A-Cd complex. The influences of various variables were examined, including pH, contact time, and extractant concentration. The Cd extraction efficiency increased with increasing pH, reaching the maximum at pH 11 and then declining at higher pH values. This pH dependence was explained by the variation in the  $\log K$  value of the STC[4]A-Cd complex along with pH change. When the STC[4]A dose was increased to an STC[4]A:Cd molar ratio of 2.5:1, Cd was exhaustively removed (up to 96.8%). The comparison experiment revealed that the Cd extraction performance of STC[4]A was almost equivalent to that of EDTA and significantly better than that of natural organic acids. STC[4]A extraction could efficiently prevent co-dissolution of soil minerals.

**Keywords:** Chemical extraction; Cadmium; *para*-Sulphonato-thiacalix[4]arene; Conditional stability constant

**K. Zhuang<sup>1</sup>, E. Ma<sup>1</sup>, Derek R. Lovley<sup>2</sup>, Radhakrishnan Mahadevan<sup>1,3,\*</sup>.** (<sup>1</sup>Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College St., Rm 326, Toronto, Ontario, Canada M5S3E5; telephone: 1-416-946-0996; fax: 1-416-978-8605, <sup>2</sup>Department of Microbiology, University of Massachusetts, Amherst, Massachusetts,

<sup>3</sup>Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada. Email: Radhakrishnan Mahadevan krishna.mahadevan@utoronto.ca.  
\*Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College St., Rm 326, Toronto, Ontario, Canada M5S3E5; telephone: 1-416-946-0996; fax: 1-416-978-8605). The design of long-term effective uranium bioremediation strategy using a community metabolic model. *Biotechnology and Bioengineering*, Volume 109(10) (2012): 2475–2483

Acetate amendment at uranium contaminated sites in Rifle, CO. leads to an initial bloom of *Geobacter* accompanied by the removal of U(VI) from the groundwater, followed by an increase of sulfate-reducing bacteria (SRBs) which are poor reducers of U(VI). One of the challenges associated with bioremediation is the decay in *Geobacter* abundance, which has been attributed to the depletion of bio-accessible Fe(III), motivating the investigation of simultaneous amendments of acetate and Fe(III) as an alternative bioremediation strategy. In order to understand the community metabolism of *Geobacter* and SRBs during artificial substrate amendment, we have created a genome-scale dynamic community model of *Geobacter* and SRBs using the previously described Dynamic Multi-species Metabolic Modeling framework. Optimization techniques are used to determine the optimal acetate and Fe(III) addition profile. Field-scale simulation of acetate addition accurately predicted the in situ data. The simulations suggest that batch amendment of Fe(III) along with continuous acetate addition is insufficient to promote long-term bioremediation, while continuous amendment of Fe(III) along with continuous acetate addition is sufficient to promote long-term bioremediation. By computationally minimizing the acetate and Fe(III) addition rates as well as the difference between the predicted and target uranium concentration, we showed that it is possible to maintain the uranium concentration below the environmental safety standard while minimizing the cost of chemical additions. These simulations show that simultaneous addition of acetate and Fe(III) has the potential to be an effective uranium bioremediation strategy. They also show that computational modeling of microbial community is an important tool to design effective strategies for practical applications in environmental biotechnology.

**Keywords:** community metabolism; metabolic modeling; optimization; bioremediation

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A biodecolorization model that considers the simultaneous mechanism of biosorption and biodegradation of a synthetic dye by immobilized white-rot fungus *Trametes subeclypus* B32 in a fixed bed bioreactor was developed. The model parameters (biokinetic, biosorption and macroscopic transport) were determined by independent experiments. The biodecolorization model was used to determine the effect of variables such as immobilized biomass content, volumetric flow of wastewater, dye feeding concentration and initial dye concentration. By

means of the model was possible to predict in the steady state, the limits of immobilized *T. subectypus* to biodecolorize polluted influent, being the model predictions similar in extent to previous reports. A dimensionless module of biosorption-bioreaction ( $\square = q_{\max} v_z / r_{\max} L$ ) was proposed to be used like criterion whenever one of the two mechanisms controls the biodecolorization. The model could be used for the designing and scaling up of fixed bed bioreactors with immobilized white-rot fungi for the biodecolorization process.

**Keywords:** Biodecolorization; Mathematical model; *Trametes subectypus*; Fixed bed bioreactor

**Hossein Zare, Ghasem Najafpour, Mostafa Rahimnejad, Ali Tardast, Saeedeh Gilani. (Biotechnology Research Lab., Faculty of Chemical Engineering, Noshirvani University of Technology, Babol, Iran). Biofiltration of ethyl acetate by *Pseudomonas putida* immobilized on walnut shell. Bioresource Technology, Volume 123 (2012): 419–423**

A biofilter packed with walnut shells was used to eliminate ethyl acetate from an air stream. The shells treated with NaOH were used as medium for immobilization of *Pseudomonas putida* PTCC 1694. At an empty bed residence time (EBRT) of 60 s, a removal efficiency of 99% was achieved at inlet concentrations lower than 430 ppm of ethyl acetate. The removal efficiency decreased below 80% with an increase in inlet concentration of ethyl acetate. When the EBRT was increased to 75 s, the removal efficiency remained above 80% even though the inlet loading rate was increased to 421 g/m<sup>3</sup> h. Michaelis–Menten type and zero-order diffusion limited models were employed and the predicted data perfectly matched the experimental data. Thus *P. putida* immobilized on walnut shell has potential for the removal of ethyl acetate from air streams.

**Keywords:** Biofilter; Ethyl acetate; Walnut shell; *Pseudomonas putida*; Mathematical model

**Kelly J. Martin, Robert Nerenberg. (Department of Civil Engineering and Geological Sciences, University of Notre Dame, 156 Fitzpatrick Hall, Notre Dame, IN 46556, USA). The membrane biofilm reactor (MBfR) for water and wastewater treatment: Principles, applications, and recent developments. Bioresource Technology, Volume 122 (2012): 83–94**

The membrane biofilm reactor (MBfR), an emerging technology for water and wastewater treatment, is based on pressurized membranes that supply a gaseous substrate to a biofilm formed on the membrane's exterior. MBfR biofilms behave differently from conventional biofilms due to the counter-diffusion of substrates. MBfRs are uniquely suited for numerous treatment applications, including the removal of carbon and nitrogen when oxygen is supplied, and reduction of oxidized contaminants when hydrogen is supplied. Major benefits include high gas utilization efficiency, low energy consumption, and small reactor footprints. The first commercial MBfR was recently released, and its success may lead to the scale-up of other applications. MBfR development still faces challenges, including biofilm management, the design of scalable reactor configurations, and the identification of cost-effective membranes. If future research and development continue to address these issues, the MBfR may play a key role in the next generation of sustainable treatment systems.

**Keywords:** MBfR; MABR; Biofilm; Membrane biofilm reactor; Hydrogen

**Kangmin Chon<sup>a,1</sup>, Ho KyongShon<sup>b,2</sup>, Jaeweon Cho<sup>a</sup>.** (<sup>a</sup> School of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), 261 Cheomdangwagi-ro, Buk-gu, Gwangju 500-712, Republic of Korea, <sup>b</sup> School of Civil and Environmental Engineering, Faculty of Engineering and Information Technology, University of Technology, Sydney (UTS), Broadway, P.O. Box 123, Sydney, Australia). **Membrane bioreactor and nanofiltration hybrid system for reclamation of municipal wastewater: Removal of nutrients, organic matter and micropollutants. *Bioresource Technology*, Volume 122( 2012): 181–188**

A membrane bioreactor (MBR) and nanofiltration (NF) hybrid system was investigated to demonstrate the performance of treating nitrogen, phosphorus and pharmaceuticals and personal care products (PPCPs) in municipal wastewater. With the MBR and NF (molecular weight cut off (MWCO): 210 Da), the concentration of total nitrogen (TN) and total phosphorus (TP) was effectively reduced by nitrification by MBR and negatively charged surface of NF (TN: 8.67 mgN/L and TP: 0.46 mgP/L). Biosorption and microbial decomposition in MBR seem to be major removal mechanisms for the removal of PPCPs. Among various parameters affecting the removal of PPCPs by NF, namely, physicochemical properties of the PPCPs (charge characteristics, hydrophobicity and  $M_w$ ) and membranes (MWCO and surface charge), the MWCO effect was found to be the most critical aspect.

**Keywords:** Membrane bioreactor; Nanofiltration; Waste water reclamation; Nutrients; Micropollutants

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In the last few years, extensive research has been dedicated to development of a novel osmotic membrane bioreactor (OMBR), which combines high-retention osmotic separation and biological reactions in a single vessel. Although promising results have been reported in the literature, some challenges associated with applications of OMBR remain unresolved at the present stage of development, including lack of a high performance forward osmosis (FO) membrane, identification of an ideal draw solute and effect of salt accumulation on biological activity. Therefore, this paper attempts to provide a comprehensive review of state of the art of OMBR for water and wastewater reclamation.

**Keywords:** Osmosis membrane bioreactor; Forward osmosis membrane; Draw solute; Salt accumulation

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**Gongshang University, Hangzhou, Zhejiang 310018, PR China, <sup>c</sup> College of Environmental Science and Engineering, Hunan University, Changsha, Hunan 410082, PR China). Tubular biofilter for toluene removal under various organic loading rates and gas empty bed residence times. *Bioresource Technology*, Volume 121(2012) : 199–204**

A tubular biofilter (TBF) which consisted of a closed chamber, a polyurethane sponge tube and a nutrient solution distributor was developed and evaluated under organic loading rates (OL) ranging from 18.7 to 149.3 g m<sup>-3</sup> h<sup>-1</sup> and gas empty bed residence times (EBRTs) of 30–5.0 s. Using toluene as model VOC, the startup of the TBF lasted approximately 7 weeks. The removal efficiency decreased from 99% to 52.2% when OL was increased from 18.7 to 149.3 g toluene m<sup>-3</sup> h<sup>-1</sup> at 15 s, but did not decline significantly when the EBRT was reduced from 30 to 5.0 s at 18.7 g m<sup>-3</sup> h<sup>-1</sup>. Biomass concentration did not increase significantly within the sponge tube during the 391 days' operation as observed through the Plexiglas pipe of the TBF. The TBF is suitable for treating waste gases with low toluene concentrations even at high gas flow and over long periods.

**Keywords:** Biofiltration; Biomass; Toluene; Tubular media; Volatile organic compound

**Yuguang Wang<sup>a</sup>, Lijun Su<sup>b</sup>, Lijuan Zhang<sup>a</sup>, Weimin Zeng<sup>a, c</sup>, Junzi Wu<sup>a</sup>, Lili Wan<sup>a</sup>, Guanzhou Qiu<sup>a, c</sup>, Xinhua Chen<sup>d</sup>, Hongbo Zhou<sup>a, c</sup>. (<sup>a</sup> School of Minerals Processing and Bioengineering, Central South University, Changsha, China, <sup>b</sup> Department of Clinic Laboratory, Changsha Medical University, Changsha, China, <sup>c</sup> Key Laboratory of Biometallurgy, Ministry of Education, Changsha, China, <sup>d</sup> Key Laboratory of Marine Biogenetic Resources, State Oceanic Administration, Xiamen, China). Bioleaching of chalcopyrite by defined mixed moderately thermophilic consortium including a marine acidophilic halotolerant bacterium. *Bioresource Technology*, Volume 121(2012): 348–354**

A defined mixed moderately thermophilic consortium including three terrestrial microorganisms (*Leptospirillum ferriphilum*, *Acidithiobacillus caldus* and *Ferroplasma thermophilum*) and a marine acidophilic halotolerant bacterium (*Sulfobacillus* sp. TPY) was constructed to evaluate its ability for bioleaching of chalcopyrite with the addition of sodium chloride (NaCl), and the community dynamics was monitored by real-time quantitative PCR (qPCR). It was found that *Sulfobacillus* sp. TPY was able to tolerate 2% (w/v) NaCl, while other three microorganisms were suppressed when the concentration of NaCl was higher than 0.35%. The results suggested that NaCl below certain concentration could improve copper extraction by using pure cultures or the consortium to bioleach chalcopyrite. Community dynamics analysis during bioleaching at 0.1% NaCl showed that *Sulfobacillus* sp. TPY was predominant species during the whole bioleaching process, *L. ferriphilum* and *A. caldus* were less at any time compared with *Sulfobacillus* sp. TPY. *F. thermophilum* had never been dominant species even in the final stage.

**Keywords:** Moderately thermophiles; Marine acidophilic halotolerant bacterium; Bioleaching; Chalcopyrite; Community dynamics

**D. Radhika, A.G. Murugesan. (Manonmaniam Sundaranar University, Sri Paramakalyani Centre of Excellence in Environmental Sciences, Alwarkurichi 627412, Tamil Nadu, India). Bioproduction, statistical optimization and characterization of microbial plastic (poly 3-hydroxy butyrate) employing various hydrolysates of water hyacinth (*Eichhornia crassipes*) as sole carbon source. *Bioresource Technology*, Volume 121(2012): 83–92**

Saccharified water hyacinth hydrolysates (acid and enzyme hydrolysate) were used for the efficient production of poly (3-hydroxybutyrate) (PHB) via the *Cupriavidus necator* bacteria. The bacterium significantly utilizes the enzymatic hydrolyzate which gave the maximum PHB concentration ( $4.3 \pm 0.4 \text{ g L}^{-1}$ ), this was greatly exceeded the value of  $2 \pm 0.1 \text{ g L}^{-1}$  obtained from the acid hydrolysate amended media. Moreover, for the optimal PHB production, response surface methodology was used through central composite rotary design method which gave improved PHB concentration in microbial cells. After 72 h,  $35 \text{ g L}^{-1}$  of reducing sugar contained water hyacinth hydrolysate and  $1.5 \text{ g L}^{-1}$  of  $(\text{NH}_4)_2\text{SO}_4$  supplementation in laboratory scale fermentor gave  $12 \text{ g L}^{-1}$  of dry cell weight and  $7 \text{ g L}^{-1}$  of PHB. The produced PHB was characterized under FTIR, GPC and DSC instruments to find out the number average molecular mass, polydispersity index and melting temperature were  $1.7 \times 10^5 \text{ kDa}$ , 1.9 and  $170 \text{ }^\circ\text{C}$  respectively.

**Keywords:** Bioconversion; Water hyacinth; Glucose; Fermentation; Hydrolysates

**A.A. Jalil<sup>a</sup>, S. Triwahyono<sup>b</sup>, M.R. Yaakob<sup>a</sup>, Z.Z.A. Azmi<sup>a</sup>, N. Sapawe<sup>a</sup>, N.H.N. Kamarudin<sup>a</sup>, H.D. Setiabudi<sup>a</sup>, N.F. Jaafar<sup>b</sup>, S.M. Sidik<sup>b</sup>, S.H. Adam<sup>a</sup>, B.H. Hameed<sup>c</sup>.** (<sup>a</sup>Institute of Hydrogen Economy, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia, <sup>b</sup> Ibnu Sina Institute for Fundamental Science Studies, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia, <sup>c</sup> School of Chemical Engineering, Engineering Campus, Universiti Sains Malaysia, 14300 Nibong Tebal, Penang, Malaysia). **Utilization of bivalve shell-treated *Zea mays* L. (maize) husk leaf as a low-cost biosorbent for enhanced adsorption of malachite green. *Bioresource Technology*, Volume 120 (2012): 218–224**

In this work, two low-cost wastes, bivalve shell (BS) and *Zea mays* L. husk leaf (ZHL), were investigated to adsorb malachite green (MG) from aqueous solutions. The ZHL was treated with calcined BS to give the BS-ZHL, and its ability to adsorb MG was compared with untreated ZHL, calcined BS and  $\text{Ca}(\text{OH})_2$ -treated ZHL under several different conditions: pH (2–8), adsorbent dosage ( $0.25$ – $2.5 \text{ g L}^{-1}$ ), contact time (10–30 min), initial MG concentration ( $10$ – $200 \text{ mg L}^{-1}$ ) and temperature (303–323 K). The equilibrium studies indicated that the experimental data were in agreement with the Langmuir isotherm model. The use of  $2.5 \text{ g L}^{-1}$  BS-ZHL resulted in the nearly complete removal of  $200 \text{ mg L}^{-1}$  of MG with a maximum adsorption capacity of  $81.5 \text{ mg g}^{-1}$  after 30 min of contact time at pH 6 and 323 K. The results indicated that the BS-ZHL can be used to effectively remove MG from aqueous media.

**Keywords:** Low-cost adsorbent; Bivalve shell; *Zea mays* L.; Malachite green; Isotherm

**Lei Wang<sup>a</sup>, Richard Templar<sup>b</sup>, Richard J. Murphy<sup>a\*</sup>** (<sup>a</sup> Department of Life Science, Imperial College London, London SW7 2AZ, UK, <sup>b</sup> Department of Chemistry, Imperial College London, London SW7 2AZ, UK). **A Life Cycle Assessment (LCA) comparison of three management options for waste papers: Bioethanol production, recycling and incineration with energy recovery. *Bioresource Technology*, Volume 120(2012) :89–98**

This study uses Life Cycle Assessment (LCA) to assess the environmental profiles and greenhouse gas (GHG) emissions for bioethanol production from waste papers and to compare them with the alternative waste management options of recycling or incineration with energy

recovery. Bioethanol production scenarios both with and without pre-treatments were conducted. It was found that an oxidative lime pre-treatment reduced GHG emissions and overall environmental burdens for a newspaper-to-bioethanol process whereas a dilute acid pre-treatment raised GHG emissions and overall environmental impacts for an office paper-to-bioethanol process. In the comparison of bioethanol production systems with alternative management of waste papers by different technologies, it was found that the environmental profiles of each system vary significantly and this variation affects the outcomes of the specific comparisons made. Overall, a number of configurations of bioethanol production from waste papers offer environmentally favourable or neutral profiles when compared with recycling or incineration.

**Keywords:** Waste paper; Bioethanol; Life Cycle Assessment; Recycling; Incineration

**Sunil Kumar<sup>a</sup>, Anisha Mathur<sup>a</sup>, Varsha Singh<sup>a</sup>, Suchismita Nandy<sup>a</sup>, Sunil Kumar Khare<sup>b</sup>, Sangeeta Negi<sup>a\*</sup>** (<sup>a</sup> Department of Biotechnology, Motilal Nehru National Institute of Technology, Allahabad 211 004, Uttar Pradesh, India, <sup>b</sup> Department of Chemistry, IIT Delhi Hauz Khas, New Delhi 110 016, India). **Bioremediation of waste cooking oil using a novel lipase produced by *Penicillium chrysogenum* SNP5 grown in solid medium containing waste grease. *Bioresource Technology*, Volume 120 (2012): 300–304**

The aim of present work was to bioremediate the waste cooking oil using a novel lipase produced in solid medium containing waste grease and wheat bran by *Penicillium chrysogenum*. Enzyme extracted with phosphate buffer was purified 10.6 and 26.28-fold after 90% ammonium sulfate precipitation and ion-exchange chromatography, respectively. The partial characterization of enzyme revealed its  $K_m$  and  $V_{max}$  value for *p*-nitrophenolpamitate as 0.4 mM and 47.61 U/ml, respectively. The relative molecular mass of lipase was 40 kDa by SDS-PAGE and confirmed by zymogram. Purified lipase was most stable at 40 °C and at 8.0 pH. Lipase activity was enhanced by metal ions such as  $Mg^{2+}$ ,  $Fe^{2+}$ ,  $Ca^{2+}$  and non-ionic surfactant TritonX-100, while suppressed in the presence of SDS. Crude lipase was applied on cooking oil waste and the acid value was 26.92 mg/g. This showed that the enzyme could be employed for the bioremediation of used cooking oil.

**Keywords:** *Penicillium chrysogenum*; Grease; Lipase; Bioremediation; Used cooking oil

**Casey Forrestal<sup>a</sup>, Pei Xu<sup>b</sup>, Peter E. Jenkins<sup>c</sup>, Zhiyong Ren<sup>a\*</sup>** (<sup>a</sup> Department of Civil Engineering, University of Colorado Denver, Denver, CO 80004, USA, <sup>b</sup> Department of Civil and Environmental Engineering, Colorado School of Mines, Golden, CO 80401, USA, <sup>c</sup> Department of Mechanical Engineering, University of Colorado Denver, Denver, CO 80004, USA). **Microbial desalination cell with capacitive adsorption for ion migration control. *Bioresource Technology*, Volume 120 (2012): 332–336**

A new microbial desalination cell with capacitive adsorption capability (cMDC) was developed to solve the ion migration problem facing current MDC systems. Traditional MDCs remove salts by transferring ions to the anode and cathode chambers, which may prohibit wastewater beneficial reuse due to increased salinity. The cMDC uses adsorptive activated carbon cloth (ACC) as the electrodes and utilizes the formed capacitive double layers for electrochemical ion adsorption. The cMDC removed an average of 69.4% of the salt from the desalination chamber through electrode adsorption during one batch cycle, and it did not add salts to the anode or cathode chamber. It was estimated that 61–82.2 mg of total dissolved solids (TDS) was adsorbed

to 1 g of ACC electrode. The cMDC provides a new approach for salt management, organic removal, and energy production. Further studies will be conducted to optimize reactor configuration and achieve *in situ* electrode regeneration.

**Keywords:** Microbial desalination cell; Microbial fuel cell; Capacitive deionization; Salt management

**S. Venkata Mohan<sup>a</sup>, P. Suresh Babu<sup>a</sup>, K. Naresh<sup>a</sup>, G. Velvizhi<sup>a</sup>, Datta Madamwar<sup>b</sup>.** (<sup>a</sup>Bioengineering and Environmental Centre (BEEC), CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad 500 607, India, <sup>b</sup> BRD School of Biosciences, Sardar Patel University, Satellite Campus, Vallabh Vidyanagar 388 120, India). **Acid azo dye remediation in anoxic-aerobic-anoxic microenvironment under periodic discontinuous batch operation: Bio-electro kinetics and microbial inventory. *Bioresource Technology*, Volume 119 (2012): 362–372**

Functional behavior of anoxic-aerobic-anoxic microenvironment on azo dye (C.I. Acid black 10B) degradation was evaluated in a periodic discontinuous batch mode operation for 26 cycles. Dye removal efficiency and azo-reductase activity ( $30.50 \pm 1$  U) increased with each feeding event until 13th cycle and further stabilized. Dehydrogenase activity also increased gradually and stabilized ( $2.0 \pm 0.2$   $\mu\text{g/ml}$ ) indicating the stable proton shuttling between metabolic intermediates providing higher number of reducing equivalents towards dye degradation. Voltammetric profiles showed drop in redox catalytic currents during stabilized phase also supports the consumption of reducing equivalents towards dye removal. Change in Tafel slopes, polarization resistance and other bioprocess parameters correlated well with the observed dye removal and biocatalyst behavior. Microbial community analysis documented the involvement of specific organism pertaining to aerobic and facultative functions with heterotrophic and autotrophic metabolism. Integrating anoxic microenvironment with aerobic operation might have facilitated effective dye mineralization due to the possibility of combining redox functions.

**Keywords:** Sequencing batch reactor; Dehydrogenase activity; Cyclic voltammeter; Wastewater treatment; Tafel analysis

**J. Plaza Cazón<sup>a</sup>, C. Bernardelli<sup>a</sup>, M. Viera<sup>a</sup>, E. Donati<sup>a</sup>, E. Guibal<sup>b</sup>.** (<sup>a</sup> Centro de Investigación y Desarrollo en Fermentaciones Industriales, CINDEFI (CCT La Plata-CONICET, UNLP), Facultad de Ciencias Exactas, 50 y 115, (1900) La Plata, Argentina, <sup>b</sup> Ecole des Mines d'Alès, Laboratoire Génie de l'Environnement Industriel, 6, avenue de Clavières, F-30319 Alès cedex, France). **Zinc and cadmium biosorption by untreated and calcium-treated *Macrocystis pyrifera* in a batch system. *Bioresource Technology*, Volume 116 (2012): 195–203**

Zinc and cadmium can be efficiently removed from solutions using the brown algae, *Macrocystis pyrifera*. Treatment with  $\text{CaCl}_2$  allowed stabilization of the biosorbent. The maximum biosorption capacities in mono-component systems were  $0.91 \text{ mmol g}^{-1}$  and  $0.89 \text{ mmol g}^{-1}$  and the Langmuir affinity coefficients were  $1.76 \text{ L mmol}^{-1}$  and  $1.25 \text{ L mmol}^{-1}$  for Zn(II) and Cd(II), respectively. In two-component systems, Zn(II) and Cd(II) adsorption capacities were reduced by 50% and 40%, respectively and the biosorbent showed a preference for Cd(II) over Zn(II).  $\text{HNO}_3$  (0.1 M) and EDTA (0.1 M) achieved 90–100% desorption of both

ions from the loaded biomass. While HNO<sub>3</sub> preserved the biomass structure, EDTA destroyed it completely. Fourier transform infrared spectra identified the contribution of carboxylic, amine and sulfonate groups on Zn(II) and Cd(II) biosorption. These results showed that biosorption using *M. pyrifer*-treated biomass could be an affordable and simple process for cadmium and zinc removal from wastewaters.

**Keywords:** *Macrocystis pyrifer*; Adsorption; Desorption; Zinc; Cadmium

**C. Acharya, P. Chandwadkar, S.K. Apte. (Molecular Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India). Interaction of uranium with a filamentous, heterocystous, nitrogen-fixing cyanobacterium, *Anabaena torulosa*. Bioresource Technology, Volume 116 (2012): 290–294**

The filamentous, heterocystous, diazotrophic cyanobacterium, *Anabaena torulosa* was found to bind uranium from aqueous solutions containing 100 µM uranyl carbonate at pH 7.8. The uranyl sequestration kinetics exhibited (a) an initial rapid phase, binding 48% uranium within 30 min resulting in a loading of 56 mg U g<sup>-1</sup> of dry wt, followed by (b) a slower phase, binding 65% uranium with resultant loading of 77.35 mg U g<sup>-1</sup> in 24 h. Energy Dispersive X-ray fluorescence spectroscopy of uranium loaded biomass revealed all components of UL X-rays (UL<sub>1</sub>, UL<sub>α</sub>, UL<sub>β1</sub> and UL<sub>β2</sub>). Heat killed cells or extracellular polysaccharides derived from live cells exhibited limited uranyl binding (~ 26%) highlighting the importance of cell viability for optimum uranyl binding. The present study revealed the involvement of acid soluble polyphosphates in uranium accumulation by this brackish water cyanobacterium.

**Keywords:** Cyanobacterium; *Anabaena torulosa*; Uranium binding; Acid soluble polyphosphates

**Ping Yan<sup>a, b</sup>, Mang Lu<sup>c</sup>, Qin Yang<sup>d</sup>, Hai-Ling Zhang<sup>d</sup>, Zhong-Zhi Zhang<sup>a</sup>, Rong Chen<sup>e</sup>. (<sup>a</sup> State Key Laboratory of Heavy Oil Processing, China University of Petroleum, Beijing 102249, China, <sup>b</sup> Dalian Petrochemical Branch Company, PetroChina, Dalian 116000, China, <sup>c</sup> School of Materials Science and Engineering, Jingdezhen Ceramic Institute, Jingdezhen 333403, Jiangxi, China, <sup>d</sup> Oil & Gas Technology Research Institute, Changqing Oilfield Company, PetroChina, Xi'an 710018, China, <sup>e</sup> Beijing Green Oil Technology Services Co., Ltd., Beijing 100028, China). Oil recovery from refinery oily sludge using a rhamnolipid biosurfactant-producing *Pseudomonas*. Bioresource Technology, Volume 116 (2012): 24–28**

In this study, a rhamnolipid biosurfactant-producing strain, *Pseudomonas aeruginosa* F-2, was used to recover oil from refinery oily sludge in laboratory and pilot-scale experiments. The optimum values of carbon to nitrogen ratio, temperature, sludge–water ratio and inoculum size for oil recovery were determined as 10, 35 °C, 1:4 and 4%, respectively. An oil recovery of up to 91.5% was obtained with the equipping of draft tubes during the field pilot-scale studies. The results showed that strain F-2 has the potential for industrial applications and may be used in oil recovery from oily sludge.

**Keywords:** Biosurfactants; *Pseudomonas aeruginosa*; Oil recovery; Oily sludge

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Portugal, <sup>b</sup> CIMAR/CIIMAR, – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, R. dos Bragas 289, 4050-123 Porto, Portugal). Potential of *Phragmites australis* for the removal of veterinary pharmaceuticals from aquatic media. *Bioresource Technology*, Volume 116 (2012): 497–501

The potential of *Phragmites australis* was evaluated for the removal of three veterinary drugs, enrofloxacin (ENR), ceftiofur (CEF) and tetracycline (TET), from aquatic mediums.

Results showed that the plant promoted the removal of 94% and 75% of ENR and TET, respectively, from wastewater. Microbial abundance estimation revealed that microorganisms were not a major participant. Occurrence of drugs adsorption to plant roots was observed in small extension. Therefore, main mechanisms occurring were drug removal by plant uptake and/or degradation. Present results demonstrated the potential of *P. australis*-planted beds to be used for removal of pharmaceuticals from livestock and slaughterhouse industries wastewater.

**Keywords:** Veterinary drugs; Constructed wetlands; Wastewater; Emergent pollutants; Remediation

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The effects of different components of real dyeing bath formulations, such as the equalizing and fixing additives—acids, salts, and surfactants—on the decolorization catalyzed by *Funalia trogii* enzymatic extracts, were investigated to understand their influence on the recalcitrance to biodegradation of this type of wastewater. The decolorization of selected dyes and dye mixtures after tissue dyeing was performed in the presence/absence of auxiliary compounds. All spent dyeing baths were enzymatically decolorized to different extents, by the addition of extracts containing laccase only or laccase plus cellobiose dehydrogenase. Whereas surfactant auxiliaries, in some instances, inhibit the decolorization of spent dyeing baths, in several occurrences the acid/salt additives favor the enzymatic process. In general, the complete spent dyeing formulations are better degraded than those containing the dyes only. The comparison of extracellular extracts obtained from spent straws from the commercial growth of *Pleurotus* sp. mushrooms with those from *F. trogii* reveals similar decolorization extents thus allowing to further reduce the costs of bioremediation.

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of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430079, China, <sup>4</sup>Beijing Bioscience Research Center, Beijing 100085, China). **Impact of an indigenous microbial enhanced oil recovery field trial on microbial community structure in a high pour-point oil reservoir. Applied Microbiology and Biotechnology, Volume 95 (3) (2012): 811-821**

Based on preliminary investigation of microbial populations in a high pour-point oil reservoir, an indigenous microbial enhanced oil recovery (MEOR) field trial was carried out. The purpose of the study is to reveal the impact of the indigenous MEOR process on microbial community structure in the oil reservoir using 16Sr DNA clone library technique. The detailed monitoring results showed significant response of microbial communities during the field trial and large discrepancies of stimulated microorganisms in the laboratory and in the natural oil reservoir. More specifically, after nutrients injection, the original dominant populations of *Petrobacter* and *Alishewanella* in the production wells almost disappeared. The expected desirable population of *Pseudomonas aeruginosa*, determined by enrichment experiments in laboratory, was stimulated successfully in two wells of the five monitored wells. Unexpectedly, another potential population of *Pseudomonas pseudoalcaligenes* which were not detected in the enrichment culture in laboratory was stimulated in the other three monitored production wells. In this study, monitoring of microbial community displayed a comprehensive alteration of microbial populations during the field trial to remedy the deficiency of culture-dependent monitoring methods. The results would help to develop and apply more MEOR processes.

**Giovanni Colica, Stefania Caparrotta, Roberto De Philippis. (Department of Agricultural Biotechnology, University of Florence, Piazzale delle Cascine 24, 50144 Firenze, Italy. Email: roberto.dephilippis@unifi.it). Selective biosorption and recovery of Ruthenium from industrial effluents with *Rhodopseudomonas palustris* strains. Applied Microbiology and Biotechnology, Volume 95 (2) (2012): 381-387**

This study demonstrated for the first time the possibility to remove and partially recover the Ruthenium contained in industrial effluents by using purple non sulfur bacteria (PNSB) as microbial biosorbents. Up to date, the biosorption was only claimed as possible tool for the removal of the platinum-group metals (PGM) but the biosorption of Ru was never experimentally investigated. The PNSBs tested have adsorbed around 40 mg g (dry biomass)<sup>-1</sup> of the Ru contained in the real industrial effluents. At the end of the bioremoval experiments, the amount of Ru recovered from the biomass ranged from 42 % to 72 % of that adsorbed by PNSB, depending by the characteristics of the Ru effluent used. In any case, the use of microbial sorbents such as PNSB for the biosorption and recovery of Ru can be considered a way to reduce both the costs and the impact on the environment of the mining activities needed to obtain the increasing amounts of this rare and precious metal requested by the industrial activities related to its use.

**Yogesh M. Kolekar, Kisan M. Kodam. (Biochemistry Division, Department of Chemistry, University of Pune, Pune, 411007, India. Email: kodam@chem.unipune.ac.in). Decolorization of textile dyes by *Alishewanella* sp. KMK6. Applied Microbiology and Biotechnology, Volume 95 (2) (2012): 521-529**

*Alishewanella* sp. strain KMK6 was isolated from textile dye-contaminated soil. The strain was able to decolorize and degrade different azo dyes and displayed high dye degradation ability and tolerance. The bacterium could completely degrade 2.5 gl<sup>-1</sup> dye, Reactive Blue 59 within 6 h.

The induction in the level of cytochrome P-450 and activities of azoreductase and NADH-dichlorophenolindophenol reductase were observed in the cells after dye decolorization indicating the role of these enzymes. The intermediates of Reactive Blue 59 degradation were identified by high-performance liquid chromatography, gas chromatography and mass spectroscopy, and Fourier transform infrared spectroscopy. The ecotoxicity has been evaluated for dye and its metabolites by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (a yellow tetrazole) and comet assay, and it revealed that the dye degradation products were nontoxic.

**Sangeeta Negi, Sunil Kumar. (Biotechnology Section, Applied Mechanics Department, Motilal Nehru National Institute of Technology, Teliarganji, Allahabad, 211 004, Uttar Pradesh, India. Email: sn5@mnnit.ac.in). Evaluation of Techniques Used for Parameters Estimation: an Application to Bioremediation of Grease Waste. Applied Biochemistry and Biotechnology, Volume 167 (6) (2012): 1613-1621**

In the present work, evaluation of different conventional techniques, i.e., chemical oxygen demand (COD), biological oxygen demand, elemental analysis, Fourier transform infrared (FTIR), and gas chromatography used for estimating biodegradation of grease waste was carried out. In this order grease waste was incubated with *Penicillium chrysogenum* for 7–30 days and analyzed percentage of degradation. After 15 days of incubation, the percentages of reduction in COD, carbon, hydrogen, and nitrogen content of grease waste were found 28, 53.5, 12.7, and 0, respectively. Further it was analyzed by FTIR and gas chromatography–mass spectroscopy (GC-MS) and observed that bifurcated peaks of grease waste at 2,926 and 2,855  $\text{cm}^{-1}$  had reduced remarkably, which corresponds to aliphatic hydrocarbons, while new broad peaks appeared at 3,400  $\text{cm}^{-1}$  indicating addition of oxygen molecule to reduced aliphatic hydrocarbon. GC-MS study also supports the results of FTIR, COD, and elemental analysis, but quantification of the percentage of degradation was difficult and limited to volatile organic content, while COD and elemental analysis were found more accurate and more informative. The current study would be helpful in the estimation of biodegradability not only of grease waste but also of other complex nonbiodegradable compounds polluting the environment.

**Ana Cristina<sup>1</sup> Borba da Cunha<sup>1</sup>, Sydney Sabedot<sup>1</sup>, Carlos Hoffmann Sampaio<sup>2</sup>, Claudete Gindri Ramos<sup>1</sup>, Alexandre Rodrigues da Silva<sup>1</sup>. (<sup>1</sup>Centro Universitário La Salle, Unilasalle, Rua Victor Barreto 2288, CEP 92010-000 Canoas, Brazil, <sup>2</sup>Universidade Federal do Rio Grande do Sul, UFRGS, P.O. Box 15021, CEP 91501-970 Porto Alegre, Brazil. Email: anacunha@unilasalle.edu.br). *Salix rubens* and *Salix triandra* Species as Phytoremediators of Soil Contaminated with Petroleum-Derived Hydrocarbons. Water, Air, & Soil Pollution, Volume 223 (8) (2012): 4723-4731**

The petroleum industry activities provide potential risks to the environment because they can contaminate ecosystems with different organic compounds in the production chain. Several accidents with transport and handling of petroleum and related products occurred in urban areas with harmful effects to the quality of life and economy. In the 1990s, bioremediation and phytoremediation technologies as economically feasible alternatives to repair the environmental damage were developed. In this study, the potential of the willows *Salix rubens* and *Salix triandra* were evaluated with regard to the phytoremediation of soils contaminated with petroleum-derived hydrocarbons (total hydrocarbons and polycyclic aromatic hydrocarbons

(PAHs)). The PAHs were quantified by extraction from soils and plants using dichloromethane under ultrasonication. The HPLC analysis was performed with GC/MSD equipment. The total hydrocarbons present in uncontaminated soil were quantified by the sum of animal/vegetable oils and greases and mineral oils and greases according to Standard Methods 5520 (1997). The two willows species *S. rubens* and *S. triandra* were resistant during the project development. In the contaminated soil, in which both species were planted, the total hydrocarbons concentration was reduced near 98 %. The PAHs content was remarkably reduced as well. Pyrene showed an initial concentration of  $23.06 \mu\text{g kg}^{-1}$ , decreasing in most cases to  $0.1 \mu\text{g kg}^{-1}$  or to undetectable levels. Chrysene decreased from  $126.27 \mu\text{g kg}^{-1}$  to undetectable levels. Benzo[k]fluoranthene and benzo[a]pyrene concentrations had also showed a decrease from 28.44 and  $3.82 \mu\text{g kg}^{-1}$ , respectively, to undetectable levels.

**Mahendra Aryal, Maria G. Ziagova, Maria Liakopoulou-Kyriakides. (Department of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece. Email: markyr@eng.auth.gr). Cu(II) Biosorption and Competitive Studies in Multi-ions Aqueous Systems by *Arthrobacter* sp. Sphe3 and *Bacillus sphaericus* Cells: Equilibrium and Thermodynamic Studies. Water, Air, & Soil Pollution, Volume 223 (8) (2012): 5119-5130**

*Arthrobacter* sp. Sphe3 and *Bacillus sphaericus* cells were used for Cu(II) biosorption. The effect of contact time, biosorbent dose, equilibrium pH, temperature and the presence of other ions on the efficiency of the process were extensively studied. Optimum pH value and biomass concentration were determined at 5.0 and 1.0 g/l, whereas contact time was found to be 5 and 10 min for *Arthrobacter* sp. Sphe3 and *Bacillus sphaericus* biomass, respectively. Equilibrium data fitted very well to Freundlich model ( $R^2 = 0.996$ ,  $n = 2.325$ ,  $K_f = 8.141$ ) using *Arthrobacter* sp. Sphe3. In the case of *B. sphaericus*, a Langmuir adsorption model [ $R^2 = 0.996$ ,  $Q_{\max} = 51.54 \text{ mg-Cu(II)/g}$ ] showed to better describe the results. Potentiometric titration and Fourier transform infrared (FTIR) spectroscopy showed that amine, carboxyl and phosphate groups participate in Cu(II)-binding. The calculated thermodynamic parameters indicated the spontaneous and feasible nature of Cu(II) biosorption on both biosorbents. Selectivity of Cu(II) biosorption was examined in binary and multi-ions systems with various anions and cations which are commonly found in municipal and industrial wastewater. A specificity towards Cu(II) was observed in binary mixtures with  $\text{Cl}^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{Mg}^{+2}$  and  $\text{Ca}^{+2}$ , and As(V) with the maximum uptake capacity remaining constant even at high competitive ion's concentrations of 200 mg/l. Desorption studies showed that Cu(II) could be completely desorbed from Cu(II)-loaded *Arthrobacter* strain Sphe3 and *B. sphaericus* biomass using 1.0 and 0.8 M HCl, respectively, and both bacterial species could be effectively reused up to five cycles, making their application in wastewater detoxification more attractive.

**Guoxin Huang<sup>1,2</sup>, Howard Fallowfield<sup>3</sup>, Huade Guan<sup>3</sup>, Fei Liu<sup>1</sup>. (<sup>1</sup>Beijing Key Laboratory of Water Resources & Environmental Engineering, China University of Geosciences (Beijing), Beijing, 100083, China, <sup>2</sup>China Meat Research Center, Beijing Academy of Food Sciences, Beijing, 100068, China, <sup>3</sup>School of the Environment, Flinders University, Sturt Road, Bedford Park, SA,, 5001, Australia. Email: Feiliu@cugb.edu.cn). Remediation of Nitrate-Nitrogen Contaminated Groundwater by a Heterotrophic-Autotrophic Denitrification Approach in an Aerobic Environment. Water, Air, & Soil Pollution, Volume 223 (7) (2012): 4029-4038**

A novel heterotrophic-autotrophic denitrification (HAD) approach supported by mixing granulated spongy iron, methanol, and mixed bacteria was proposed for the remediation of nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) contaminated groundwater in a dissolved oxygen (DO)-rich environment. The HAD process involves biological deoxygenation, chemical reduction (CR) of  $\text{NO}_3\text{-N}$  and DO, heterotrophic denitrification (HD), and autotrophic denitrification (AD). Batch experiments were performed to: (1) investigate deoxygenation capacities of HAD; (2) determine the contributions of AD, HD, and CR to the overall  $\text{NO}_3\text{-N}$  removal in the HAD; and (3) evaluate the effects of environmental parameters on the HAD. There were 174, 205, and 2,437 min needed to completely reduce DO by the HAD, spongy iron-based CR, and by the mixed bacteria, respectively. The HAD depended on abiotic and biotic effects to remove DO. CR played a dominant role in deoxygenation in the HAD. After 5 days, approximately 100, 63.0, 20.1, and 9.7 % of the initial  $\text{NO}_3\text{-N}$  was removed in the HAD, HD, AD + CR, and CR incubations, respectively. CR, HD, and AD all contributed to the overall  $\text{NO}_3\text{-N}$  removal in the HAD. HD was the most important  $\text{NO}_3\text{-N}$  degradation mechanism in the HAD. There existed symbiotic, synergistic, and promotive effects of CR, HD, and AD within the HAD. The decrease in  $\text{NO}_3\text{-N}$  and the production of nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ) and ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) in the HAD were closely related to the C to N weight ratio. The C to N ratio of 3.75:1 was optimal for complete denitrification. Denitrification rate at 27.5°C was 1.36 times higher than at 15.0°C.

**Jian Lu<sup>1,2</sup>, Jun Wu<sup>1</sup>, Tianhu Chen<sup>1</sup>, P. Chris Wilson<sup>2</sup>, Jiazhong Qian<sup>1</sup>, Xiangyang Hao<sup>3</sup>, Chang Liu<sup>1</sup>, Yu Su<sup>1</sup> and Xin Jin<sup>1</sup>.** (<sup>1</sup>School of Resources and Environmental Engineering, Hefei University of Technology, Hefei, 230009, Anhui Province, People's Republic of China, <sup>2</sup>Indian River Research and Education Center, University of Florida, Fort Pierce, FL 34945-3138, USA, <sup>3</sup>School of Material and Chemical Engineering, China University of Geosciences, Beijing, 100083, People's Republic of China). **Valuable Metal Recovery During the Bioremediation of Acidic Mine Drainage Using Sulfate Reducing Straw Bioremediation System. *Water, Air, & Soil Pollution*, Volume 223 (6) (2012): 3049-3055**

Recovery of valuable metals from acidic mine drainage (AMD) during bioremediation using straw bioremediation system was investigated, with observation of efficient metal recovery. The recovery loading rates of Cu and Zn were  $46.19 \pm 6.13$  and  $43.86 \pm 6.76 \text{ mg m}^{-3} \text{ h}^{-1}$ , respectively. More than 97.0% of Cu and more than 87.0% of Zn were recovered from AMD during bioremediation. The recovery loading rate of Cu increased by  $4.54 \text{ mg m}^{-3} \text{ h}^{-1}$  for each  $1 \text{ mg L}^{-1}$  increase in influent concentration while that of Zn increased by  $4.08 \text{ mg m}^{-3} \text{ h}^{-1}$ . Heavy metal toxic effect on the metal recovery in the straw bioremediation system could be neglected in most cases. Low pH could severely decrease recovery rate of Zn, while it had no influence on that of Cu. The recovery loading rate of Zn decreased by almost 70% when the influent pH decreased from 7.0 to 3.0. Cu could be recovered efficiently even at a short hydraulic residence time (HRT) of 18 h, while Zn could only be efficiently recovered at a relatively long HRT of 80 h, indicating that selective metal recovery might be achieved with relatively short HRT and bioreactor process optimization. These findings suggested the feasibility of using sulfate-reducing straw bioremediation system to recover valuable metals during bioremediation of AMD

**Budhadev Basumatary<sup>1</sup>, Sabitry Bordoloi<sup>1</sup>, Hari Prasad Sarma<sup>2</sup>.** (<sup>1</sup>Life Science Division, Institute of Advanced Study in Science and Technology, Guwahati, 781035, India,

**<sup>2</sup>Environmental Science Department, Gauhati University, Guwahati, 781014, India). Crude Oil-Contaminated Soil Phytoremediation by Using *Cyperus brevifolius* (Rottb.) Hassk. Water, Air, & Soil Pollution, Volume 223 (6) (2012): 3373-3383**

The degradation of total oil and grease (TOG) in crude oil-contaminated soil in the presence of *Cyperus brevifolius* (Rottb.) Hassk was investigated in a net house study. *C. brevifolius* plants were transplanted in to spiked soil containing 8% (w/w) crude oil. The capability of plant for enhancing the biodegradation process was tested in pots containing fertilized and unfertilized soil over a 360-day period. Analysis of the degradation of hydrocarbon contaminants, plant growth, and biomass was conducted at 60-day interval. In the presence of contaminants, plant biomass and height were significantly reduced. The specific root surface area was reduced under the effects of crude oil. Concerning TOG content in soil, *C. brevifolius* could decrease up to 86.2% in TA (crude oil-contaminated soil with fertilizer) and 61.2% in TC (crude oil-contaminated soil without fertilizer). In the unvegetated pots, the reduction of TOG was 13.7% in TB (crude oil-contaminated soil with fertilizer) and 12.5% in TD (crude oil-contaminated soil without fertilizer). However, biodegradation was significantly more in vegetated pots than in unvegetated pots ( $p = 0.05$ ). The addition of fertilizer had positive effect on TOG degradation in the presence of *C. brevifolius* compared to the unfertilized treatments. Thus, there was evidence of *C. brevifolius* enhancing the biodegradation of crude oil in soil under the conditions of this experiment.

**Einstine Opiso<sup>1,2</sup>, Atsushi Asai<sup>1,3</sup>, Tsutomu Sato<sup>1</sup>, Tetsuro Yoneda<sup>1</sup>, Xiaoji Liu<sup>1</sup>. (<sup>1</sup>Life Science Division, Institute of Advanced Study in Science and Technology, Guwahati, 781035, India, <sup>2</sup>Environmental Science Department, Gauhati University, Guwahati, 781014, India, Laboratory of Environmental Geology, Graduate School of Engineering, Hokkaido University, Sapporo 060-8628, Japan, <sup>2</sup>Present address: Geo-environmental Engineering Group, College of Engineering, Central Mindanao University, Musuan, Bukidnon, 8710, Philippines, <sup>3</sup>Present address: ECO-SYSTEM OKAYAMA CO., Ltd. 3-1, 1-chome, Kaigandori, Minami-ku, Okayama-City Okayama, 702-8506, Japan). Sorption Behavior of Arsenate by Mg-Bearing Minerals at Hyperalkaline Condition: Implications for Oxyanions Sequestration During the Use and Disposal of Alkaline Wastes. Water, Air, & Soil Pollution, Volume 223 (6) (2012): 3471-3483**

The utilization and disposal of alkaline waste materials such as slag and coal fly ash as cement aggregates and raw materials in cement manufacturing can pose environmental and health hazards because these waste materials usually contain elevated concentration of toxic elements. This study examined the possibility of controlling the pore water chemistry of these waste materials in order to induce the secondary mineral formation of Mg-bearing minerals as major sorbing solids for oxyanions during the utilization and disposal of alkaline wastes. The formation of Mg-bearing minerals was examined at ambient temperature and alkaline pH conditions in the Mg–Si–Al system. The interaction of Mg-bearing minerals with oxyanions using arsenate as an analog was examined during and after mineral formation. The results revealed that the generated Mg-bearing mineral phases were smectite and brucite in Mg–Si system and hydrotalcite and serpentine in Mg–Si–Al system. Moreover, hydrotalcite, serpentine, brucite, and smectite phases formed under low Si ratio showed high sorption capacity for arsenate, but only high Al content hydrotalcite and serpentine showed substantial irreversible fraction of sorbed arsenate. Hence, the generation of these kinds of hydrotalcite and serpentine phases as scavengers for oxyanions must be considered during the utilization and disposal of alkaline wastes.

**Ashutosh Kumar Verma, Chandralata Raghukumar, Rajesh Ramnath Parvatkar, Chandrakant Govind Naik. (National Institute of Oceanography (Council of Scientific and Industrial Research), Dona Paula, Goa, 403-004, India). A Rapid Two-Step Bioremediation of the Anthraquinone Dye, Reactive Blue 4 by a Marine-Derived Fungus. Water, Air, & Soil Pollution, Volume 223 (6) (2012): 3499-3509**

A rapid two-step technique for bioremediation of the anthraquinone dye, the Reactive Blue 4 (RB4) by a marine-derived fungus is reported here. In the first step, 1,000 mg L<sup>-1</sup> of this dye treated with partially purified laccase of this fungus resulted in 61 % color removal and twofold decrease in chemical oxygen demand by 12 h. The metabolites formed during the enzymatic degradation were characterized by mass spectrometry, ultra performance liquid chromatography, and UV/visible spectroscopy. These analyses confirmed changes in the aromatic character of the parent dye and formation of low molecular weight phenolic compounds as the final products of the enzymatic degradation. Based on these results, the probable degradation products of RB4 were 2-formylbenzoic acid, 1,2,4,5-tetrahydroxy-3-benzoic acid, 2,3,4-trihydroxybenzenesulfonic acid, and 1,2,3,4-pentahydroxybenzene. In the second step, the enzyme-transformed dye solution subjected to sorption on the powdered fungal biomass resulted in a further reduction in color up to 93 % within 10 min. Sorption of the degraded dye was confirmed by the changes in the pattern of Fourier transform infrared spectroscopy spectrum. The two-step treatment resulted in a decrease of 29 % in total carbon accompanied by twofold decrease in toxicity. This is the first report on decolorization, detoxification, and mineralization of RB4 by laccase from a marine-derived fungus.

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Cyanobacteria have been found to be potential biosorbents of metal ions from waste water. The Pb<sup>2+</sup> removal capacity of growing cells of indigenous cyanobacterium *Oscillatoria laete-virens* (Crouan and Crouan) Gomont was studied under batch experiments and it was found capable of removing Pb<sup>2+</sup> of lower concentrations (below 100 mg L<sup>-1</sup>). The effects of different concentrations of Pb<sup>2+</sup>, on the growth rate of alga were also evaluated. The research parameters include the pH of the solution, contact time, initial concentration of Pb<sup>2+</sup>, and culture density. Of the parameters studied, the pH of the solution was found to be the most crucial. The removal of Pb<sup>2+</sup> peaked at an initial pH of 5. The data obtained from the equilibrium experiments were found well fitting with the Langmuir isotherm with a maximum sorptive capacity ( $q_{max}$ ) of 20.36 mg g<sup>-1</sup>, indicating a good biosorbitive potential of growing cells. This was confirmed using scanning electron microscope and energy dispersive X-ray analysis, which showed the adsorption of lead on the surface of the cell. The species could tolerate a concentration as high as 60 mg L<sup>-1</sup> of Pb<sup>2+</sup>. It was observed that the removal obeyed the pseudo-second-order kinetics. The percentage removal was found to decrease with increasing metal concentration, from 10 to 100 mg L<sup>-1</sup>. FTIR analysis indicates the involvement of amino, carboxylic and amide groups in

the sorption process. Among the desorbing agents evaluated, an efficient recovery of 90.2 % was achieved by HCl, in 24 h. Thus *Oscillatoria laete-virens* (Crouan and Crouan) Gomont seems to be a promising metal biosorbent for the treatment of  $Pb^{2+}$ , in waste waters.

**Madhumita Talukdar<sup>1</sup>, Aparajita Duarah<sup>1</sup>, Shruti Talukdar<sup>1</sup>, Manorama Bura Gohain<sup>1</sup>, Rajal Debnath<sup>1</sup>, Archana Yadav<sup>1</sup>, Dhruva K. Jha<sup>2</sup>, Tarun C. Bora<sup>1</sup>.** (<sup>1</sup>Biotechnology Division, CSIR-North East Institute of Science and Technology, Jorhat, 785006, India, <sup>2</sup>Microbial Ecology Laboratory, Department of Botany, Gauhati University, Guwahati, 781014, India. Email: tcbora@yahoo.com). **Bioprospecting *Micromonospora* from Kaziranga National Park of India and their anti-infective potential. World Journal of Microbiology and Biotechnology, Volume 28 (8) (2012): 2703-2712**

Large number of strains was isolated from soils of Kaziranga National Park of North-East India using selective isolation procedure. They were assigned to the genus *Micromonospora* on the basis of their typical colonial and pigmentation features. The taxonomic identities of the isolates were confirmed on the basis of their molecular characters (16SrDNA). A total of one hundred *Micromonospora* strains were isolated during the present investigation. The diagnostic cell wall sugar and amino acids were determined from these *Micromonospora* strains. After preliminary screening most of the isolates exhibited excellent anti-infective activity against human bacterial pathogens *Staphylococcus aureas*, *Bacillus subtilis*, *Proteus vulgaris*, *Echerichia coli*, *Pseudomonas aeruginosa* and fungal pathogens *Aspergillus niger*, *Fusarium oxysporum* and *Candida albicans*. Among these isolates one strain designated as HK-10 showed promising activity against human pathogens *S. aureas*, *B. subtilis*, *P. vulgaris* and *P. aeruginosa*.

**Sibel Yalçın, Semih Sezer, Reşat Apak. (Faculty of Engineering, Chemistry Department, Istanbul University, Avcılar, 34320 Istanbul, Turkey. Email: sibelyal@istanbul.edu.tr). Characterization and lead(II), cadmium(II), nickel(II) biosorption of dried marine brown macro algae *Cystoseira barbata*. Environmental Science and Pollution Research, Volume 19 (8) (2012): 3118-3125**

The objectives of this research are to identify the functional groups and determine corresponding  $pK_a$  values of the acidic sites on dried brown algae *Cystoseira barbata* using FTIR and potentiometric titrations, and to investigate the biosorption ability of biomass towards divalent nickel, cadmium, and lead ions. Adsorption was studied as a function of solution pH and contact time, and experimental data were evaluated by the Langmuir isotherm model.  $CaCl_2$  pretreatment was applied to the sorbent for enhancing the metal uptake capacity. The effect of solution pH on biosorption equilibrium was investigated in the pH range of 1.5–5.0. Individual as well as competitive adsorption capacity of the sorbent were studied for metal cations and mixtures. The retention of the tested metal ions was mostly influenced from pH in the range of 1.5–2.5, then stayed almost constant up to 5.0, while Ni(II) uptake showed the highest variation with pH. Potentiometric titrations were performed to find the number of strong and weak acidic groups and their acidity constants. The density of strong and weak acidic functional groups in the biomass were found to be 0.9 and 2.26 mmol/g, respectively. The FTIR spectra of the sorbent samples indicated various functionalities on the biomass surface including carboxyl, hydroxyl, and amino and sulphonate groups which are responsible for the binding of metal ions. The capacity of the biomass for single metal ions (around 1 mmol/g) was increased to 1.3 mmol/g in competitive adsorption, Pb(II) showing the highest Langmuir intensity

constant. Considering its extremely high abundance and low cost, *C. barbata* may be potentially important in metal ion removal from contaminated water and industrial effluents.

**Linda B. L. Lim<sup>1</sup>, Namal Priyantha<sup>2</sup>, D. T. B. Tennakoon<sup>1</sup>, Muhd Khairud Dahri<sup>1</sup>.** (<sup>1</sup>Department of Chemistry, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE 1410 Negara, Brunei Darussalam, <sup>2</sup>Department of Chemistry, Univerisity of Peradeniya, Peradeniya, Sri Lanka. Email: namal.priyantha@yahoo.com). **Biosorption of cadmium(II) and copper(II) ions from aqueous solution by core of *Artocarpus odoratissimus*. Environmental Science and Pollution Research, Volume 19 (8) (2012): 3250-3256**

This research is on the evaluation of biosorption capability of the core of *Artocarpus odoratissimus* (Tarap), grown in Brunei Darussalam, towards Cd(II) and Cu(II) ions present in synthetic solutions, and to characterize the surface of Tarap particles. Thermogravimetric analysis and surface titrations were conducted to characterize the surface of dried Tarap core particles. Atomic absorption spectroscopic measurements were conducted to determine the extent of removal of Cd(II) and Cu(II) under different experimental conditions. Mass reductions associated with many exothermic reaction peaks were observed beyond 200°C up to 650°C indicating the combustion of organic matter in Tarap. Dried particles of core of Tarap bear a negative surface charge promoting strong interaction towards positively charged ions, such as Cu(II) and Cd(II). Biosorption of the two metal ions on Tarap, which is relatively high beyond pH = 4, occurs within a short period of exposure time. The extent of biosorption is enhanced by acid treatment of the biosorbent, and further it does not significantly depend on the presence of nonreacting ions up to an ionic strength of 2.0 M. Strong attraction between each metal ion and the biosorbent is attributed to the negative surface charge on the biosorbent within a broad pH range. Acid treatment of the biosorbent improves sorption characteristics, suggesting that ion exchange plays an important role in the metal ion—biosorbent interaction process.

**Yunhai Wu<sup>1</sup>, Yajun Wen<sup>2</sup>, Jianxin Zhou<sup>2</sup>, Qi Dai<sup>2</sup>, Yunying Wu<sup>3</sup>.** (<sup>1</sup>Key Laboratory of Integrated Regulation and Resources Development of Shallow Lakes, Ministry of Education, Hohai University, Nanjing, 210098, China, <sup>2</sup>College of Environment, Hohai University, Nanjing, 210098, China, <sup>3</sup>Department of Chemistry, Hanshan Normal University, Chaozhou, China. Email: wenkevin@hhu.edu.cn). **The characteristics of waste *Saccharomyces cerevisiae* biosorption of arsenic(III). Environmental Science and Pollution Research, Volume 19 (8) (2012): 3371-3379**

The potential of using waste *Saccharomyces cerevisiae* as adsorbent for the adsorption of As(III) from aqueous solution was assessed. The biosorbent was characterized by Fourier transform infrared (FTIR) spectroscopy analysis. Various parameters including pH, biosorbent dosage, contact time, and temperature were systematically investigated. The FTIR results of *S. cerevisiae* biomass showed that biomass has different functional groups, and these functional groups are able to react with metal ion in aqueous solution. Several biosorption isotherms were used to fit the equilibrium data, showing sorption to be monolayer on the heterogeneous surface of the biosorbent. The maximum biosorption capacity calculated using Langmuir model was found to be 62.908 µg/g at pH 5.0, biosorbent dosage 5 g/L, contact time 240 min, and temperature 35 °C. The kinetic studies indicated that the biosorption process of the As(III) followed well the pseudo-second-order equation. The intraparticle diffusion and Richenberg

models were applied to the data, and we found that the biosorption of As(III) was governed by film diffusion followed by intraparticle diffusion. The thermodynamics constants indicated that the biosorption of As(III) onto *S. cerevisiae* was spontaneous and endothermic under examined conditions. Biosorbent could be regenerated using 0.5 M NaOH solution, with up to 75 % recovery.

**Hanzhong Jia<sup>1</sup>, Cheng Gu<sup>2</sup>, Hui Li<sup>2</sup>, Xiaoyun Fan<sup>1</sup>, Shouzhu Li<sup>1</sup>, Chuanyi Wang<sup>1</sup>.** (<sup>1</sup>Laboratory of Eco-Materials and Sustainable Technology (LEMST), Xinjiang Technical Institute of Physics & Chemistry, Chinese Academy of Sciences, Urumqi, Xinjiang, 830011, China, <sup>2</sup>Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48823, USA. Email: jiahz@ms.xjb.ac.cn). **Effect of groundwater geochemistry on pentachlorophenol remediation by smectite-templated nanosized Pd<sup>0</sup>/Fe<sup>0</sup>. Environmental Science and Pollution Research, Volume 19 (8) (2012): 3498-3505**

Zero-valent iron holds great promise in treating groundwater, and its reactivity and efficacy depend on many surrounding factors. In the present work, the effects of solution chemistry such as pH, humic acid (HA), and inorganic ions on pentachlorophenol (PCP) dechlorination by smectite-templated Pd<sup>0</sup>/Fe<sup>0</sup> were systematically studied. Smectite-templated Pd<sup>0</sup>/Fe<sup>0</sup> was prepared by saturating the negatively charged sites of smectite clay with Fe(III) and a small amount of Pd(II), followed by borohydride reduction to convert Fe(III) and Pd(II) into zero-valent metal clusters. Batch experiments were conducted to investigate the effects of water chemistry on PCP remediation. The PCP dechlorination rate critically depends on the reaction pH over the range 6.0~10.0; the rate constant ( $k_{obs}$ ) increases with decreasing the reaction pH value. Also, the PCP remediation is inhibited by HA, which can be attributed to the electron competition of HA with H<sup>+</sup>. In addition, the reduction of PCP can be accelerated by various anions, following the order: Cl<sup>-</sup> > HCO<sub>3</sub><sup>-</sup> > SO<sub>4</sub><sup>2-</sup> ~no anion. In the case of cations, Ca<sup>2+</sup> and Mg<sup>2+</sup> (10 mM) decrease the dechlorination rate to 0.7959 and 0.7798 from 1.315 h<sup>-1</sup>, respectively. After introducing HA into the reaction systems with cations or/and anions, the dechlorination rates are similar to that containing HA alone. This study reveals that low pH and the presence of some anions such as Cl<sup>-</sup> facilitate the PCP dechlorination and induce the rapid consumption of nanosized zero-valent iron simultaneously. However, the dechlorination rate is no longer correlated to the inhibitory or accelerating effects by cations and anions in the presence of 10 mg/L HA.

**Nabeel Khan Niazi<sup>1,4</sup>, Balwant Singh<sup>1</sup>, Lukas Van Zwieten<sup>2</sup>, Anthony George Kachenko<sup>3</sup>.** (<sup>1</sup>Faculty of Agriculture Food and Natural Resources, The University of Sydney, Sydney, NSW, 2006, Australia, <sup>2</sup>Wollongbar Primary Industries Institute, NSW Department of Primary Industries, Wollongbar, NSW, 2477, Australia, <sup>3</sup>Nursery and Garden Industry Australia, Epping, Sydney, NSW, 2121, Australia, <sup>4</sup>Present address: Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Faisalabad, 38000, Pakistan. Email: nabeelkniazi@gmail.com). **Phytoremediation of an arsenic-contaminated site using *Pteris vittata* L. and *Pityrogramma calomelanos* var. *austroamericana*: a long-term study. Environmental Science and Pollution Research, 19 (8) (2012): 3506-3515**

This field study investigated the phytoremediation potential of two arsenic (As) hyperaccumulating fern species, *Pityrogramma calomelanos* var. *austroamericana* and *Pteris vittata* over 27-month duration at a disused As-contaminated cattle-dip site located at Wollongbar, NSW, Australia. Ferns planted in January 2009 were harvested following 10, 22 and 27 months of growth. A detailed soil sampling was undertaken in June 2009 (initial,  $n = 42$

per plot) and limited sampling in April 2011 (after 27 months,  $n = 15$  per plot) to measure total and phosphate-extractable As concentrations in soil at 0 – 20-, 20 – 40- and 40 – 60-cm depths. The choice of the limited number of samples was considered sufficient to estimate the changes in soil As concentration following phytoremediation based on a geostatistical model. The average frond dry biomass, As concentration and As uptake were significantly ( $P < 0.001 - 0.05$ ) greater in *P. calomelanos* var. *austroamericana* than *P. vittata*, at all three harvests (1.6 – 4.3, 1.3 – 1.5 and 2.2–5.7 times, respectively). After 27-months of growth, *P. calomelanos* var. *austroamericana* removed 8,053 mg As (i.e. cumulative over three harvests) in plot B (25.4 kg As ha<sup>-1</sup>) that was 2.65 times higher than that depleted by *P. vittata* (3,042 mg As in plot A (9.7 kg As ha<sup>-1</sup>)). The cumulative frond As uptake data of the two fern species revealed that *P. calomelanos* var. *austroamericana* extracted 1.7–3.9 % and *P. vittata* removed 0.53–1.5 % of total As from soil at three depths. However, for the surface (0–20 cm) and subsurface (40–60 cm) layers, the (post-experiment) soil As data indicated that total As concentration in soil was reduced by 49 and 63 % ( $P < 0.05$ ), respectively, using *P. calomelanos* var. *austroamericana*; and 17 and 15 % ( $P > 0.05$ ), respectively, by *P. vittata*. Our results show that phytoremediation time based on observed changes in soil As based on limited sampling is not reliable; hence, it is recommended that the frond As uptake should be considered in order to evaluate the phytoremediation efficiency of the two fern species at the experimental site. Using As uptake of the two fern species, we estimate that with *P. calomelanos* var. *austroamericana* it would take 55–125 years to decrease mean total As content below the ecological investigation level (20 mg kg<sup>-1</sup>) in the surface and subsurface soils, whereas with *P. vittata* 143–412 years would be required to achieve this target.

**Guang-Jie Zhou, Fu-Qiang Peng, Li-Juan Zhang, Guang-Guo Ying. (State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, 510640, People's Republic of China. Email: guangguo.ying@gmail.com). Biosorption of zinc and copper from aqueous solutions by two freshwater green microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. Environmental Science and Pollution Research, Volume 19 (7) (2012): 2918-2929**

The objective of this study was to determine the removal of zinc and copper by two freshwater green microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliquus* and to investigate changes of algal ultrastructure and photosynthetic pigment. Algal cells were exposed for 8 days to different initial zinc or copper concentrations. Heavy metal concentrations were detected by an atomic absorption spectrophotometer. Algal growth, ultrastructure, and photosynthetic pigment were analyzed by a microplate reader, transmission electron microscope, and spectrophotometer, respectively. Low zinc and copper concentrations induced increase in algal growth, whereas application of high zinc and copper concentrations suppressed the growth of both algae. High metal concentrations also decreased the photosynthetic pigments and destroyed algal cell ultrastructure. The zinc removal efficiency by both algae increased rapidly during the first day and thereafter remained nearly constant throughout the experiment. The copper removal efficiency by both algae increased slowly during the whole experimental periods. In all cultures, the quantity of both metals removed intracellularly was much lower than the adsorbed quantity on the cell surface. Both strains of the microalgae had proven effective in removing zinc and copper from aqueous solutions, with the highest removal efficiency being near 100%. In addition, *C. pyrenoidosa* appeared to be more efficient than *S. obliquus* for removing copper ions.

On the contrary, *S. obliquus* appeared to be more efficient than *C. pyrenoidosa* for removing zinc ions.

**T. Baykal Ozer<sup>1</sup>, I. Acikgoz Erkaya<sup>2</sup>, Abel U. Udoh<sup>2</sup>, D. Yalcin Duygu<sup>2</sup>, Aydın Akbulut<sup>3</sup>, Gulay Bayramoglu<sup>4</sup>, M. Yakup Arica<sup>3,4</sup>.** (<sup>1</sup>Department of Biology, Faculty of Arts and Science, Ahi Evran University, Kirsehir, Turkey, <sup>2</sup>Department of Biology Education, Faculty of Education, Gazi University, 06500 Teknikokullar, Ankara, Turkey, <sup>3</sup>Department of Biology, Faculty of Science, Gazi University, 06500 Teknikokullar, Ankara, Turkey, <sup>4</sup>Biochemical Processing and Biomaterial Research Laboratory, Faculty of Sciences, Gazi University, 06500 Teknikokullar, Ankara, Turkey. Email: akbuluta@gazi.edu.tr). **Biosorption of Cr(VI) by free and immobilized *Pediastrum boryanum* biomass: equilibrium, kinetic, and thermodynamic studies. Environmental Science and Pollution Research, Volume 19 (7) (2012): 2983-2993**

The biosorption of Cr(VI) from aqueous solution has been studied using free and immobilized *Pediastrum boryanum* cells in a batch system. The algal cells were immobilized in alginate and alginate–gelatin beads via entrapment, and their algal cell free counterparts were used as control systems during biosorption studies of Cr(VI). The changes in the functional groups of the biosorbents formulations were confirmed by Fourier transform infrared spectra. The effect of pH, equilibrium time, initial concentration of metal ions, and temperature on the biosorption of Cr(VI) ion was investigated. The maximum Cr(VI) biosorption capacities were found to be 17.3, 6.73, 14.0, 23.8, and 29.6 mg/g for the free algal cells, and alginate, alginate–gelatin, alginate–cells, and alginate–gelatin–cells at pH 2.0, which are corresponding to an initial Cr(VI) concentration of 400 mg/L. The biosorption of Cr(VI) on all the tested biosorbents (*P. boryanum* cells, alginate, alginate–gelatin, and alginate–cells, alginate–gelatin–cells) followed Langmuir adsorption isotherm model. The thermodynamic studies indicated that the biosorption process was spontaneous and endothermic in nature under studied conditions. For all the tested biosorbents, biosorption kinetic was best described by the pseudo-second-order model.

**Melina A. Talano, Débora C. Busso, Cintia E. Paisio, Paola S. González, Silvia A. Purro, María I. Medina, Elizabeth Agostini.** (Departamento de Biología Molecular, FCEFYQ, Universidad Nacional de Río Cuarto, Ruta Nacional 36 Km 601, CP 5800, Río Cuarto, Córdoba, Argentina. Corresponding author Email: melinatalano@hotmail.com). **Phytoremediation of 2,4-dichlorophenol using wild type and transgenic tobacco plants. Environmental Science and Pollution Research, Volume 19 (6) (2012): 2202-2211**

Transgenic plant strategies based on peroxidase expression or overexpression would be useful for phenolic compound removal since these enzymes play an important role in phenolic polymerizing reactions. Thus, double transgenic (DT) plants for basic peroxidases were obtained and characterized in order to compare the tolerance and efficiency for 2,4-dichlorophenol (2,4-DCP) removal with WT and simple transgenic plants expressing TPX1 or TPX2 gene. Several DT plants showed the expression of both transgenes and proteins, as well as increased peroxidase activity. DT lines showed higher tolerance to 2,4-DCP at early stage of development since their germination index was higher than that of WT seedlings exposed to 25 mg/L of the pollutant. High 2,4-DCP removal efficiencies were found for WT tobacco plants. TPX1 transgenic plants and DT (line *d*) reached slightly higher removal efficiencies for 10 mg/L of 2,4-DCP than WT plants, while DT plants (line *A*) showed the highest removal efficiencies (98%). These plants showed an increase of 21% and 14% in 2,4-DCP removal efficiency for solutions containing 10 and 25 mg/L 2,4-DCP, respectively, compared with WT plants. In

addition, an almost complete toxicity reduction of postremoval solutions using WT and DT plants was obtained through AMPHITOX test, which indicates that the 2,4-DCP degradation products would be similar for both plants. These results are relevant in the field of phytoremediation application and, moreover, they highlight the safety of using DT tobacco plants because nontoxic products were formed after an efficient 2,4-DCP removal.

**Dhara Shukla, Padma S. Vankar. (Indian Institute of Technology, Facility for Ecological and Analytical Testing (FEAT), 204 A, Southern Block, Kanpur, 208016, India. Email: psv@iitk.ac.in). Efficient biosorption of chromium(VI) ion by dry *Araucaria* leaves. *Environmental Science and Pollution Research* Volume 19 (6) (2012): 2321-2328**

In this paper, batch removal of hexavalent chromium from aqueous solutions by *Araucaria heterophylla* leaves was investigated. The batch experiments were conducted to study the adsorption of metal species and effect of different pH, contact time, metal concentration, biosorbent concentration, and adsorption capacity. Freundlich and Langmuir's isotherm model were used to describe the adsorption behavior, and the experimental results fitted Freundlich model well. The adsorption efficiency observed for all chromium concentrations, i.e., 1, 3, 5, and 10 mg/L was 100% and the equilibrium was achieved in 30 min for 1 and 3 mg/L, whereas for 5 and 10 mg/L, it was less than 60 min. FTIR spectra was taken to identify functional groups involved in the biosorption. Thus, *Araucaria* leaves can be considered as one of the cheap and efficient biosorbent for toxic hexavalent chromium removal from natural or wastewaters.

**M. H. Khani<sup>1</sup>, H. Pahlavanzadeh<sup>2</sup>, K. Alizadeh<sup>2</sup>. (<sup>1</sup>Nuclear Science Research School, Nuclear Science and Technology Research Institute, P.O. Box 14395, 836 Tehran, Iran, <sup>2</sup>Department of Chemical Engineering, Faculty of Chemical Engineering, Tarbiat Modares University, P.O. Box 14115, 114 Tehran, Iran. Email: pahlavzh@modares.ac.ir). Biosorption of strontium from aqueous solution by fungus *Aspergillus terreus*. *Environmental Science and Pollution Research*, Volume 19 (6) (2012): 2408-2418**

The biosorption characteristics of strontium ions using fungus *Aspergillus terreus* were investigated. Experimental parameters affecting the biosorption process such as pH, contact time, initial metal concentration, and temperature were studied. Fungus *A. terreus* exhibited the highest strontium uptake capacity at 15°C at an initial strontium ion concentration of 876 mg L<sup>-1</sup> and an initial pH of 9. Biosorption capacity increased from 219 to 308 mg g<sup>-1</sup> with a decrease in temperature from 45°C to 15°C at this initial strontium concentration. The equilibrium data fitted very well to the Langmuir adsorption model in the concentration range of strontium ions and at all the temperatures studied. Evaluation of the experimental data in terms of biosorption dynamics showed that the biosorption of strontium onto fungus followed the pseudo-second-order dynamics well ( $R^2 > 0.985$ ). The calculated thermodynamics parameters ( $-1.64 < \Delta G^\circ < -1.93$  kJ mol<sup>-1</sup> at temperatures of 45–15°C,  $\Delta H^\circ = -4.83$  kJ mol<sup>-1</sup> and  $\Delta S^\circ = -0.01$  kJ mol<sup>-1</sup> K<sup>-1</sup>) showed that the biosorption of strontium ions were feasible, spontaneous, and exothermic at the temperature ranges of 15–45°C.

**Md. Mezbaul Bahar<sup>1,2</sup>, Mallavarapu Megharaj<sup>1,2</sup>, Ravi Naidu<sup>1,2</sup>. (<sup>1</sup>Centre for Environmental Risk Assessment and Remediation (CERAR), University of South Australia, Building X, Room X2-03, Mawson Lakes Boulevard, Mawson Lakes, SA, 5095, Australia, <sup>2</sup>Cooperative Research Centre for Contamination Assessment and Remediation**

**of Environment (CRC CARE), Mawson Lakes Boulevard, Mawson Lakes, SA, 5095, Australia. Email: bahmm001@mymail.unisa.edu.au). Arsenic bioremediation potential of a new arsenite-oxidizing bacterium *Stenotrophomonas* sp. MM-7 isolated from soil. *Biodegradation*, Volume 23 (6) (2012): 803-812**

A new arsenite-oxidizing bacterium was isolated from a low arsenic-containing ( $8.8 \text{ mg kg}^{-1}$ ) soil. Phylogenetic analysis based on 16S rRNA gene sequencing indicated that the strain was closely related to *Stenotrophomonas panacihumi*. Batch experiment results showed that the strain completely oxidized  $500 \mu\text{M}$  of arsenite to arsenate within 12 h of incubation in a minimal salts medium. The optimum initial pH range for arsenite oxidation was 5–7. The strain was found to tolerate as high as 60 mM arsenite in culture media. The arsenite oxidase gene was amplified by PCR with degenerate primers. The deduced amino acid sequence showed the highest identity (69.1 %) with the molybdenum containing large subunit of arsenite oxidase derived from *Bosea* sp. Furthermore the amino acids involved in binding the substrate arsenite, were conserved with the arsenite oxidases of other arsenite oxidizing bacteria such as *Alcaligenes faecalis* and *Herminimonas arsenicoxydans*. To our knowledge, this study constitutes the first report on arsenite oxidation using *Stenotrophomonas* sp. and the strain has great potential for application in arsenic remediation of contaminated water.

**Palanisami Thavamani<sup>1,2</sup>, Mallavarapu Megharaj<sup>1,2</sup>, Ravi Naidu<sup>1,2</sup>. (<sup>1</sup>Centre for Environmental Risk Assessment and Remediation (CERAR), University of South Australia, Mawson Lakes, SA, 5095, Australia, <sup>2</sup>Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), Mawson Lakes, SA, Australia. Email: megharaj.mallavarapu@unisa.edu.au). Bioremediation of high molecular weight polyaromatic hydrocarbons co-contaminated with metals in liquid and soil slurries by metal tolerant PAHs degrading bacterial consortium. *Biodegradation*, Volume 23 (6) (2012): 823-835**

Bioremediation of polyaromatic hydrocarbons (PAH) contaminated soils in the presence of heavy metals have proved to be difficult and often challenging due to the ability of toxic metals to inhibit PAH degradation by bacteria. In this study, a mixed bacterial culture designated as consortium-5 was isolated from a former manufactured gas plant (MGP) site. The ability of this consortium to utilise HMW PAHs such as pyrene and BaP as a sole carbon source in the presence of toxic metal Cd was demonstrated. Furthermore, this consortium has proven to be effective in degradation of HMW PAHs even from the real long term contaminated MGP soil. Thus, the results of this study demonstrate the great potential of this consortium for field scale bioremediation of PAHs in long term mix contaminated soils such as MGP sites. To our knowledge this is the first study to isolate and characterize metal tolerant HMW PAH degrading bacterial consortium which shows great potential in bioremediation of mixed contaminated soils such as MGP.

**Simi Sudharshan<sup>1,2</sup>, Ravi Naidu<sup>1,2</sup>, Megharaj Mallavarapu<sup>1,2</sup>, Nanthi Bolan<sup>1,2</sup>. (<sup>1</sup>Centre for Environmental Risk Assessment and Remediation (CERAR), University of South Australia, Mawson Lakes, SA, Australia, <sup>2</sup>Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), Mawson Lakes, SA, Australia. Email: Ravi.Naidu@unisa.edu.au). DDT remediation in contaminated soils: a review of recent studies. *Biodegradation*, Volume 23 (6) (2012): 851-863**

Over the past few decades significant progress has been made in research on DDT degradation in the environment. This review is an update of some of the recent studies on the degradation and biodegradation pathways of DDT and its metabolites, particularly in soils. The latest reports on human toxicity shows that DDT intake is still occurring even in countries that banned its use decades ago. Ageing, sequestration and formation of toxic metabolites during the degradation processes pose environmental challenges and result in difficulties in bioremediation of DDT contaminated soils. Degradation enhancement strategies such as the addition of chelators, low molecular organic acids, co-solvent washing and the use of sodium and seaweeds as ameliorant have been studied to accelerate degradation. This review describes and discusses the recent challenges and degradation enhancement strategies for DDT degradation by potentially cost effective procedures based on bioremediation.

**Paula M. Tribelli, Carla Di Martino, Nancy I. López, Laura J. Raiger Iustman. (Dpto. de Qca. Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Guiraldes 2160 Pab 2 Piso 4., Buenos Aires, Argentina. Email: lri@qb.fcen.uba.ar). Biofilm lifestyle enhances diesel bioremediation and biosurfactant production in the Antarctic polyhydroxyalkanoate producer *Pseudomonas extremaustralis*. *Biodegradation*, Volume 23 (5) (2012): 645-651**

Diesel is a widely distributed pollutant. Bioremediation of this kind of compounds requires the use of microorganisms able to survive and adapt to contaminated environments. *Pseudomonas extremaustralis* is an Antarctic bacterium with a remarkable survival capability associated to polyhydroxyalkanoates (PHAs) production. This strain was used to investigate the effect of cell growth conditions—in biofilm versus shaken flask cultures—as well as the inocula characteristics associated with PHAs accumulation, on diesel degradation. Biofilms showed increased cell growth, biosurfactant production and diesel degradation compared with that obtained in shaken flask cultures. PHA accumulation decreased biofilm cell attachment and enhanced biosurfactant production. Degradation of long-chain and branched alkanes was observed in biofilms, while in shaken flasks only medium-chain length alkanes were degraded. This work shows that the PHA accumulating bacterium *P. extremaustralis* can be a good candidate to be used as hydrocarbon bioremediation agent, especially in extreme environments.

**Mónica Martins<sup>1</sup>, Rita Taborda<sup>1</sup>, Gonçalo Silva<sup>2</sup>, Ana Assunção<sup>1</sup>, António Pedro Matos<sup>3,4</sup>, Maria Clara Costa<sup>1</sup>. (<sup>1</sup>Departamento de Química e Farmácia, Faculdade de Ciências e de Tecnologia, Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal, <sup>2</sup>Faculdade de Ciências e de Tecnologia, Centro de Biodiversidade, Genómica Integrativa e Funcional (BioFIG), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal, <sup>3</sup>Serviço de Anatomia Patológica, Hospital Curry Cabral, Campo Grande, Lisboa, Portugal, <sup>4</sup>Faculdade de Ciências, Centro de Estudos do Ambiente e do Mar (CESAM)–Polo de Lisboa, Universidade de Lisboa, Campo Grande, Lisboa, 1749-016, Portugal. Email: mcorada@ualg.pt). Aluminum and sulphate removal by a highly Al-resistant dissimilatory sulphate-reducing bacteria community. *Biodegradation*, Volume 23 (5) (2012): 693-703**

A highly Al-resistant dissimilatory sulphate-reducing bacteria community was isolated from sludge of the wetland of Urgeiriça mine (community W). This community showed excellent sulphate removal at the presence of Al<sup>3+</sup>. After 27 days of incubation, 73, 86 and 81% of

sulphate was removed in the presence of 0.48, 0.90 and 1.30 mM of  $Al^{3+}$ , respectively. Moreover,  $Al^{3+}$  was simultaneously removed: 55, 85 and 78% of metal was removed in the presence of 0.48, 0.90 and 1.30 mM of  $Al^{3+}$ , respectively. The dissociation of aluminium-lactate soluble complexes due to lactate consumption by dissimilatory sulphate-reducing bacteria can be responsible for aluminum removal, which probably precipitates as insoluble aluminium hydroxide. Phylogenetic analysis of 16S rRNA gene showed that this community was mainly composed by bacteria closely related to *Desulfovibrio desulfuricans*. However, bacteria affiliated to *Proteus* and *Ralstonia* were also present in the community.

**Laura Carmen Apostol<sup>1</sup>, Luciana Pereira<sup>2</sup>, Raquel Pereira<sup>2</sup>, Maria Gavrilescu<sup>1</sup>, Maria Madalena Alves<sup>2</sup>. (<sup>1</sup>Department of Environmental Engineering and Management, Faculty of Chemical Engineering and Environmental Protection, “Gheorghe Asachi” Technical University of Iasi, 73 Prof.dr.docent Dimitrie Mangeron Street, 700050 Iasi, Romania, <sup>2</sup>IBB-Instituto Biotecnologia e Bioengenharia, Centro Engenharia Biológica, Universidad do Minho, Campus de Gualtar, 4710-057 Braga, Portugal. Email: mgav@tuiasi.ro). Biological decolorization of xanthene dyes by anaerobic granular biomass. *Biodegradation*, Volume 23 (5) (2012): 725-737**

Biodegradation of a xanthene dyes was investigated for the first time using anaerobic granular sludge. On a first screening, biomass was able to decolorize, at different extents, six azo dye solutions: acid orange 7, direct black 19, direct blue 71, mordant yellow 10, reactive red 2 and reactive red 120 and two xanthene dyes—Erythrosine B and Eosin Y. Biomass concentration, type of electron donor, induction of biomass with dye and mediation with activated carbon (AC) were variables studied for Erythrosine B (Ery) as model dye. Maximum color removal efficiency was achieved with 4.71 g VSS L<sup>-1</sup>, while the process rates were independent of the biomass concentration above 1.89 g VSS L<sup>-1</sup>. No considerable effects were observed when different substrates were used as electron donors (VFA, glucose or lactose). Addition of Ery in the incubation period of biomass led to a fivefold increase of the decolorization rate. The rate of Ery decolorization almost duplicated in the presence of commercial AC (0.1 g L<sup>-1</sup> AC<sub>0</sub>). Using different modified AC samples (from the treatment of AC<sub>0</sub>), a threefold higher rate was obtained with the most basic one, ACH<sub>2</sub>, as compared with non-mediated reaction. Higher rates were obtained at pH 6.0. Chemical reduction using Na<sub>2</sub>S confirmed the recalcitrant nature of this dye. The results attest that decolorization of Ery is essentially due to enzymatic and adsorption phenomena.

**Ivar Zekker<sup>1</sup>, Kristel Kroon<sup>1</sup>, Ergo Rikmann<sup>1</sup>, Toomas Tenno<sup>1</sup>, Martin Tomingas<sup>1</sup>, Priit Vabamäe<sup>1</sup>, Siegfried E. Vlaeminck<sup>2</sup>, Taavo Tenno<sup>1</sup>. (<sup>1</sup>Institute of Chemistry, University of Tartu, 14a Ravila St., 50411 Tartu, Estonia, <sup>2</sup>Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, 9000 Ghent, Belgium. Email: Ivar.Zekker@gmail.com). Accelerating effect of hydroxylamine and hydrazine on nitrogen removal rate in moving bed biofilm reactor. *Biodegradation*, Volume 23 (5) (2012): 739-749**

In biological nitrogen removal, application of the autotrophic anammox process is gaining ground worldwide. Although this field has been widely researched in last years, some aspects as the accelerating effect of putative intermediates (mainly N<sub>2</sub>H<sub>4</sub> and NH<sub>2</sub>OH) need more specific investigation. In the current study, experiments in a moving bed biofilm reactor (MBBR) and batch tests were performed to evaluate the optimum concentrations of anammox process intermediates that accelerate the autotrophic nitrogen removal and mitigate a decrease in the

anammox bacteria activity using anammox (anaerobic ammonium oxidation) biomass enriched on ring-shaped biofilm carriers. Anammox biomass was previously grown on blank biofilm carriers for 450 days at moderate temperature  $26.0 (\pm 0.5) ^\circ\text{C}$  by using sludge reject water as seeding material. FISH analysis revealed that anammox microorganisms were located in clusters in the biofilm. With addition of  $1.27$  and  $1.31 \text{ mg N L}^{-1}$  of each  $\text{NH}_2\text{OH}$  and  $\text{N}_2\text{H}_4$ , respectively, into the MBBR total nitrogen (TN) removal efficiency was rapidly restored after inhibitions by  $\text{NO}_2^-$ . Various combinations of  $\text{N}_2\text{H}_4$ ,  $\text{NH}_2\text{OH}$ ,  $\text{NH}_4^+$ , and  $\text{NO}_2^-$  were used as batch substrates. The highest total nitrogen (TN) removal rate with the optimum  $\text{N}_2\text{H}_4$  concentration ( $4.38 \text{ mg N L}^{-1}$ ) present in these batches was  $5.43 \text{ mg N g}^{-1} \text{ TSS h}^{-1}$ , whereas equimolar concentrations of  $\text{N}_2\text{H}_4$  and  $\text{NH}_2\text{OH}$  added together showed lower TN removal rates. Intermediates could be applied in practice to contribute to the recovery of inhibition-damaged wastewater treatment facilities using anammox technology.

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A lead resistant bacterial strain isolated from effluent of lead battery manufacturing company of Goa, India has been identified as *Enterobacter cloacae* strain P2B based on morphological, biochemical characters, FAME profile and 16S rDNA sequence data. This bacterial strain could resist lead nitrate up to  $1.6 \text{ mM}$ . Significant increase in exopolysaccharide (EPS) production was observed as the production increased from  $28$  to  $108 \text{ mg/L}$  dry weight when exposed to  $1.6 \text{ mM}$  lead nitrate in Tris buffered minimal medium. Fourier-transformed infrared spectroscopy of this EPS revealed presence of several functional groups involved in metal binding viz. carboxyl, hydroxyl and amide groups along with glucuronic acid. Gas chromatography coupled with mass spectrometry analysis of alditol-acetate derivatives of acid hydrolysed EPS produced in presence of  $1.6 \text{ mM}$  lead nitrate demonstrated presence of several neutral sugars such as rhamnose, arabinose, xylose, mannose, galactose and glucose, which contribute to lead binding hydroxyl groups. Scanning electron microscope coupled with energy dispersive X-ray spectrometric analysis of this lead resistant strain exposed to  $1.6 \text{ mM}$  lead nitrate interestingly revealed mucous EPS surrounding bacterial cells which sequestered  $17 \%$  lead (as weight %) extracellularly and protected the bacterial cells from toxic effects of lead. This lead resistant strain also showed multidrug resistance. Thus these results significantly contribute to better understanding of structure, function and environmental application of lead-enhanced EPSs produced by bacteria. This lead-enhanced biopolymer can play a very important role in bioremediation of several heavy metals including lead.

**R. Harish, Jastin Samuel, R. Mishra, N. Chandrasekaran, A. Mukherjee. (Centre for Nanobiotechnology, School of Biosciences and Technology, VIT University, Vellore, 632014, India. Email: amit.mookerjee@gmail.com). Bio-reduction of Cr(VI) by exopolysaccharides (EPS) from indigenous bacterial species of Sukinda chromite mine, India. Biodegradation, Volume 23 (4) (2012): 487-496**

Chrome mining activity has contributed intensively towards pollution of hexavalent chromium around Sukinda Valley, Orissa, India. In an attempt to study the specific contribution of

exopolysaccharides (EPS) extracted from indigenous isolates towards Cr(VI) reduction, three chromium (VI) tolerant strains were isolated from the effluent mining sludge. Based on the tolerance towards Cr(VI) and EPS production capacity, one of them was selected for further work. The taxonomic identity of the selected strain was confirmed to be *Enterobacter cloacae* (showing 98% similarity in BLAST search to *E. cloacae*) through 16S rRNA analysis. The EPS production was observed to increase with increasing Cr(VI) concentration in the growth medium, highest being 0.078 at 100 mg/l Cr(VI). The extracted EPS from *Enterobacter cloacae* SUKCr1D was able to reduce 31.7% of Cr(VI) at 10 mg/l concentration, which was relevant to the prevailing natural concentrations at Sukinda mine effluent sludge. The FT-IR spectral studies confirmed the surface chemical interactions of hexavalent chromium with EPS.

**Randhir P. Deo<sup>1</sup>, Bruce E. Rittmann<sup>2</sup>. (<sup>1</sup>Chemistry Department, Division of Natural Sciences, College of Natural and Applied Sciences, University of Guam, Mangilao, GU 96923, USA, <sup>2</sup>Center for Environmental Biotechnology, Biodesign Institute at Arizona State University, Tempe, AZ 85287-5701, USA. Email: rdeo@uguam.uog.edu). A biogeochemical framework for bioremediation of plutonium(V) in the subsurface environment. *Biodegradation*, Volume 23 (4) (2012): 525-534**

Accidental release of plutonium (Pu) from storage facilities in the subsurface environment is a concern for the safety of human beings and the environment. Given the complexity of the subsurface environment and multivalent state of Pu, we developed a quantitative biogeochemical framework for bioremediation of Pu(V)O<sub>2</sub><sup>+</sup> in the subsurface environment. We implemented the framework in the biogeochemical model CCBATCH by expanding its chemical equilibrium for aqueous complexation of Pu and its biological sub-models for including Pu's toxicity and reduction reactions. The quantified framework reveals that most of the Pu(V) is speciated as free Pu(V)O<sub>2</sub><sup>+</sup> (aq), which is a problem if the concentration of free Pu(V)O<sub>2</sub><sup>+</sup> is  $\geq 28 \mu\text{M}$  (the half-maximum toxicity value for bacteria able to reduce Pu(V) to Pu(III)PO<sub>4(am)</sub>) or  $\geq 250 \mu\text{M}$  (the full-toxicity value that takes the bioreduction rate to zero). The framework includes bioreduction of Fe<sup>3+</sup> to Fe<sup>2+</sup>, which abiotically reduces Pu(V)O<sub>2</sub><sup>+</sup> to Pu(IV) and then to Pu(III). Biotic (enzymatic) reduction of Pu(V)O<sub>2</sub><sup>+</sup> directly to Pu(III) by *Shewanella alga* (*S. alga*) is also included in the framework. Modeling results also reveal that for formation of Pu(III)PO<sub>4(am)</sub>, the desired immobile product, the concentration of coexisting model strong ligand—nitrilotriacetic acid (NTA)—should be less than or equal to the concentration of total Pu(III).

## **Biotransformation**

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Biotransformation of an irregular monoterpene alcohol, ( $\pm$ )-lavandulol [( $\pm$ )-5-methyl-2-(1-methylethenyl)-4-hexen-1-ol] (**I**) and its tetrahydro derivative, ( $\pm$ )-tetrahydrolavandulol [( $\pm$ )-2-isopropyl-5-methylhexan-1-ol] (**II**) were studied using a soil isolated fungal strain *Rhizopus oryzae*. Five metabolites, 2-((3,3-dimethyloxiran-2-yl)methyl)-3-methylbut-3-en-1-ol (**Ia**), 2-methyl-5-(prop-1-en-2-yl)hex-2-ene-1,6-diol (**Ib**), 2-methyl-5-(prop-1-en-2-yl)hexane-1,6-diol (**Ic**), 2-(3-methylbut-2-enyl)-3-methylenebutane-1,4-diol (**Id**), 5-methyl-2-(2-methyloxiran-2-yl)hex-4-en-1-ol (**Ie**) have been isolated from the fermentation medium and characterized with lavandulol as a substrate. When tetrahydrolavandulol used as a substrate, two metabolites 2-isopropyl-5-methylhexane-1,5-diol (**IIa**) and 2-isopentyl-3-methylbutane-1,3-diol (**IIb**) have been isolated from the fermentation medium. Biotransformation studies with *R. oryzae* clearly indicate that the organism initiates the transformation either by hydroxylation at allylic methyl groups or epoxidation of double bond. GC and GCMS analyses indicated that both (*R*)- and (*S*)-enantiomers of **I** and **II** have been transformed into corresponding hydroxylated or epoxy derivatives, when racemic **I** and **II** were used as substrates.

**Keywords:** Biotransformation; Lavandulol; Fungus; *Rhizopus oryzae*; Tetrahydrolavandulol

**D. Muñoz Solano, P. Hoyos, M.J. Hernáiz, A.R. Alcántara, J.M. Sánchez-Montero.** (Biotransformations Group, Department of Organic and Pharmaceutical Chemistry, Faculty of Pharmacy, Complutense University, Plaza de Ramón y Cajal, s/n, Ciudad Universitaria, E-28040 Madrid, Spain). **Industrial biotransformations in the synthesis of building blocks leading to enantiopure drugs. Bioresource Technology, Volume 115 (2012): 196–207**

Due to the growing demand of enantiomerically pure compounds, as well as the increasing strict safety, quality and environmental requirements of industrial synthetic processes, the development of more sustainable, healthy and economically attractive strategies for the synthesis of chiral biologically active molecules is still an open challenge in the pharmaceutical industry. In this context, the biotransformations field has emerged as a real alternative to traditional synthetic routes, because of the exquisite chemo-, regio- and enantioselectivities commonly displayed by enzymes; thus, biocatalysis is becoming a widespread methodology for the synthesis of chiral compounds, not only at laboratory scale, but also at industrial scale. As hydrolases and oxido-reductases are the most employed enzymes, this review is focused on describing several industrial processes based on the use of these enzymes for obtaining chiral compounds useful for the pharmaceutical industry.

**Keywords:** Biocatalysis; Hydrolases; Oxido-reductases; Industrial scale; Chiral drugs

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The environmental fate of transformation products from organic pollutants such as drugs has become a new research area of increasing interest over the last few years. Whereas in the past mainly parent compounds or their major human metabolites were studied, new questions have arisen what compounds could be formed during incomplete degradation in the aquatic environment and what effects the resulting transformation products might have on nature and mankind. Psychiatric drugs are among the most important prescription drugs worldwide, but so far only little data is provided upon their degradation behavior. This especially accounts for tricyclic antipsychotic drugs of the phenothiazine class. Therefore, the degradation of such drugs was investigated in this study. In this study the aerobic *Closed Bottle test* (The Organisation for Economic Co-operation and Development (OECD) 301D) was used to assess the ready biodegradability of three trifluoromethylated phenothiazine drugs: fluphenazine, triflupromazine, and trifluoperazine. As it is known from literature that phenothiazine drugs can easily form various photolytic transformation products under light exposure, photochemical transformation was also investigated. Since transformation products are usually not available commercially, the calculation of environmental parameters with the aid of quantitative structure activity relationship (QSAR) software was used for first evaluation of these compounds. According to the OECD test guideline, all trifluoromethylated phenothiazines had to be classified as not readily biodegradable. Chromatographic data revealed the formation of some transformation products. Comparing retention time and mass spectrometric data with the analytical results of the light exposure experiments, we found peaks with the same retention time and mass spectra. So these transformation products were not of bacterial, but photolytic, origin and are formed very quickly even under low light doses. A special chromatographic column and solvent gradient along with multiple stage mass spectrometric fragmentation experiments uncovered the presence of, in total, nine photolytic transformation products and allowed for their structural elucidation. Typical modifications of the molecules were sulfoxidation, exocyclic *N*-oxidation, and transformation of the trifluoromethyl to a carboxylic moiety. The obtained results of the QSAR calculations show that all transformation products are highly mobile in the aquatic environment and elimination through biotic or abiotic pathways cannot be expected. Transformation products of trifluoromethylated phenothiazine drugs have to be expected in the aquatic environment, yet nothing is known about their toxicological properties. Therefore, further risk assessment upon these drugs and their fate is strongly recommended.

### **Biomarker**

**Katelyn J. Edge<sup>1,2</sup>, Emma L. Johnston<sup>1</sup>, Anthony C. Roach<sup>2</sup>, Amy H. Ringwood<sup>3</sup>.** (<sup>1</sup>Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, 2052, Australia, <sup>2</sup>Centre for Ecotoxicology, NSW Office of Environment and Heritage, Lidcombe, NSW, Australia, <sup>3</sup>Department of Biology, University of North Carolina at Charlotte, 9201 University City Boulevard, Charlotte, NC 28223, USA). **Indicators of environmental stress: cellular biomarkers and reproductive responses in the Sydney rock oyster (*Saccostrea glomerata*). *Ecotoxicology*, Volume 21(5) (2012): 1415-1425**

We measured a suite of common biomarker responses for the first time in the Sydney rock oyster *Saccostrea glomerata* to evaluate their utility as biological effects measures for pollution monitoring. To examine the relationship between biomarker responses and population level effects, fertilisation and embryo development assays were also conducted. Adult oysters were

deployed in two contaminated estuaries and a reference estuary in Sydney, Australia. The concentrations of various contaminants (metals and polycyclic aromatic hydrocarbon, PAHs) were quantified in oyster's tissue from each site and both metals and total PAHs were significantly elevated in contaminated estuaries relative to the reference estuary. Lysosomal membrane destabilisation, lipid peroxidation levels and glutathione (GSH) concentrations were measured in the digestive gland of oysters. Of all biomarkers measured, lysosomal membrane destabilisation proved to be the most useful indicator of oysters facing anthropogenic stress and we suggest this may be an especially useful biomarker for incorporation into local environmental monitoring programs. Moreover, lysosomal membrane destabilisation showed good correlations with fertilisation, normal embryo development and estuary status. GSH and lipid peroxidation were not as valuable for distinguishing between estuaries exposed to differing levels of anthropogenic stress, but did provide additional valuable information regarding overall health status of the oysters.

### **Biofertilizer**

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Biofertilizers are becoming increasingly popular in many countries and for many crops, but very few studies on their effect on grain yield have been conducted in rice. Therefore, we evaluated three different biofertilizers (based on *Azospirillum*, *Trichoderma*, or unidentified rhizobacteria) in the Philippines during four cropping seasons between 2009 and 2011, using four different fertilizer rates (100% of the recommended rate [RR], 50% RR, 25% RR, and no fertilizer as Control). The experiments were conducted under fully irrigated conditions in a typical lowland rice environment. Significant yield increases due to biofertilizer use were observed in all experimental seasons with the exception of the 2008/09 DS. However, the effect on rice grain yield varied between biofertilizers, seasons, and fertilizer treatments. In relative terms, the seasonal yield increase across fertilizer treatments was between 5% and 18% for the best biofertilizer (*Azospirillum*-based), but went up to 24% in individual treatments. Absolute grain yield increases due to biofertilizer were usually below 0.5 t·ha<sup>-1</sup>, corresponding to an estimated additional N uptake of less than 7.5 kg N ha<sup>-1</sup>. The biofertilizer effect on yield did not significantly interact with the inorganic fertilizer rate used but the best effects on grain yield were achieved at low to medium fertilizer rates. Nevertheless, positive effects of the biofertilizers even occurred at grain yields up to 5 t·ha<sup>-1</sup>. However, the trends in our results seem to indicate that biofertilizers might be most helpful in rainfed environments with limited inorganic fertilizer input. However, for use in these target environments, biofertilizers need to be evaluated under conditions with abiotic stresses typical of such systems such as drought, soil acidity, or low soil fertility.

**Keywords:** *Azospirillum*; biofertilizer; grain yield; inorganic fertilizer; PGPR; plant growth-promoting rhizobacteria; rice; *Trichoderma*

**Mehrab Yadegari<sup>1\*</sup>, G. H. N., Farahani<sup>2</sup> and Z. Mosadeghzad<sup>2</sup>.** (<sup>1</sup>Department of Agriculture, Faculty of Agriculture and Natural Resources, Islamic Azad University, Shahrekord Branch, 166 Shahrekord, Iran, <sup>2</sup>Department of Chemistry, Faculty of Science, Islamic Azad University, Shahrekord Branch, 166 Shahrekord, Iran. \*Corresponding author. E-mail: mehrabyadegari@gmail.com. Tel: 0098-9133814318). **Biofertilizers effects on quantitative and qualitative yield of Thyme (*Thymus vulgaris*). African Journal of Agricultural Research, Volume 7(34) (2012): 4716-4723**

A factorial experiment in randomized complete block design was conducted in order to evaluate the effects of bio and chemical fertilizers on essence, dry and fresh weight production in Thyme (*Thymus vulgaris*) in a field study in Shahrekord, Iran. The applied fertilizers were: (a) phosphate-solubilizing bacterium, (b) frowzy manure from mutton, (c) Nitroxin biofertilizer and (d) phosphate from super phosphate triple at level of 0, 20, 40 and 60 kg/ha. Control experiments were carried out without soil fortification with any fertilizers. Significant differences were observed in dry and fresh weight, essential oil production and number of lateral shoots while soils were fortified with one of the chemical or bio fertilizers or combination of both. Results of Duncan's multiple range test showed the best treatment was simultaneous application of phosphate-solubilizing bacterium, frowzy manure from mutton, nitroxin bio fertilizer and 40 kg/ha of super phosphate triple (essence production = 0.66% of plant dry weight). However, in control experiments without soil fortification with any fertilizers, the minimum production of the essence and other parameters of plants were obtained. The essence production in this treatment was 0.047% of plant dry weight. The application of biofertilizers (phosphate-solubilizing bacterium, nitroxin and frowzy manure) caused the highest biomass and essence production. It was found that soil fortification with biofertilizers make more plant production comparing to usage of chemical fertilizer.

**Keywords:** Essence production, frowzy manure from mutton, nitroxin, phosphate-solubilizing bacterium, super phosphate triple, Thyme (*Thymus vulgaris* L.).

**Mona E. Eleiwa<sup>1\*</sup>, Eman R. Hamed<sup>2</sup>, Heba Sh. Shehata<sup>3</sup>.** (<sup>1</sup>Department of Botany, Faculty of Science, Cairo University, Giza, Egypt, <sup>2</sup>Department of Chemistry of Natural and Microbial Products, National Research center, Giza, Egypt, <sup>3</sup>Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt). **Biofertilizers and/or some micronutrients role on wheat plants grown on newly reclaimed soil. African Journal of Ecology, Volume 50 (4) (2012): 464-475**

Pot experiment was conducted to study the effect of biofertilizers (inoculation with different bacterial isolates), foliar spraying with some micronutrients (MN, ZN, FE and MN+ZN+FE) and their interaction on growth, physiological parameters and nutrients content of wheat plants grown on reclaimed soil. Pot experiment was conducted in the greenhouse of National Research center, the experimental design was split plot with four replicates. Four biofertilizer treatments (un-inoculated, *Bacillus polymyxa*, *Azotobacter chroococcum* or *Azospirillum brasilense*) were used and randomly distributed in the main pots. The foliar treatments with micronutrients were randomly distributed in the sub plots. The growth parameters (plant height, leaf area, roots, shoots and whole plant dry weights and number of tillers & leaves per plant); some physiological parameters (soluble sugar %, protein %, polysaccharide %, chl. A+b  $\mu\text{g cm}^{-1}$  leaf per plant,

carotenoids  $\mu\text{g g}^{-1}$ , IAA  $\text{mg kg}^{-1}$  and psll mol DCPIP reduced per mg chl. per h) and nutrient contents (N, P, K, MG, MN, ZN and CU) of wheat plants were significantly increased by inoculating wheat grains with different bacteria as compared with un-inoculated plants (control). The highest values of all the mentioned parameters were obtained by using *Azospirillum brasilense* followed by *Azotobacter chroococcum* and *Bacillus polymyxa* in decreasing order. Foliar spraying treatments significantly increased the growth parameters, physiological parameters as well as nutrients content of wheat plants as compared with control. Highest values were obtained by using (MN+FE+ZN) treatment followed by ZN, FE and MN in decreasing order. Micronutrients in wheat plants differed as the foliar treatments were differed, so application of any micronutrient individually significantly increased its content and enhanced the content of other micronutrients in wheat. Interaction between the used biofertilizers and foliar spraying with micronutrients significantly affected all the studied parameters of wheat plants, the highest were obtained by inoculating wheat grains with *Azospirillum brasilense* and spraying the plants with (MN+FE+ZN) treatment, while the lowest values were attained by un-inoculated grains (control) and spraying the wheat plants with tap water (control). Effective microorganisms in combination with micronutrients could be recommended to farmers to lead higher wheat yield.

**Keywords:** bacterial isolates; biofertilizers; foliar spray; micronutrient; nutrient content; physiological parameters; wheat plants

### **Biocomposting**

**Tejinder Pal Khaket, Mangal Singh, Suman Dhanda, Talwinder Singh, Jasbir Singh. (Department of Biochemistry, Kurukshetra University, Kurukshetra 136119, India). Biochemical characterization of consortium compost of toxic weeds *Parthenium hysterophorus* and *Eichhornia crassipe*. Bioresource Technology, Volume 123 (2012): 360–365**

*Parthenium hysterophorus* and *Eichhornia crassipes* are two uncontrolled weeds with high concentration of N, P, K, Zn and Fe that makes them suitable for composting. Three types of compost viz. *Parthenium* and *Eichhornia* each alone as well as combined were prepared. Biochemical and enzymatic analysis of the compost in addition to seed germination was performed. Phenols, organic carbon, C/N and C/P ratios were found to decrease significantly while N, P, K, polyphenol oxidase increased significantly in combined compost. Furthermore, seed germination test of *Vigna radiata* and *Triticum* seeds, revealed a significant increase in root, shoot length and germination index in 60 days old combined compost. It can be concluded that combined composting of *Parthenium* with *Eichhornia* not only reduces the allelopathic effect but also increases its nutrient quality and thus could be promising for organic farming and bioremediation.

**Keywords:** *Parthenium hysterophorus*; *Eichhornia crassipes*; Compost, Phenols; Allelopathy

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**104 Sturgis Hall, Baton Rouge, LA 70803, USA). Nutrient transformations during composting of pig manure with bentonite. Bioresource Technology, Volume 121 (2012): 362–368**

This work aimed to evaluate the influence of different amounts of bentonite on nutrients transformation during pig manure composting process. The results showed that bentonite had no significant effects on compost temperature and pH changes. While, EC, moisture, OM, TN and  $\text{NO}_3^-$ -N were notably influenced by BT addition. The adding of BT could facilitate OM degradation, increase TKN content and decrease the C/N ratio. Increasing the proportion of bentonite in pig manure compost to reduce extractable heavy metal content is feasible. However, *potherb mustard* seed GI decreased with the proportion of added bentonite increasing. The results suggest that a proportion of less than 2.5% bentonite is recommended for addition to pig manure compost, and examining the additive ratio in a comprehensive waste composting project is a worthwhile direction for future research.

**Keywords:** Compost; Bentonite; Pig manure; Nutrient transformations; Heavy metal availability

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This work illustrates the vermistabilization of wastewater sludge from a milk processing industry (MPIS) unit spiked with cow dung (CD), sugarcane trash (ST) and wheat straw (WS) employing earthworms *Eisenia fetida*. A total of nine experimental vermibeds were established and changes in chemical parameters of waste material have been observed for 90 days. Vermistabilization caused significant reduction in pH, organic carbon and C:N ratio and substantial increase in total N, available P and exchangeable K. The waste mixture containing MPIS (60%) + CD (10%) + ST (30%) and MPIS (60%) + CD (10%) + WS (30%) had better waste mineralization rate among waste mixtures studied. The earthworm showed better biomass and cocoon numbers in all vermibeds during vermicomposting operation. Results, thus suggest the suitability of *E. fetida* for conversion of noxious industrial waste into value-added product for land restoration programme.

**Keywords:** Vermicomposting; Dairy wastewater sludge; C:N ratio; *Eisenia fetida*; Industrial waste

### **Biopesticides**

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Bacteria play a significant role in water contamination. Chemicals are mostly used for the treatment of bacteriologically contaminated water. The use of bacterial interactions is a new approach to limit the pathogens' growth. Detection of antimicrobial substances produced by lactic acid bacteria against the waterborne pathogens is the objective of this work. Microbiological and biochemical methods were used to identify lactic acid bacteria having an antimicrobial activity. Evaluation of antimicrobial activity with growth kinetic measurements was performed. Four isolates of lactic acid bacteria obtained from whey and curd were identified. The predominant species belonging to the *Lactobacillus* genera are: *Lactobacillus rhamnosus*, *Lactobacillus sakei*, *Lactobacillus paracasei*, and *Lactobacillus paraplantarum*. The present study revealed that the *Lactobacillus* consortium is able to inhibit *Staphylococcus aureus*'s growth along with *Escherichia coli* and *Vibrio* species. In mixed culture, after 24 h, the *Lactobacillus* consortium reduces the growth of *S. aureus* by 2.03 log; moreover, the growth of the latter bacteria totally ceased after 72 h of incubation. The protein produced by the *Lactobacillus* consortium was responsible for arresting the growth of *S. aureus*.

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The combination of chemical oxidation (Fenton reaction) and biological treatment processes is a promising technique aiming to reduce recalcitrant wastewater loads. Preliminary tests were carried out on two widely used toxic and non-biodegradable pesticides, namely, Dazomet and Fenamiphos. The chemical reaction was employed as a pre-treatment step for the conversion of the substrates into oxygenated intermediates that were easily removed by means of a final biological treatment. In the combined action, the mineralisation activity of a selected microbial consortium was used to degrade residual volatile and non-volatile organic compounds into CO<sub>2</sub> and biomass.

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We studied the diversity and biocontrol potential of 100 fungal endophytes isolated from *Espeletia* spp., endemic plant species from the Paramo in the Andean mountain range. Our sample was genotypically highly diverse at all ITS similarity levels. The antagonistic properties of these isolates were tested against common crop pathogens in Colombia, including *Pectobacterium carotovorum*, *Ralstonia solanacearum*, *Pseudomonas syringae*, *Xanthomonas campestris*, *Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Phytophthora infestans*. All endophytic isolates were able to significantly inhibit the growth of at least one of the plant pathogens tested ( $P < 0.05$ ). Three main types of endophyte/pathogen interactions were observed. However, only those endophytes that produced an evident inhibition halo were further

studied using their crude extracts to confirm that the inhibitory effect was due to the production of endophytic bioactive metabolites. From these experiments, nine promising isolates were selected for co-inoculation tests with *R. solani* in tomato plants. The isolates identified as *Aureobasidium pullulans* and *Paraconiothyrium sporulosum* not only protected the plants against this pathogen but also allowed them to exhibit similar growth and development as the uninoculated control. This work explores new alternatives for disease management without the application of chemical pesticides.

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*Pythium ultimum* (Trow) is one of the main causes of damping-off disease in many parts of the world. Control of the disease depends mainly on application of chemical fungicides. However, soil treatments with fungicides are not always feasible due to economical and ecological reasons. Soil-borne, non-pathogenic bacteria of the genus *Pseudomonas fluorescens* with the ability to antagonise fungal phytopathogens, represent a realistic alternative to chemical fungicides and show great promise with respect to protect plant roots from fungal-induced diseases. In an attempt to find an integrated control system of damping-off disease in tomato, fungicides including azoxystrobin, metalaxyl-M and pyraclostrobin were applied alone and in combination with *P. fluorescens* isolate CW2. The fungicides were tested in vitro for their antagonistic potential against *P. ultimum* and for compatibility with CW2. It was found that the fungicides were fungitoxic to *P. ultimum*, but did not inhibit the growth of the *P. fluorescens*. The efficacy of the fungicides alone and in combination with CW2 was also tested in greenhouse experiments against damping-off disease on tomato. Two concentrations (5 and 10 µg ml<sup>-1</sup>) were applied. Damping-off incidence of tomato seedlings in Humosoil<sup>®</sup>:sand mixture infested with *P. ultimum* was reduced following seed treatment with the fungicides. However, the degree of control obtained varied significantly depending on the fungicide used. Combined seed treatment with *P. fluorescens* and the fungicides resulted in a significant improvement in disease control and improved plant growth as indicated by shoot and root dry weights. Metalaxyl-M treatment applied alone or in combination with *P. fluorescens*, significantly protected tomato seedlings against damping-off. Strobilurin fungicides stimulated plant growth compared to metalaxyl-M. Combined treatment of tomato seeds with strobilurin fungicides and CW2 showed a moderate to good disease control and an increase in shoot and root dry weights.

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Predation potential of the larval odonates *Ceriagrion coromandelianum* and *Brachydiplax chalybea chalybea* on the II and IV instar larvae of *Culex quinquefasciatus* was evaluated under simulated natural conditions in the laboratory. A type II functional response was exhibited by the odonates, with the attack rate and handling time differing significantly between prey sizes for *C. coromandelianum*. The per capita prey consumption varied between vegetated and open habitat

conditions and between the days as reflected through the Clearance Rate (CR). Results of univariate ANOVA revealed that prey consumption varied significantly ( $P < 0.05$ ) with the prey and predator densities for both the odonate predators, whereas habitat structure had significant effects only in case of *B. chalybea chalybea*. Thus, the use of larvae of *C. coromandelianum* and *B. chalybea chalybea* can facilitate conservation and biological control simultaneously under suitable habitat conditions.

**Keywords:** *Ceriagrion coromandelianum*; *Brachydiplax chalybea chalybea*; Odonata; Mosquito larvae; Functional response

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A *Bacillus* isolate (B014) demonstrating strong inhibition against the pathogen *Xanthomonas axonopodis* pv. *dieffenbachiae* (XAD) was obtained from healthy tissue of an Anthurium plant and was selected for further characterization. Based on the sequence analysis of the 16S rDNA gene and the *gyrA* gene, the *Bacillus* B014 isolate was identified as *Bacillus amyloliquefaciens*. *Bacillus* B014 was shown to produce anti-XAD metabolites that have a high resistance to a wide range of pH values, high temperatures and digestion by proteinase K. PCR-based detection showed that *Bacillus* B014 can potentially coproduce iturins and surfactins. Applying the *Bacillus* B014 cell suspension or its cell-free culture filtrate to the leaves of an Anthurium pot plant before challenge with the pathogen XAD decreased the percentage of leaves with lesions to 17.86% and 28.57%, the percentage of the leaf area with lesions to  $7.86 \pm 1.25\%$  and  $18.14 \pm 1.44\%$ , respectively, when compared to 96.30% and  $49.85 \pm 1.58\%$  in control Anthurium plants challenged only with the bacterial pathogen. The *Bacillus* B014 cell suspension or its cell-free culture filtrate also induced an increase in the activities of the defense-related enzymes phenylalanine ammonia lyase, peroxidase and polyphenol oxidase, when compared to control Anthurium plants challenged only with the pathogen. The *Bacillus* B014 cell-free culture filtrate also inhibited further disease development of Anthurium plants already demonstrating disease symptoms caused by XAD. In conclusion, the newly isolated *B. amyloliquefaciens* B014 is a promising candidate as a biological agent to control bacterial blight caused by XAD, particularly in the Anthurium plant.

**Keywords:** Anthurium; Bacterial blight; *Xanthomonas axonopodis* pv. *dieffenbachiae*; *Bacillus amyloliquefaciens* B014; Biocontrol

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**Evaluation of biological control agents against *Ralstonia* wilt on ginger. *Biological Control*, Volume 62 (3) (2012): 144–151**

Of the 420 bacterial strains isolated from rhizosphere soil and the plant surface as well as the stem, leaf, and root tissue of ginger, 85 were selected for amplified ribosomal DNA restriction analysis (ARDRA) fingerprint analysis based on their antagonistic activity *in vitro* against *Ralstonia solanacearum* and on their ability to produce certain enzymes and metabolites. Of these 85, 19 antagonists from different clusters were assessed for their antagonism in greenhouse against *Ralstonia* wilt of ginger and biocontrol efficacies of these strains were 26.09–69.17%. In order to find out the relationship between potential and practical biocontrol ability we established an assessment system that evaluated their *in vitro* activity. The correlation coefficient between the assessments *in vitro* and biocontrol efficacies was 0.87, which indicates that this selection strategy can be used in screening biocontrol agents against plant diseases. In the greenhouse, the following strains showed biocontrol efficacies greater than 50%: *Bacillus subtilis* strain 1JN2, *Myroides odoratimimus* 3YW8, *Bacillus amyloliquefaciens* 5YN8, and *Stenotrophomonas maltophilia* 2JW6. This is the first report of using *Myroides* sp. and *Stenotrophomonas* sp. as biocontrol agents against ginger wilt caused by *R. solanacearum*, and these strains show promise as commercial biocontrol agents.

**Keywords:** Biocontrol; *Ralstonia solanacearum*; Screening strategy; *Zingiber officinale*

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A leaf-feeding geometrid, *Chiasmia assimilis* (Warren), was introduced into northern Queensland from South Africa in 2002 as a biological control agent for the invasive woody weed, prickly acacia, *Acacia nilotica* subsp. *indica* (Bentham) Brenan. The insect established in infestations in coastal areas between the townships of Ayr and Bowen where the larvae periodically cause extensive defoliation at some localities during summer and autumn. The impact of this herbivory on a number of plant parameters, including shoot length, basal stem diameter, root length, number of leaves, number of branches, and above and below ground biomass was investigated at one coastal site through an insect exclusion trial using potted seedlings and regular spray applications of a systemic insecticide to exclude the biological control agent. Half the seedlings, both sprayed and unsprayed, were placed beneath the prickly acacia canopy, the other half were placed in full sunlight. Larvae of *C. assimilis* were found on unsprayed seedlings in both situations. The effects of herbivory, however, were significant only for seedlings grown beneath the canopy. At the end of the five-month trial period, shoot length of these seedlings was reduced by 30%, basal stem diameter by 44%, root length by 15%, number of leaves by 97%, above ground biomass by 87%, and below ground biomass by 77% when compared to sprayed seedlings. Implications are that the insect, where established, may reduce seedling growth beneath existing canopies and in turn may help limit the formation of dense infestations.

**Keywords:** Prickly acacia; *Acacia nilotica* subsp. *indica*; *Chiasmia assimilis*; Exclusion trial; Herbivory

**D.M. O’Connell, S.D. Wratten, A.R. Pugh, A-M. Barnes. (Bio-Protection Research Centre, PO Box 84, Lincoln University, Lincoln 7647, New Zealand). ‘New species association’ biological control? Two coccinellid species and an invasive psyllid pest in New Zealand. Biological Control, Volume 62(2) (2012): 86–92**

The ‘new species association’ biological control approach is based on the ecological principle in which a natural enemy is used that has not coevolved with a pest. The recent incursion of the tomato–potato psyllid (TPP), *Bactericera cockerelli* to New Zealand offered a unique opportunity to investigate the potential of a new species association for biological control of this pest. This laboratory-based study investigated the potential for a new species association between two New Zealand naturalized coccinellids, *Cryptolaemus montrouzieri* and *Cleobora mellyi*, and TPP. A third naturalized New Zealand coccinellid, *Scymnus loewii*, was compared as a potential ‘old association’. We conducted two experiments to determine consumption rate and feeding behavior, respectively, of adults and fourth-instar larvae of the three coccinellid species on mixed instars of TPP nymphs, using tomato and potato leaflets, and a no-leaflet control. *C. montrouzieri* consumed up to 30 psyllids over 24 h, depending on leaflet type and predator life stage. *C. mellyi* adults and larvae were the most voracious predators of psyllids, consuming up to 100 TPP over 24 h. Adult *C. mellyi* spent more time feeding on psyllids in the control (56%) and potato leaflets (30%) than on tomato leaflets. Larvae spent 79% more time feeding on the no-leaflet control, compared to the potato (24%) and tomato leaflet (14%) treatments. *S. loewii* consumed relatively few psyllids on all three substrates (<10 over 24 h), and spent less time feeding compared to the other two species. By demonstrating that these predators will consume this psyllid, our results suggest that a new species association may potentially exist between *C. montrouzieri*, *C. mellyi* and the TPP.

**Keywords:** *Scymnus loewii*; *Cryptolaemus montrouzieri*; *Cleobora mellyi*; *Bactericera cockerelli*; Agriculture; Predator; Arthropod; Prey; Biological control

**Sergio de los Santos-Villalobos, Guadalupe Coyolxauhqui Barrera-Galicia, Mario Alberto Miranda-Salcedo, Juan José Peña-Cabriales. (Centro de Investigación y de Estudios Avanzados-IPN, Unidad Irapuato, 36500, Irapuato, Guanajuato, Mexico). *Burkholderia cepacia* XXVI siderophore with biocontrol capacity against *Colletotrichum gloeosporioides*. World Journal of Microbiology and Biotechnology, Volume 28 (8) (2012): 2615-2623**

*Colletotrichum gloeosporioides* is the causal agent of anthracnose in mango. *Burkholderia cepacia* XXVI, isolated from mango rhizosphere and identified by 16S rDNA sequencing as a member of *B.cepacia* complex, was more effective than 6 other mango rhizosphere bacteria in inhibiting the model mango pathogen, *C. gloeosporioides* ATCC MYA 456. Biocontrol of this pathogen was demonstrated on Petri-dishes containing PDA by > 90 % reduction of surface colonization. The nature of the biocontrol metabolite(s) was characterized via a variety of tests. The inhibition was almost exclusively due to production of agar-diffusile, not volatile, metabolite(s). The diffusible metabolite(s) underwent thermal degradation at 70 and 121 °C (1 atm). Tests for indole acetic acid production and lytic enzyme activities (cellulase, glucanase and chitinase) by *B. cepacia* XXVI were negative, indicating that these metabolites were not involved in the biocontrol effect. Based on halo formation and growth inhibition of the pathogen on the diagnostic medium, CAS-agar, as well as colorimetric tests we surmised that

strain XXVI produced a hydroxamate siderophore involved in the biocontrol effect observed. The minimal inhibitory concentration test showed that  $0.64 \mu\text{g ml}^{-1}$  of siderophore (Deferoxamine mesylate salt-equivalent) was sufficient to achieve 91.1 % inhibition of the pathogen growth on Petri-dishes containing PDA. The biocontrol capacity against *C. gloeosporioides* ATCC MYA 456 correlated directly with the siderophore production by *B. cepacia* XXVI: the highest concentration of siderophore production in PDB on day 7,  $1.7 \mu\text{g ml}^{-1}$  (Deferoxamine mesylate salt-equivalent), promoted a pathogen growth inhibition of 94.9 %. The growth of 5 additional strains of *C. gloeosporioides* (isolated from mango “Ataulfo” orchards located in the municipality of Chahuities, State of Oaxaca in Mexico) was also inhibited when confronted with *B. cepacia* XXVI. Results indicate that *B. cepacia* XXVI or its siderophore have the potential to be used as a biological control agent against *C. gloeosporioides*; thus diminishing environmental problems caused by the current practices to control this disease.

### **Biodegradation**

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Bensulfuron-methyl (BSM) is a new kind of sulfonylurea herbicide widely used to control broad-leaf weeds in rice paddies. The aim of this work was to study BSM biodegradation in paddy soils with BSM-degrading bacteria *Bacillus megaterium* L1 and *Brevibacterium* sp. BH and its effect on the structures of soil bacterial community. More than 90 % of BSM could be degraded in paddy soils with  $0.0355 \text{ mg kg}^{-1}$  BSM concentration. Addition of BSM-degrading bacterial strains *Bacillus megaterium* L1 into BSM contaminated paddy soil could have the half-life time of BSM compared to treatment without *Bacillus megaterium* L1 inoculation. Denaturing gradient gel electrophoresis and principle component analysis indicated that the diversity of the soil microbial community structure changed along with the addition of BSM, which recovered at the end of the experiment (5 weeks). Addition of BSM-degrading bacteria *Bacillus megaterium* L1 enriched the diversity of soil microbial community structure in paddy soils. This study provides information on the biodegradation of BSM and BSM’s influences on the soil bacteria microbial community structures.

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and Technology, Tsukuba, Ibaraki 305-8566, Japan). **Estrogenic activity of biodegradation products of C-heavy oil revealed by gene-expression profiling using an oligo-DNA microarray system. *Environmental Pollution*, Volume 168 (2012) : 10–14**

Degradation of heavy oil by bacteria to decompose organic compounds such as aliphatic and aromatic hydrocarbons has been used in bioremediation. However, the biological and environmental effects of the degradation products including intermediates are still not clear. Here, we monitored the degradation of C-heavy oil by analyzing the products formed in cultures with oil-degrading bacteria (complex microbes or a single bacterial strain). Furthermore, proliferation assays using breast cancer MCF-7 cells and gene-expression profiling of MCF-7 cells using oligonucleotide-DNA microarrays were performed to evaluate the estrogenic activity of the degradation products. While the products did not show any significant cell-proliferative activity, the oil samples cultured for longer periods (2–3 months), whether cultured with mixed microbes or a single bacterial strain, showed gene-expression profiles similar to that of 17 $\beta$ -estradiol (E<sub>2</sub>). These results suggest that oil-degradation products have estrogenic activity, and estrogen-like components could possibly be produced during the degradation process.

**Keywords:** Bioremediation; C-heavy oil; Gene-expression profiling; DNA microarray; Estrogen

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A quantitative knowledge of the fate of deicing chemicals in the subsurface can be provided by joint analysis of lab experiments with numerical simulation models. In the present study, published experimental data of microbial degradation of the deicing chemical propylene glycol (PG) under flow conditions in soil columns were simulated inversely to receive the parameters of degradation. We evaluated different scenarios of an advection-dispersion model including different terms for degradation, such as zero order, first order and inclusion of a growing and decaying biomass for their ability to explain the data. The general break-through behavior of propylene glycol in soil columns can be simulated well using a coupled model of solute transport and degradation with growth and decay of biomass. The susceptibility of the model to non-unique solutions was investigated using systematical forward and inverse simulations. We found that the model tends to equifinal solutions under certain conditions.

**Keywords:** Propylene glycol; Monod kinetics; Column experiment; Airfield soil; Equifinality; Airport winter operation; Biodegradation

**Ruiyu Zuo<sup>1</sup>, Juan Chang<sup>1</sup>, Qingqiang Yin<sup>1,2\*</sup>, Ping Wang<sup>2</sup>, Wei Cheng<sup>3</sup>, Xiao Wang<sup>2</sup>, Junxi Liu<sup>2</sup> and Qihong Zheng<sup>1</sup>.** (<sup>1</sup>College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China, <sup>2</sup>Henan Engineering and Technology Research Center of Feed Microbes, Zhoukou 466000, China, <sup>3</sup>Zhengzhou College of Animal Husbandry Engineering, Zhengzhou 450008, China. \*Corresponding author. E-

mail: QQZ22@yahoo.com.cn. Tel: 86 0371 63558180. Fax: 86 0371 63558998).  
**Inhibiting *Aspergillus flavus* growth and degrading aflatoxin B<sub>1</sub> by combined beneficial microbes. African Journal of Biotechnology, Vol. 11(65) (2012): 12903-12909**

Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is a type of toxin produced by *Aspergillus flavus*, which has a negative effect on animal production and economic profits. In order to inhibit *A. flavus* growth and degrade aflatoxin, the optimal proportion of beneficial microbes such as *Lactobacillus casei*, *Bacillus subtilis* and *Pichia anomala* were selected. The results show that AFB<sub>1</sub> production and mycelium weight of *A. flavus* was decreased by more than 34 folds (161.05 vs. 4.69 µg/L) and 7.7 folds (6.98 vs. 0.90 mg/ml) with the free-cell supernatants of *L. casei* and *B. subtilis* (P<0.05), respectively. The optimal proportion of *L. casei*, *B. subtilis* and *P. anomala* was 2:1:2 for inhibiting *A. flavus* growth determined by 3×3 orthogonal design. Based on the optimal proportion of three microbial species, the maximum AFB<sub>1</sub> degradation was during 24 to 48 h incubation (P<0.05). When three species of beneficial microbes were mixed with yeast cell wall and oligosaccharide, both of them could not help the microbes in AFB<sub>1</sub> degradation. The combined microbial incubation showed that AFB<sub>1</sub> contents in the supernatant and cells were 10.25 (P<0.05) and 3.34 µg/L, lower than the control group (68.55 µg/L), indicating that most of the AFB<sub>1</sub> were degraded by the microbes and only a little of them were absorbed and deposited in microbial cells.

**Keywords:** *Aspergillus flavus*, aflatoxin B<sub>1</sub> detoxification, beneficial microbes, yeast cell wall, oligosaccharide.

**Ji-Quan Sun<sup>a, b</sup>, Lian Xu<sup>a, b</sup>, Yue-Qin Tang<sup>a</sup>, Fu-Ming Chen<sup>b</sup>, Xiao-Lei Wu<sup>a</sup>.** (<sup>a</sup> College of Engineering, Peking University, Beijing 100871, PR China, <sup>b</sup> Research Institute of Tsinghua University in Shenzhen, Shenzhen 518057, PR China). **Simultaneous degradation of phenol and *n*-hexadecane by *Acinetobacter* strains. Bioresource Technology, Volume 123 (2012): 664–668**

Three phenol- and alkanes-degrading bacterial strains were isolated from a freshwater sample. Upon the 16S rRNA gene analysis, phenotype and physiological features, the three strains were designated as *Acinetobacter* sp. with both phenol hydroxylase gene (*phe*) and alkane monooxygenase gene (*alkM*) detected. They could simultaneously degrade phenol and *n*-hexadecane for growth, but prefer phenol than *n*-hexadecane. Between phenol (400 mg l<sup>-1</sup>) and *n*-hexadecane (400 mg l<sup>-1</sup>), *n*-hexadecane enhanced phenol degradation in mineral salt medium (MSM), while phenol affects negatively the *n*-hexadecane degradation. However, combination of phenol (400 mg l<sup>-1</sup>) and *n*-hexadecane (400 mg l<sup>-1</sup>) in MSM led to higher growth of the strains than the phenol and *n*-hexadecane separately. The transcription levels of *phe* and *alkM* genes supported the physiological properties of the strains.

**Keywords:** Biodegradation; *Acinetobacter* spp; Phenol; *n*-Hexadecane; Co-contamination

**Naresh K. Sharma, Ligy Philip, S. Murty Bhallamudi.** (Environmental and Water Resources Engineering Division, Department of Civil Engineering, IIT Madras, Chennai 600 036, India). **Aerobic degradation of phenolics and aromatic hydrocarbons in presence of cyanide. Bioresource Technology, Volume 121(2012): 263–273**

Present study focused on the degradation of a mixture of phenol, cresol, xylene, quinoline, and indole along with cyanide, commonly found in coke oven wastewater, using aerobic mixed

culture. It was found that xylenol and indole were difficult to degrade, when the concentrations were above 250 mg/L. It was observed that free cyanide (2.5 mg/L and above) has the potency to holdup the oxidation of organics (250 mg/L) until the cyanide concentration drops to a minimum level. Final TOC in the mixed pollutant system was less than 4 mg/L, indicating the absence of other organic byproducts. Experimental results highlight effect of free cyanide on removal of organics and the combined toxic influence of cyanide and organics on the microbes treating coking wastewater. The proposed mathematical model was able to predict the biodegradation of mixed pollutant system satisfactorily.

**Keywords:** Activated sludge; Phenolics; Aromatic hydrocarbons; Cyanide; Coking wastewater

**Soumitra Ghorai<sup>a</sup>, Apurba Sinhamahapatra<sup>b</sup>, Asish Sarkar<sup>a</sup>, Asit Baran Panda<sup>b</sup>, Sagar Pal<sup>a</sup>.** (<sup>a</sup> Polymer Chemistry Laboratory, Department of Applied Chemistry, Indian School of Mines, Dhanbad 826 004, India, <sup>b</sup> Discipline of Inorganic Materials and Catalysis, Central Salt and Marine Chemicals Research Institute (CSIR-CSMCRI), Bhavnagar 364021, Gujarat, India). **Novel biodegradable nanocomposite based on XG-g-PAM/SiO<sub>2</sub>: Application of an efficient adsorbent for Pb<sup>2+</sup> ions from aqueous solution. Bioresource Technology, Volume 119(2012): 181–190**

This article highlights the development of a novel nanocomposite based on nanosilica filled modified natural polymer (i.e. xanthan gum grafted with polyacrylamide:XG-g-PAM) for removal of Pb<sup>2+</sup> ions from aqueous solution. The chemical, structural, textural, and rheological characteristics of the nanocomposite (XG-g-PAM/SiO<sub>2</sub>) revealed stronger interaction of silica nanoparticles with polymer matrix and showed maximum adsorption capacity ( $Q_{\max} = 537.634 \text{ mg g}^{-1}$ ) of Pb<sup>2+</sup> ion, which is significantly higher than other reported adsorbents. This developed novel material also finds potential application as an efficient adsorbent for the treatment of battery industry wastewater. The enhanced adsorption efficiency may be because of its higher hydrodynamic radius and hydrodynamic volume. The adsorption kinetic parameters were best described by pseudo-second-order model. The adsorption equilibrium data fitted well with Langmuir isotherm. The thermodynamic studies confirm that the adsorption is spontaneous and endothermic. Desorption studies affirmed the regenerative efficacy of loaded Pb<sup>2+</sup>.

**Keywords:** Adsorption; Biodegradable; Graft copolymer; Nanocomposite; Xanthan gum

**P.J. Welz<sup>a</sup>, J.-B. Ramond<sup>b</sup>, D.A. Cowan<sup>b</sup>, S.G. Burton<sup>a</sup>.** (<sup>a</sup> Biocatalysis and Technical Biology (BTB) Research Group, Cape Peninsula University of Technology, Bellville Campus, P.O. Box 1906, Bellville 7535, South Africa, <sup>b</sup> Institute for Microbial Biotechnology and Metagenomics, University of the Western Cape, Bellville 7535, South Africa). **Phenolic removal processes in biological sand filters, sand columns and microcosms. Bioresource Technology, Volume 119(2012): 262–269**

This study evaluated the removal processes involved in the removal of the phenolic component of winery wastewater in biological sand filters, sand columns and sand microcosms. It was found that at low influent phenolic concentrations, complete organic removal was accomplished, but at high concentrations, there was incomplete substrate removal and an accumulation of potentially toxic metabolites, including catechol. The sand provided a suitable substrate for the treatment of

phenolic-laden waste, and both biotic (48%) and abiotic (52%) removal mechanisms effected the removal of model phenolics. Prior acclimation of microbial communities increased the biodegradation rate of phenolic acids significantly.

**Keywords:** Biological sand filter; Constructed wetland; Organic; Phenolic; Winery wastewater

**Sergio D. Ríos<sup>a, b</sup>, Joan Salvadó<sup>a, b</sup>, Xavier Farriol<sup>a</sup>, Carles Torras<sup>b</sup>. ( <sup>a</sup> Departament d'Enginyeria Química, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain, <sup>b</sup> Catalonia Institute for Energy Research, IREC, Marcel·lí Domingo 2, 43007 Tarragona, Catalonia, Spain). Antifouling microfiltration strategies to harvest microalgae for biofuel. *Bioresource Technology*, Volume 119 (2012) : 406–418**

Microalgae are microorganisms that can fix CO<sub>2</sub> by using the energy from the sun and transforming it into organic molecules such as lipids (i.e. feedstock for biodiesel production). Microfiltration is a promising method to be considered in the harvesting step. In this study, two antifouling methods were tested in order to minimize permeability decrease over time, at low trans-membrane pressure filtration.

Preliminary experiments were performed to find optimum conditions of transmembrane pressure, rotational speed and membrane pore size. Pilot experiments were carried out in the optimal conditions using microalgae obtained from the culture step and from a previous concentration process based on sedimentation. Fouling was significantly minimized, and the permeability plateau increased up to 600 L/h/m<sup>2</sup>/bar.

Three microalgae species were tested: *Phaeodactylum tricornutum* (Pht), *Nannochloropsis gaditana* (Nng) and *Chaetoceros calcitrans* (Chc).

An economic assessment was also performed, which demonstrated that dynamic filtration is economically more efficient than tangential cross-flow filtration.

**Keywords:** Microfiltration; Fouling; Microalgae; Economic assessment

**Mohan Pal, Gautam Srivastava, Lomary S. Moon<sup>1</sup>, Ravinder S. Jolly. (Institute of Microbial Technology (Council of Scientific and Industrial Research), Sector 39, Chandigarh 160036, India). Bioreduction of methyl heteroaryl and aryl heteroaryl ketones in high enantiomeric excess with newly isolated fungal strains. *Bioresource Technology*, Volume 118(2012): 306–314**

Enantioenriched heteroaryl ethanols and aryl heteroarylmethanols are important intermediates and structural motifs in medicinal chemistry. Asymmetric biocatalytic reduction of corresponding ketones provides a straightforward approach for preparation of these compounds. Accordingly, three newly isolated fungal strains have been described, which produced the desired heteroaryl alcohols in high enantiomeric excess (ee). A broad substrate specificity was observed within these limited number of biocatalysts as demonstrated by preparation of a variety of heteroaryl alcohols, including (*S*)-5-(1-hydroxyethyl)furo[2,3-*c*]pyridine, a key intermediate for HIV-1 reverse transcriptase inhibitor, (*S*)-phenyl(pyridin-2-yl)methanol, an analgesic and (*S,S*)-2,6-bis(1-hydroxyethyl)pyridine, a chiral building block, mostly in >99% ee and 80–92% yield. Micro-morphologically, one of the isolate was found to be similar to *Penicillium funiculosum*. However, its  $\beta$ -tubulin sequence showed only 88% sequence identity with the

known  $\beta$ -tubulin sequences of *Penicillium*. It may, therefore, represent a new species of *Penicillium*. The other biocatalysts were identified as *Alternaria alternata* and *Talaromyces flavus*.

**Keywords:** Bioreduction; Heteroaryl ethanols; Aryl heteroarylmethanols; (S)-5-(1-hydroxyethyl)furo[2,3-c]pyridine; (S)-phenyl(pyridin-2-yl)methanol

**Bhargav C. Patel, Devayani R. Tipre, Shailesh R. Dave. (Department of Microbiology and Biotechnology, School of Sciences, Gujarat University, Ahmedabad 380 009, Gujarat, India). Development of *Leptospirillum ferriphilum* dominated consortium for ferric iron regeneration and metal bioleaching under extreme stresses. Bioresource Technology, Volume 118(2012): 483–489**

Activated iron oxidizing consortium SR-BH-L enriched from Rajpardi lignite mine soil sample gave iron oxidation rate 1954 mg/L/h. Developed novel polystress resistant consortium oxidized ferrous iron under 11cP viscosity, 7.47 M ionic strength, 2.3 pH and g/L of 0.50 cadmium, 3.75 copper, 0.20 lead, 92.00 zinc, 6.4 sodium, 5.5 chloride, 154 sulphate and 393.8 TDS. The developed consortium showed 78.0% and 70.0% copper and zinc extraction from polymetallic bulk concentrate in monophasic bioleaching process. The bioregenerated ferric by the consortium in leachate showed 80.81% and 54.0% copper and zinc leaching in only 30 and 90 min. The DGGE analysis indicated the presence of 11 OTUs in the consortium. 16S rRNA gene sequence (JN797729) of the dominant band on DGGE shared >99% similarity with *Leptospirillum ferriphilum*. RE digestion analysis of the total 16S rRNA gene also illustrated the dominance of *L. ferriphilum* in the consortium.

**Keywords:** *Leptospirillum ferriphilum*; Bioleaching; DGGE; Ferric regeneration; Restriction digestion

**Weizhong Wu, Feifei Yang, Luhua Yang. (Department of Environmental Science, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, PR China). Biological denitrification with a novel biodegradable polymer as carbon source and biofilm carrier. Bioresource Technology, Volume 118(2012): 136–140**

A novel biodegradable polymer composed of PHBV and PLA was prepared for advanced wastewater treatment. It could serve as both biofilm carrier and carbon source for denitrification. Results of batch test showed the average denitrification rate was 0.07 mg NO<sub>3</sub>-N/(g h). The kinetic study demonstrated that when nitrate concentration was above 10.00 mg/L, DOC could not be detected in the effluent. In continuous packed-bed reactor, the average nitrogen removal efficiency was 94.11%. Nitrite concentration throughout the experiment was below 0.15 mg/L. The formation of NH<sub>4</sub>-N was observed, though small. DOC released in the effluent did not exceed 16.00 mg/L in the whole process, and it finally dropped below 1.20 mg/L.

**Keywords:** Biodegradable polymer; Carbon source; Biofilm carrier

**Qiong Jin, Zhongce Hu, Zanfeng Jin, Lequan Qiu, Weihong Zhong, Zhiyan Pan. (College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, PR China). Biodegradation of aniline in an alkaline environment by a**

**novel strain of the halophilic bacterium, *Dietzia natronolimnaea* JQ-AN. *Bioresource Technology*, Volume 117(2012) : 148–154**

*Dietzia natronolimnaea* JQ-AN was isolated from industrial wastewater containing aniline. Under aerobic conditions, the JQ-AN strain degraded 87% of the aniline in a 300 mg L<sup>-1</sup> aniline solution after 120 h of shake flask incubation in a medium containing sodium acetate. This strain had an unusually high salinity tolerance in minimal medium (0–6% NaCl, w/v). The optimal pH for microbial growth and aniline biodegradation was pH 8.0. Two liters of simulated aniline wastewater was created in a reactor at pH 8.0 and 3% NaCl (w/v), and biodegradation of aniline was tested over 7 days at 30 °C. For the initial concentrations of 100, 300, and 500 mg L<sup>-1</sup>, 100%, 80.5% and 72% of the aniline was degraded, respectively. Strain JQ-AN may use an *ortho*-cleavage pathway for dissimilation of the catechol intermediate.

**Keywords:** *Dietzia*; Biodegradation; Aniline; Salinity; Alkalinity

**Alessandro Spagni, Stefania Casu, Selene Grilli. (ENEA, Italian National Agency for New Technologies, Energy and the Sustainable Economic Development, Environment Department, Water Resource Management Laboratory, via M.M. Sole 4, 40129 Bologna, Italy). Decolourisation of textile wastewater in a submerged anaerobic membrane bioreactor. *Bioresource Technology*, Volume 117(2012) :180–185**

Azo dye decolourisation can be easily achieved by biological reduction under anaerobic conditions. The aim of this study was to evaluate the applicability of submerged anaerobic membrane bioreactors (SAMBRs) for the decolourisation of dyeing wastewater containing azo dyes. The reactive orange 16 was used as model of an azo dye. The results demonstrated that very high decolourisation (higher than 99%) can be achieved by SAMBRs. Although decolourisation was not significantly influenced by the azo dye concentrations up to 3.2 g L<sup>-1</sup>, methane production was greatly inhibited (up to 80–85%). Since volatile fatty acids accumulated in the treatment system with the azo dye concentration increase, methanogenes seem to be the most sensitive microbial populations of the anaerobic ecological community. The results demonstrated that anaerobic process combined with membrane filtration can deal with highly concentrated wastewaters that result from stream separation of industrial discharges.

**Keywords:** Decolourisation; Azo dye; Anaerobic digestion; Membrane bioreactor; High strength textile wastewater

**Eldon R. Rene, Balsam T. Mohammad, María C. Veiga, Christian Kennes. (Chemical Engineering Laboratory, Faculty of Sciences, Rúa da Fraga 10, University of La Coruña, 15008 La Coruña, Spain). Biodegradation of BTEX in a fungal biofilter: Influence of operational parameters, effect of shock-loads and substrate stratification, *Bioresource Technology*, Volume 116, (2012): 204–213**

The effect of relative humidity (RH: 30% to >95%) of a gas-phase mixture composed of benzene, toluene, ethylbenzene and *para*-, *meta*- and *ortho*-xylenes (BTEX), inlet concentrations (0.2–12.6 g m<sup>-3</sup>), and empty bed residence times (EBRTs) (48–144 s) was tested in a fungi-dominant biofilter. A maximum elimination capacity (EC<sub>max</sub>) of 244.2 gBTEX m<sup>-3</sup> h<sup>-1</sup> was achieved at a total inlet loading rate (ILR<sub>T</sub>) of 371.2 gBTEX m<sup>-3</sup> h<sup>-1</sup> (RH: 65%). The transient-state response was tested by increasing the ILR<sub>T</sub>, in two steps, from ~ 50 to 850 g m<sup>-3</sup> h<sup>-1</sup> and

from  $\sim 50$  to  $320 \text{ g m}^{-3} \text{ h}^{-1}$ , at a constant EBRT of 41.7 s. Increasing the ILR<sub>T</sub> reduced the total BTEX removal efficiency (RE<sub>T</sub>) from >97% to 35%, and from >90% to 60% during medium and high shock-load, respectively. When subjected to short (4 d) and long-term (7 d) shut-down periods, the biofilter was able to recover high EC<sub>max</sub> of, respectively, 200 and 72 gBTEX m<sup>-3</sup> h<sup>-1</sup> after resuming operation.

**Keywords:** Fungi; Relative humidity; pH; Gas to liquid (G/L) ratio; Transient-operations

**Junwei Qian<sup>a, b, c, d</sup>, Daping Li<sup>b, c, d</sup>, Guoqiang Zhan<sup>b, c, d</sup>, Liang Zhang<sup>b, c, d</sup>, Wentao Su<sup>b, c, d</sup>, Ping Gao<sup>a</sup>.** (<sup>a</sup>College of Life Sciences, Sichuan University, Chengdu 610064, China, <sup>b</sup> Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China, <sup>c</sup> Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu 610041, China, <sup>d</sup> Key Laboratory of Environmental and Applied Microbiology, Chinese Academy of Sciences, Chengdu 610041, China). **Simultaneous biodegradation of Ni–citrate complexes and removal of nickel from solutions by *Pseudomonas alcaliphila*. Bioresource Technology, Volume 116(2012) : 66–73**

The objective of this study was to study the simultaneous biodegradation of Ni–citrate complexes and removal of Ni from solutions by *Pseudomonas alcaliphila*. Adding excess citrate to 1:1 Ni–citrate complexes promoted the degradation of the complexes and removal of Ni. The alkaline pH generated by the metabolism of excess citrate caused partial dissociation of citrate from the Ni–citrate complexes, allowing degradation, and the released Ni was removed through bioaccumulation and precipitation. Addition of Fe<sup>3+</sup> enhanced the degradation of Ni–citrate complexes and removal of Ni from solutions. The displacement of Ni from recalcitrant Ni–citrate complexes by Fe<sup>3+</sup> and subsequent biodegradation of the degradable Fe(III)–citrate complex resulted in complete metabolism of citrate. The almost complete removal of Ni (>98%) can be attributed to the combination of coprecipitation with Fe<sup>3+</sup>, bioaccumulation and precipitation. *P. alcaliphila* potentially could be applied in the treatment of effluent containing Ni–citrate complexes.

**Keywords:** *Pseudomonas alcaliphila*; Ni–citrate complex; Biodegradation; Removal mechanism

**Yao Lu, Xian-Yong Wei, Jing-Pei Cao, Peng Li, Fang-Jing Liu, Yun-Peng Zhao, Xing Fan, Wei Zhao, Liang-Ce Rong, Yan-Bin Wei, Shou-Ze Wang, Jun Zhou, Zhi-Min Zong.** (Key Laboratory of Coal Processing and Efficient Utilization (Ministry of Education), China University of Mining & Technology, Xuzhou 221116, Jiangsu, China). **Characterization of a bio-oil from pyrolysis of rice husk by detailed compositional analysis and structural investigation of lignin. Bioresource Technology, Volume 116 (2012): 114–119**

Detailed compositional analysis of a bio-oil (BO) from pyrolysis of rice husk was carried out. The BO was extracted sequentially with *n*-hexane, CCl<sub>4</sub>, CS<sub>2</sub>, benzene and CH<sub>2</sub>Cl<sub>2</sub>. In total, 167 organic species were identified with GC/MS in the extracts and classified into alkanes, alcohols, hydroxybenzenes, alkoxybenzenes, dioxolanes, aldehydes, ketones, carboxylic acids, esters, nitrogen-containing organic compounds and other species. The benzene ring-containing species (BRCCs) were attributed to the degradation of lignin while most of the rests were derived from the degradation of cellulose and hemicellulose. Along with guaiacyl and *p*-hydroxyphenyl units

as the main components, a new type of linkage was suggested, i.e., C<sub>ar</sub>-CH<sub>2</sub>-C<sub>ar</sub> in 4,4'-methylenebis(2,6-dimethoxyphenol). Based on the species identified, a possible macromolecular structure of the lignin and the mechanism for its pyrolysis are proposed. The BO was also extracted with petroleum ether in ca. 17.8% of the extract yield and about 82.1% of the extracted components are BRCCs.

**Keywords:** Rice husk; Pyrolysis; Compositional analysis; Sequential extraction; Lignin

**Simone Larcher, Viviane Yargeau. (Department of Chemical Engineering, McGill University, 3610 University Street, Montréal, QC, Canada, H3A 2B2). Biodegradation of sulfamethoxazole: current knowledge and perspectives. Applied Microbiology and Biotechnology, Volume 96 (2) (2012): 309-318**

Antibiotic compounds, like sulfamethoxazole (SMX), have become a concern in the aquatic environment due to the potential development of antibacterial resistances. Due to extensive consumption, excretion and disposal, SMX has been frequently detected in wastewaters and surface waters. This has led to numerous studies investigating the nature of SMX, with many researchers focusing on the biodegradation and persistence of SMX during wastewater treatment and in the environment. This review provides a summary of recent developments, outlines the discrepancies in observations and results, and demonstrates the need for further research to determine optimal biological removal strategies for SMX and other antibiotics.

**Guoqiang Zhan<sup>1,2,3</sup>, Daping Li<sup>1,2</sup>, Liang Zhang<sup>1,2,3</sup>. (<sup>1</sup>Key Laboratory of Environmental and Applied Microbiology, Chengdu Institute of Biology, Chinese Academy of Sciences, 9 South Renmin Road, 4th Section, Chengdu, Sichuan, 610041, China, <sup>2</sup>Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu, 610041, China, <sup>3</sup>Graduate University of the Chinese Academy of Sciences, Beijing, 100049, China). Aerobic bioreduction of nickel(II) to elemental nickel with concomitant biomineralization. Applied Microbiology and Biotechnology, Volume 96 (1) (2012): 273-281**

Although microorganisms have the potential to reduce metals, products with elementary forms are unusual. In the present study, a strain of *Pseudomonas* sp. MBR was tested for its ability to reduce metal ions to their elementary forms coupled to biomineralization under aerobic conditions. The *Pseudomonas* sp. MBR strain was able to reduce metals such as Fe(III), Mn(II), Cu(II), Ni(II), Cd(II), Co(II), Al(III), Se(IV), and Te(IV) as electron acceptors to elementary forms using citrate, lactate, pyruvate, succinate, malate, glucose, or ethanol as electron donors. Growth and reduction during biomineralization occurred within the pH range of 6.0 to 11.0 and temperature range of 4 to 40 °C, with an optimum growth temperature of 28 °C. The resistance of Ni(II) varied from 0.5 to 5 mM. Ni(II) reduction was still observed when nitrate was present in addition to oxygen as a potential electron acceptor. The Ni(II) reduction efficiency was related with the molar ratio of the electron donor to Ni(II). Unlike other dissimilatory metal-reducing bacteria, which oxidizes organic matter with Fe(III) or Mn(IV) as the sole electron acceptor coupled to energy production under facultative anaerobic conditions, this strain used oxygen as an electron acceptor combined with metal reduction. The aerobic metal reduction may relate to a co-metabolic reduction. Transmission electron microscopy images demonstrated that the cells had the ability to accumulate heavy metals, and that the detoxicity mechanism was intracellular metal reduction. These results suggested that the use of *Pseudomonas* sp. MBR could be promising for toxic heavy metal bioremediation and biological metallurgy.

Jean-Patrick Toussaint<sup>1,2</sup>, Thi Thanh My Pham<sup>1</sup>, Diane Barriault<sup>1</sup>, Michel Sylvestre<sup>1</sup>. (<sup>1</sup>Institut National de la Recherche Scientifique, INRS-Institut Armand-Frappier, 531 Boul. des Prairies, Laval, QC, H7V 1B7, Canada, <sup>2</sup>Present address: Fondation David Suzuki, 50 rue Sainte-Catherine Ouest, Montréal, QC, H2X 3 V4, Canada. Corresponding author, Email: michel.sylvestre@iaf.inrs.ca). Plant exudates promote PCB degradation by a rhodococcal rhizobacteria. *Applied Microbiology and Biotechnology*, Volume 95(6) (2012): 1589-1603

*Rhodococcus erythropolis* U23A is a polychlorinated biphenyl (PCB)-degrading bacterium isolated from the rhizosphere of plants grown on a PCB-contaminated soil. Strain U23A *bphA* exhibited 99% identity with *bphA1* of *Rhodococcus globerulus* P6. We grew *Arabidopsis thaliana* in a hydroponic axenic system, collected, and concentrated the plant secondary metabolite-containing root exudates. Strain U23A exhibited a chemotactic response toward these root exudates. In a root colonizing assay, the number of cells of strain U23A associated to the plant roots ( $5.7 \times 10^5$  CFU g<sup>-1</sup>) was greater than the number remaining in the surrounding sand ( $4.5 \times 10^4$  CFU g<sup>-1</sup>). Furthermore, the exudates could support the growth of strain U23A. In a resting cell suspension assay, cells grown in a minimal medium containing *Arabidopsis* root exudates as sole growth substrate were able to metabolize 2,3,4'- and 2,3',4-trichlorobiphenyl. However, no significant degradation of any of congeners was observed for control cells grown on Luria-Bertani medium. Although strain U23A was unable to grow on any of the flavonoids identified in root exudates, biphenyl-induced cells metabolized flavanone, one of the major root exudate components. In addition, when used as co-substrate with sodium acetate, flavanone was as efficient as biphenyl to induce the biphenyl catabolic pathway of strain U23A. Together, these data provide supporting evidence that some rhodococci can live in soil in close association with plant roots and that root exudates can support their growth and trigger their PCB-degrading ability. This suggests that, like the flagellated Gram-negative bacteria, non-flagellated rhodococci may also play a key role in the degradation of persistent pollutants.

Anouk F. Duque<sup>1</sup>, Syed A. Hasan<sup>2</sup>, Vânia S. Bessa<sup>1</sup>, Maria F. Carvalho<sup>1</sup>, Ghufrana Samin<sup>2</sup>, Dick B. Janssen<sup>2</sup>, Paula M. L. Castro<sup>1</sup>. (<sup>1</sup>CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Oporto, Portugal, <sup>2</sup>Laboratory of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands. Corresponding author, Email: plcastro@esb.ucp.pt). Isolation and characterization of a *Rhodococcus* strain able to degrade 2-fluorophenol. *Applied Microbiology and Biotechnology*, Volume 95 (2) (2012): 511-520

A pure bacterial culture able to utilize 2-fluorophenol (2-FP) as sole carbon and energy source was isolated by selective enrichment from sediments collected from a contaminated site in Northern Portugal. 16S rRNA gene analysis showed that the organism (strain FP1) belongs to the genus *Rhodococcus*. When grown aerobically on 2-FP, growth kinetics of strain FP1 followed the Luong model. An inhibitory effect of increasing 2-FP concentrations was observed with no growth occurring at 2-FP levels higher than ca. 4 mM. *Rhodococcus* strain FP1 was able to degrade a range of other organofluorine compounds, including 2-fluorobenzoate, 3-fluorobenzoate, 4-fluorobenzoate, 3-fluorophenol, 4-fluorophenol, 3-fluorocatechol, and 4-fluorocatechol, as well as chlorinated compounds such as 2-chlorophenol and 4-chlorophenol.

Experiments with cell-free extracts and partially purified enzymes indicated that the first step of 2-fluorophenol metabolism was conversion to 3-fluorocatechol, suggesting an unusual pathway for fluoroaromatic metabolism. To our knowledge, this is the first time that utilization of 2-FP as a growth substrate by a pure bacterial culture is reported.

**Rashmi Paliwal, Anand Prabha Rawat, Monica Rawat, J. P. N. Rai. (G.B.Pant. University of Agriculture and Technology, Udham Singh Nagar, Uttarakhand, India. J. P. N. Rai, Email: jamesraionline@gmail.com). Bioligninolysis: Recent Updates for Biotechnological Solution. Applied Biochemistry and Biotechnology, Volume 167(7) (2012): 1865-1889**

Bioligninolysis involves living organisms and/or their products in degradation of lignin, which is highly resistant, plant-originated polymer having three-dimensional network of dimethoxylated (syringyl), monomethoxylated (guaiacyl), and non-methoxylated (*p*-hydroxyphenyl) phenylpropanoid and acetylated units. As a major repository of aromatic chemical structures on earth, lignin bears paramount significance for its removal owing to potential application of bioligninolytic systems in industrial production. Early reports illustrating the discovery and cloning of ligninolytic biocatalysts in fungi was truly a landmark in the field of enzymatic delignification. However, the enzymology for bacterial delignification is hitherto poorly understood. Moreover, the lignin-degrading bacterial genes are still unknown and need further exploration. This review deals with the current knowledge about ligninolytic enzyme families produced by fungi and bacteria, their mechanisms of action, and genetic regulation and reservations, which render them attractive candidates in biotechnological applications.

**Raj Boopathy<sup>1</sup>, Sara Shields<sup>2</sup>, Siva Nunna<sup>1</sup>. (<sup>1</sup>Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310, USA, <sup>2</sup>Department of Biological Sciences, Mississippi State University, Starkville, MS 39762, USA. Raj Boopathy, Email: Ramaraj.Boopathy@nicholls.edu). Biodegradation of Crude Oil from the BP Oil Spill in the Marsh Sediments of Southeast Louisiana, USA. Applied Biochemistry and Biotechnology, Volume 167(6) (2012): 1560-1568**

The significant challenges presented by the April 20, 2010 explosion, sinking, and subsequent oil spill of the Deepwater Horizon drilling platform in Canyon Block 252 about 52 miles southeast of Venice, LA, USA greatly impacted Louisiana's coastal ecosystem including the sea food industry, recreational fishing, and tourism. The short-term and long-term impact of this oil spill are significant, and the Deepwater Horizon spill is potentially both an economic and an ecological disaster. Microbes present in the water column and sediments have the potential to degrade the oil. Oil degradation could be enhanced by biostimulation method. The conventional approach to bioremediation of petroleum hydrocarbon is based on aerobic processes. Anaerobic bioremediation has been tested only in a very few cases and is still considered experimental. The currently practiced conventional in situ bioremediation of petroleum-contaminated soils and ground water relies on the supply of oxygen to the subsurface to enhance natural aerobic processes to remediate the contaminants. However, anaerobic microbial processes can be significant in oxygen-depleted subsurface environments and sediments that are contaminated with petroleum-based compounds such as oil-impacted marshes in Louisiana. The goal of this work was to identify the right conditions for the indigenous anaerobic bacteria present in the contaminated sites to enhance degradation of petroleum hydrocarbons. We evaluated the ability of microorganisms under a variety of electron acceptor conditions to degrade petroleum hydrocarbons. Researched microbial systems include sulfate-, nitrate-reducing bacteria, and fermenting bacteria. The results indicated that anaerobic bacteria are viable candidates for

bioremediation. Enhanced biodegradation was attained under mixed electron acceptor conditions, where various electron-accepting anaerobes coexisted and aided in degrading complex petroleum hydrocarbon components of marsh sediments in the coastal Louisiana. Significant degradation of oil also occurred under sulfate-reducing and nitrate-reducing conditions.

**E. Suja, Y. Venkata Nancharaiah, Vayalam P. Venugopalan. (Biofouling and Biofilm Processes Section, Water and Steam Chemistry Division, Bhabha Atomic Research Centre, Kalpakkam, 603102, Tamil Nadu, India. Vayalam P. Venugopalan, Email: vpv@igcar.gov.in). *p*-Nitrophenol Biodegradation by Aerobic Microbial Granules. *Applied Biochemistry and Biotechnology*, Volume 167 (6) (2012): 1569-1577**

Mixed microbial consortia in the form of aerobic microbial granules (AMG) capable of xenobiotic degradation can be developed from activated sludge or by adaptation of microbial granules pre-grown on labile carbon sources. Both of these approaches were investigated for the cultivation of AMG capable of *p*-nitrophenol (PNP) biodegradation. Attempts to cultivate AMG from activated sludge using PNP as the sole carbon source were not successful due to poor microbial growth and washout of the inoculated activated sludge. As part of the second approach, parallel sequencing batch reactors (SBRs) were inoculated with pre-grown AMG and operated by feeding both acetate and PNP together (RA), PNP alone (RB) or acetate alone (RC). Acetate/PNP mineralization and nitrification were monitored in the three SBRs. PNP biodegradation was quickly established in both RA and RB. PNP removal rates were found to be 47 and 55 mg g VSS<sup>-1</sup> h<sup>-1</sup> in RA and RB, respectively. PNP biodegradation during the SBR cycle consisted of distinct lag, exponential and deceleration phases. However, with higher concentrations of PNP (>50 mg l<sup>-1</sup>), disintegration of granules was observed in RA and RB. When PNP was the sole carbon source, it inhibited the development of aerobic granules from activated sludge and caused disintegration of pre-cultivated aerobic granules. When PNP was the co-substrate along with acetate, the structural and functional integrity (including nitrification) of the granular sludge was maintained. This report highlights the importance of a labile co-substrate for maintaining the physical and functional integrity of granular sludge, when used for toxic waste degradation.

**Tatoba R. Waghmode<sup>1</sup>, Mayur B. Kurade<sup>2</sup>, Harshad S. Lade<sup>1</sup>, Sanjay P. Govindwar<sup>1</sup>. (<sup>1</sup>Department of Biochemistry, Shivaji University, Kolhapur, 416004, India, <sup>2</sup>Department of Biotechnology, Shivaji University, Kolhapur, 416004, India. Corresponding author, Email: spg\_biochem@unishivaji.ac.in). Decolorization and Biodegradation of Rubine GFL by Microbial Consortium GG-BL in Sequential Aerobic/Microaerophilic Process. *Applied Biochemistry and Biotechnology*, Volume 167(6) (2012): 1578-1594**

This study represents the development of a new batch method by consortium GG-BL using two microbial cultures viz., *Galactomyces geotrichum* MTCC 1360 and *Brevibacillus laterosporus* MTCC 2298, by varying environmental conditions for the decolorization and biodegradation of Rubine GFL. Consortium was found to give better decolorization and degradation of Rubine GFL as compared to the individual microorganism at aerobic/microaerophilic process. The consortial metabolic activity of these strains lead to 100% decolorization of Rubine GFL (50 mg/L) within 30 h with significant reduction in chemical oxygen demand (79%) and total organic carbon (68%). Induction in the activities of laccase, veratryl alcohol oxidase, tyrosinase,

azo reductase, and riboflavin reductase suggested their role in the decolorization process. Nondenaturing polyacrylamide gel electrophoresis analysis showed differential induction pattern of oxidoreductive enzymes during decolorization of the dye at different incubation temperatures. The degradation of Rubine GFL into different metabolites by individual organism and in consortium was confirmed using high performance thin layer chromatography, high performance liquid chromatography, Fourier transform infrared spectroscopy, and gas chromatography-mass spectrometry analysis. Phytotoxicity studies revealed nontoxic nature of the metabolites of Rubine GFL.

**Garima Mathur<sup>1</sup>, Ramasare Prasad<sup>2</sup>. (<sup>1</sup>Department of Biotechnology, Jaypee Institute of Information Technology, Noida, India, <sup>2</sup>Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee, 247667, India. Corresponding author, Email: rapdyfbs@iitr.ernet.in). Degradation of Polyurethane by *Aspergillus flavus* (ITCC 6051) Isolated from Soil. *Applied Biochemistry and Biotechnology*, Volume 167(6) (2012): 1595-1602**

The present study deals with the isolation of fungi from soil with the ability to degrade polyurethane (PU). A pure fungal isolate was analyzed for its ability to utilize PU as a sole carbon source in shaking culture for 30 days. Incubation of PU with *Aspergillus flavus* resulted in 60.6% reduction in weight of PU. The scanning electron microscopy and Fourier transform infrared spectroscopy (FTIR) results showed certain changes on the surface of PU film and formation of some new intermediate products after polymer breakdown. Thermogravimetric curves showed changes between the thermal behavior of the samples that were inoculated with *A. flavus* and control. FTIR spectra showed detectable changes in control and incubated samples, suggesting that degradation occurs, with the decreased intensity of band at 1,715 cm<sup>-1</sup>, corresponding to ester linkages. We have identified an extracellular esterase activity which might be responsible for the polyurethanolytic activity.

**Prajeesh Gangadharan Puthiya Veetil, Anupama Vijaya Nadaraja, Arya Bhasi, Sudheer Khan, Krishnakumar Bhaskaran. (Environmental Technology, National Institute for Interdisciplinary Science & Technology (CSIR-India) Industrial estate (PO), Thiruvananthapuram, 695 019, India. Email: krishna@niist.res.in). Degradation of Triclosan under Aerobic, Anoxic, and Anaerobic Conditions. *Applied Biochemistry and Biotechnology*, Volume 167 (6) (2012): 1603-1612**

Triclosan (2, 4, 4'-trichloro-2'-hydroxyl diphenyl ether) is a broad-spectrum antimicrobial agent present in a number of house hold consumables. Aerobic and anaerobic enrichment cultures tolerating triclosan were developed and 77 bacterial strains tolerating triclosan at different levels were isolated from different inoculum sources. Biodegradation of triclosan under aerobic, anoxic (denitrifying and sulphate reducing conditions), and anaerobic conditions was studied in batch cultures with isolated pure strains and enrichment consortium developed. Under aerobic conditions, the isolated strains tolerated triclosan up to 1 g/L and degraded the compound in inorganic-mineral-broth and agar media. At 10 mg/L level triclosan, 95±1.2% was degraded in 5 days, producing phenol, catechol and 2, 4-dichlorophenol as the degradation products. The strains were able to metabolize triclosan and its degradation products in the presence of monooxygenase inhibitor 1-pentyne. Under anoxic/anaerobic conditions highest degradation (87%) was observed in methanogenic system with acetate as co-substrate and phenol, catechol, and 2, 4-dichlorophenol were among the products. Three of the isolated strains tolerating 1 g/L triclosan were identified as *Pseudomonas* sp. (BDC 1, 2, and 3).

Sanghyun Jeong<sup>1</sup>, Lan Hee Kim<sup>2</sup>, Sung-Jo Kim<sup>2</sup>, Tien Vinh Nguyen<sup>1</sup>, Saravanamuthu Vigneswaran<sup>1</sup>, In S. Kim<sup>2</sup>. (<sup>1</sup>Faculty of Engineering and IT, University of Technology, Sydney (UTS), P.O. Box 123, Broadway, NSW 2007 Sydney, Australia, <sup>2</sup>School of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), 261 Oryong-dong, Buk-gu, Gwangju, 500-712, Korea. Email: s.vigneswaran@uts.edu.au). **Biofouling Potential Reductions Using a Membrane Hybrid System as a Pre-treatment to Seawater Reverse Osmosis. Applied Biochemistry and Biotechnology, Volume 167(6) (2012): 1716-1727**

Biofouling on reverse osmosis (RO) membranes is the most serious problem which affects desalination process efficiency and increases operation cost. The biofouling cannot be effectively removed by the conventional pre-treatment traditionally used in desalination plants. Hybrid membrane systems coupling the adsorption and/or coagulation with low-pressure membranes can be a sustainable pre-treatment in reducing membrane fouling and at the same time improving the feed water quality to the seawater reverse osmosis. The addition of powder activated carbon (PAC) of 1.5 g/L into submerged membrane system could help to remove significant amount of both hydrophobic compounds (81.4%) and hydrophilic compounds (73.3%). When this submerged membrane adsorption hybrid system (SMAHS) was combined with FeCl<sub>3</sub> coagulation of 0.5 mg of Fe<sup>3+</sup>/L, dissolved organic carbon removal efficiency was excellent even with lower dose of PAC (0.5 g/L). Detailed microbial studies conducted with the SMAHS and the submerged membrane coagulation-adsorption hybrid system (SMCAHS) showed that these hybrid systems can significantly remove the total bacteria which contain also live cells. As a result, microbial adenosine triphosphate (ATP) as well as total ATP concentrations in treated seawater and foulants was considerably decreased. The bacteria number in feed water prior to RO reduced from 5.10E<sup>+06</sup> cells/mL to 3.10E<sup>+03</sup> cells/mL and 9.30E<sup>+03</sup> cells/mL after SMAHS and SMCAHS were applied as pre-treatment, respectively. These led to a significant reduction of assimilable organic carbon (AOC) by 10.1 µg/L acetate-C when SMCAHS was used as a pre-treatment after 45-h RO operation. In this study, AOC method was modified to measure the growth of bacteria in seawater by using the *Pseudomonas P.60* strain.

**C. Juárez-Ramírez, R. Velázquez-García, N. Ruiz-Ordaz, J. Galíndez-Mayer, O. Ramos Monroy. (Departamento de Ingeniería Bioquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Carpio y Plan de Ayala, Col Santo Tomás, CP 11340 Mexico, D.F., Mexico. Email: cmayer@encb.ipn.mx). Degradation kinetics of 4-amino naphthalene-1-sulfonic acid by a biofilm-forming bacterial consortium under carbon and nitrogen limitations. Journal of Industrial Microbiology & Biotechnology, Volume 39 (8) (2012): 1169-1177**

By decolorization of azo dyes, caused by reductive cleavage of the azo linkage, toxic or recalcitrant amines are generated. The present study deals with the effect of the inflowing medium composition (C:N ratio) on the kinetic behavior of a bacterial biofilm-forming consortium, able to use as carbon, nitrogen and sulfur source, the molecule of 4-aminonaphthalene-1-sulfonic acid (4ANS), which is one of the most recalcitrant byproducts generated by decolorization of azo dyes. All the experiments were carried out at room temperature in a lab-scale packed-bed biofilm reactor. Because environmental conditions affect the bioreactor performance, two mineral salts media containing 4ANS, with distinct C:N ratios;

0.68 (carbon as the limiting nutrient) and 8.57 (nitrogen as the limiting nutrient) were used to evaluate their effect on 4ANS biodegradation. By HPLC and COD measurements, the 4ANS removal rates and removal efficiencies were determined. The cultivable bacterial strains that compose the consortium were identified by their 16S rDNA gene sequence. With the enrichment technique used, a microbial consortium able to use efficiently 4ANS as the sole carbon source and energy, nitrogen and sulfur, was selected. The bacterial strains that constitute the consortium were isolated and identified. They belong to the following genera: *Bacillus*, *Arthrobacter*, *Microbacterium*, *Nocardioides*, and *Oleomonas*. The results obtained with this consortium showed, under nitrogen limitation, a remarkable increase in the 4ANS removal efficiency  $\eta_{\text{ANS}}$ , and in the 4ANS volumetric removal rates  $R_{\text{V,4ANS}}$ , as compared to those obtained under carbon limitation. Differences observed in bioreactor performance after changing the nutrient limitation could be caused by changes in biofilm properties and structure.

**Aju K. Asok\*, M. S. Jisha. (School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, 686560, India. Email: jishams@mgu.ac.in). Biodegradation of the Anionic Surfactant Linear Alkylbenzene Sulfonate (LAS) by Autochthonous *Pseudomonas* sp. Water, Air, & Soil Pollution, Volume 223 (8) (2012): 5039-5048**

Anionic surfactants, the earliest and the most common surfactants in detergent and cosmetic product formulations contribute significantly to the pollution profile of the ecosystem. Linear alkylbenzene sulfonates (LAS), a major chemical constituent of detergents, forms an imperative group of anionic surfactants. Bioremediation of LAS by conventional processes such as activated sludge is ineffective due to the low kinetics of degradation by unsuitable organisms and foam production. Hence this study was focused on isolating and characterizing indigenous LAS-degrading bacteria from soil. Twenty different LAS-degrading bacteria were isolated from detergent-contaminated soil by enrichment culture technique and degradation efficiency was assessed by Methylene Blue Active Substances (MBAS) assay and by reverse-phase high-performance liquid chromatography (HPLC) analysis. The most efficient LAS-degrading isolates, L9 (81.33±0.7) and L12 (81.81±0.8), were selected and identified as *Pseudomonas nitroreducens* (MTCC 10463) and *Pseudomonas aeruginosa* (MTCC 10462). The 16S rDNA sequences of the isolates were deposited in NCBI GenBank under the accession numbers HQ 271083 (L9) and HQ 271084 (L12). The isolates were capable of degrading 0.05 g/l LAS at 25 °C and pH 7.0–7.5. Presence of a solid support caused biofilm formation which in turn enhanced LAS degradation. The isolates tend to display diauxic growth with alternate carbon source such as dextrose. These isolates also have the capability to degrade other xenobiotics like hydrocarbons and pesticides. Since xenobiotic pollutants in nature occur as a mixture of compounds rather than single pollutants, the potential of these two indigenous LAS-degrading isolates to degrade multiple xenobiotics gains relevance.

**Che Hasnam Che Noraini, Norhashimah Morad, Ismail Norli, Tjoon Tow Teng, Chimezie Jason Ogugbue. (Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia. Email: nhashima@usm.my). Methylene Blue Degradation by *Sphingomonas paucimobilis* under Aerobic Conditions. Water, Air, & Soil Pollution, Volume 223(8) (2012): 5131-5142**

The presence of synthetic dyes in industrial wastewaters may create serious environmental problems due to their mutagenicity and toxicity to aquatic life and humans. In this study, the decolourization and degradation of methylene blue (MB) by a *Sphingomonas paucimobilis* strain isolated from industrial wastewater was investigated under aerobic conditions. Decolourization

extent of MB in medium was over 85 % when the bacterium was grown on a high concentration of the dye (1,000 mg/L) after a retention time of 5 days, while reduction in COD was 92.99 % suggesting mineralization of dyes as a result of microbial activities. The bacterium retained decolourizing activity over a wide range of pH (2–10), with peak activity obtained at pH 9. Analysis of samples extracted from decolourized culture flasks at pH 9 using UV–visible and Fourier transform infrared (FTIR) spectroscopy confirmed that the mechanism of colour removal was due to biodegradation rather than adsorption of dye on cells. Scanning and transmission electron microscopy revealed the secretion of exopolysaccharides (EPS) by *S. paucimobilis* cells on exposure to MB—a probable physiological defence mechanism to ensure controlled diffusion of dye molecules into cellular structures. Biokinetic coefficients, namely, growth yield,  $Y$ ; specific biomass decay,  $K_d$ ; maximum specific substrate rate,  $k$ ; saturation constant for substrate,  $K_s$ ; and maximum specific biomass growth rate,  $\mu_{max}$ , were determined by the Monod type kinetic equation. Results indicate that *S. paucimobilis* holds a promise as a good candidate for the biological treatment of industrial effluent containing high concentrations of synthetic dyes.

**Brijesh K. Yadav<sup>1,2\*</sup>, Shristi R. Shrestha<sup>1</sup>, S. Majid Hassanizadeh<sup>1</sup>.** (<sup>1</sup>Environmental Hydrogeology Group, Faculty of Geosciences, University of Utrecht, Budapestlaan 4, P.O. Box 80021, 3508, TA, Utrecht, The Netherlands, <sup>2</sup>Department of Civil Engineering, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, 110016, India. Email: brijeshy@gmail.com). **Biodegradation of Toluene Under Seasonal and Diurnal Fluctuations of Soil-Water Temperature. *Water, Air, & Soil Pollution*, Volume 223 (7) (2012): 3579-3588,**

An increasing interest in bioremediation of hydrocarbon polluted sites raises the question of the influence of seasonal and diurnal changes on soil-water temperature on biodegradation of BTEX, a widespread group of (sub)-surface contaminants. Therefore, we investigated the impact of a wide range of varying soil-water temperature on biodegradation of toluene under aerobic conditions. To see the seasonal impact of temperature, three sets of batch experiments were conducted at three different constant temperatures: 10°C, 21°C, and 30°C. These conditions were considered to represent (1) winter, (2) spring and/or autumn, and (3) summer seasons, respectively, at many polluted sites. Three additional sets of batch experiments were performed under fluctuating soil-water temperature cases (21↔10°C, 30↔21°C, and 10↔30°C) to mimic the day–night temperature patterns expected during the year. The batches were put at two different temperatures alternatively to represent the day (high-temperature) and night (low-temperature) times. The results of constant- and fluctuating-temperature experiments show that toluene degradation is strongly dependent on soil-water temperature level. An almost two-fold increase in toluene degradation time was observed for every 10°C decrease in temperature for constant-temperature cases. Under fluctuating-temperature conditions, toluene degraders were able to overcome the temperature stress and continued thriving during all considered weather scenarios. However, a slightly longer time was taken compared to the corresponding time at daily mean temperature conditions. The findings of this study are directly useful for bioremediation of hydrocarbon-polluted sites having significant diurnal and seasonal variations of soil-water temperature.

**Tian Li<sup>\*</sup>, Xin-Ping Deng, Jin-Jun Wang, Hui Zhao, Lei Wang, Kun Qian. (Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing, 400715, People's Republic of China. Email: jjwang7008@yahoo.com). Biodegradation of 3,4-Dichloroaniline by a Novel *Myroides odoratimimus* Strain LWD09 with Moderate Salinity Tolerance. Water, Air, & Soil Pollution, Volume 223(6) (2012) : 3271-3279**

A Gram-negative bacterium strain LWD09, capable of growing aerobically on 3,4-dichloroaniline (DCA) as the sole carbon and energy source, was isolated from the farm field. This bacterium was identified as *Myroides odoratimimus* strain by morphological, physiological, and biochemical characteristics as well as 16S rDNA sequence. Analysis of culture pH, temperature, cells growth, and DCA concentration demonstrated that strain LWD09 could effectively degrade DCA without a lag phase. The kinetics of DCA degradation was well described using the Andrews equation, and the kinetic parameters were as follows:  $q_{\max}=1.74 \text{ h}^{-1}$ ,  $K_s=43.5 \text{ mg L}^{-1}$ , and  $K_i=230.3 \text{ mg L}^{-1}$ . In addition, strain LWD09 was found to be moderately halophilic and showed the highest power of DCA degradation in 5% NaCl (*w/w*, %). With initial concentrations of 30, 100, and 200  $\text{mg L}^{-1}$ , 100%, 80.4%, and 33.2% of DCA were transformed after 96 h in 5% NaCl, respectively. These results suggest that strain LWD09 has the potential to degrade DCA in saline wastewater. To date, this is the first report on the degradation of DCA by a *M. odoratimimus* strain with moderate salinity tolerance.

**Qammer Zaib<sup>1\*</sup>, Ifthekeer A. Khan<sup>1</sup>, Navid B. Saleh<sup>1</sup>, Joseph R. V. Flora<sup>1</sup>, Yong-Gyun Park<sup>2</sup>, Yeomin Yoon<sup>1</sup>. (<sup>1</sup>Department of Civil and Environmental Engineering, University of South Carolina, Columbia, SC 29208, USA, <sup>2</sup>Environmental and Energy Research Team, GS E&C Research Institute, 417-1 Deokseong-ri Ydong-myeon, Cheoin-gu Yongin-si, Gyeonggi-do, 449-831, South Korea. Email: yoony@cec.sc.edu). Removal of Bisphenol A and 17 $\beta$ -Estradiol by Single-Walled Carbon Nanotubes in Aqueous Solution: Adsorption and Molecular Modeling. Water, Air, & Soil Pollution, Volume 223 (6) (2012): 3281-3293**

Single-walled carbon nanotubes, both before (SWNTs) and after treatment (t-SWNTs) with acidified ammonium persulfate, were successfully used to adsorb bisphenol A (BPA) and 17 $\beta$ -estradiol (E2) from aqueous systems. The surface characteristics of the SWNTs and t-SWNTs were analyzed by measuring their surface charge and by imaging their morphological properties through transmission electron microscopy. The extent of defects on the SWNT scaffold generated through acid etching was analyzed by Raman spectroscopy. A total of 19.4, 15.4, and 14.3  $\text{mg/g}$  of BPA was adsorbed on SWNTs, while a total of 8.0, 6.4, and 5.1  $\text{mg/g}$  was adsorbed on t-SWNTs with a 72-h contact time at 280, 295, and 315 K, respectively. A significantly high fraction of E2 (27.2  $\text{mg/g}$ ) was absorbed by both SWNTs and t-SWNTs, as compared to BPA. The adsorption kinetics was analyzed using a pseudo-second-order model. Sorption experiments showed that t-SWNTs adsorbed less than half as much BPA as SWNTs, but their E2 adsorption was similar. The sorption mechanism was investigated by performing molecular-level calculations. Adsorption energies calculated using density functional theory show preferential sorption of E2 to SWNTs and graphene ( $-26.2 \text{ kcal/mol}$  on SWNT and  $-34.1 \text{ kcal/mol}$  on graphene) compared to BPA ( $-17.1 \text{ kcal/mol}$  on SWNT and  $-22.5 \text{ kcal/mol}$  on graphene), which were consistent with the experimental findings. Thus, ab initio calculations can mechanistically explain the adsorption differences of BPA and E2 on SWNTs.

**Raquel Simarro<sup>1\*</sup>, Natalia González<sup>1</sup>, L. Fernando Bautista<sup>2</sup>, M. Carmen Molina<sup>1</sup>, Emanuele Schiavi<sup>3</sup>. (<sup>1</sup>Department of Biology and Geology, ESCET, Universidad Rey Juan**

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For a bioremediation process to be effective, we suggest to perform preliminary studies in laboratory to describe and characterize physicochemical and biological parameters (type and concentration of nutrients, type and number of microorganisms, temperature) of the environment concerned. We consider that these studies should be done by taking into account the simultaneous interaction between different factors. By knowing the response capacity to pollutants, it is possible to select and modify the right treatment conditions to enhance bioremediation.

**Fan Li<sup>1\*</sup>, Dan Yu<sup>2</sup>, Xiumei Lin<sup>1</sup>, Dongbo Liu<sup>1</sup>, Hongmei Xia<sup>1</sup>, Shan Chen<sup>1</sup>. (<sup>1</sup>School of Life Sciences, Northeast Normal University, Changchun, 130024, China, <sup>2</sup>Changchun Medical College, Changchun, 130013, China. Email: lifan0302@163.com). Biodegradation of poly( $\epsilon$ -caprolactone) (PCL) by a new *Penicillium oxalicum* strain DSYD05-1. *World Journal of Microbiology and Biotechnology, Volume 28 (10) (2012): 2929-2935***

In this study, fungi isolated from soil were screened for their ability to form clear zones on agar plates with emulsified poly( $\epsilon$ -caprolactone) (PCL). The most active strain, designated as DSYD05, was identified as *Penicillium oxalicum* on the basis of morphological characteristics and phylogenetic analysis. Mutant DSYD05-1, obtained by ultraviolet-light mutagenesis from strain DSYD05, was more effective in PCL degradation. In liquid cultures of the mutant strain with PCL emulsion, DSYD05-1 showed the highest PCL-degrading activity after 4 days of cultivation. The products of PCL degradation were analysed by mass spectrometry; the results indicated that 6-hydroxyhexanoic acid was produced and assimilated during cultivation. The degradation of PCL film by DSYD05-1 was observed by scanning electron microscopy, and was indicative of a three-stage degradation process. The degradation of amorphous parts of the film preceded that of the crystalline center and then the peripheral crystalline regions. In addition, DSYD05-1 showed a wide range of substrate specificity, with capability to degrade PCL, poly( $\beta$ -hydroxybutyrate), and poly(butylene succinate), but not poly(lactic acid), indicating that the strain could have potential for application in the treatment or recycling of bio-plastic wastes.

**P. V. Suresh\*. (Department of Meat, Fish and Poultry Technology, CSIR-Central Food Technological Research Institute, Mysore, 570020, India. Email: drsureshpv@hotmail.com). Biodegradation of shrimp processing bio-waste and concomitant production of chitinase enzyme and *N*-acetyl-D-glucosamine by marine bacteria: production and process optimization. *World Journal of Microbiology and Biotechnology, Volume 28 (10) (2012): 2945-2962***

A total of 250 chitinolytic bacteria from 68 different marine samples were screened employing enrichment method that utilized native chitin as the sole carbon source. After thorough

screening, five bacteria were selected as potential cultures and identified as; *Stenotrophomonas* sp. (CFR221 M), *Vibrio* sp. (CFR173 M), *Phyllobacteriaceae* sp. (CFR16 M), *Bacillusadius*(CFR198 M) and *Bacillus* sp. (CFR188 M). All five strains produced extracellular chitinase and GlcNAc in SSF using shrimp bio-waste. Scanning electron microscopy confirmed the ability of these marine bacteria to adsorb onto solid shrimp bio-waste and to degrade chitin microfibers. HPLC analysis of the SSF extract also confirmed presence of 36–65 % GlcNAc as a product of the degradation. The concomitant production of chitinase and GlcNAc by all five strains under SSF using shrimp bio-waste as the solid substrate was optimized by ‘one factor at a time’ approach. Among the strains, *Vibrio* sp. CFR173 M produced significantly higher yields of chitinase (4.8 U/g initial dry substrate) and GlcNAc (4.7 µmol/g initial dry substrate) as compared to other cultures tested. A statistically designed experiment was applied to evaluate the interaction of variables in the biodegradation of shrimp bio-waste and concomitant production of chitinase and GlcNAc by *Vibriosp.* CFR173 M. Statistical optimization resulted in a twofold increase of chitinase, and a 9.1 fold increase of GlcNAc production. These results indicated the potential of chitinolytic marine bacteria for the reclamation of shrimp bio-waste, as well as the potential for economic production of chitinase and GlcNAc employing SSF using shrimp bio-waste as an ideal substrate.

**Atipan Saimmai<sup>1</sup>, Onkamon Rukadee<sup>2</sup>, Theerawat Onlamool<sup>2</sup> Vorasan Sobhon<sup>2</sup>, Suppasil Maneerat<sup>2\*</sup>. (1Faculty of Agricultural Technology, Phuket Rajabhat University, Phuket, 83000, Thailand, 2Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand. Email: suppasil.m@psu.ac.th). Isolation and functional characterization of a biosurfactant produced by a new and promising strain of *Oleomonas sagaranensis* AT18. World Journal of Microbiology and Biotechnology, Volume 28 (10) (2012): 2973-2986**

Biosurfactant-producing bacteria were isolated from mangrove sediment in southern Thailand. Isolates were screened for biosurfactant production by using the surface tension test. The highest reduction of surface tension was achieved with a bacterial strain which was identified by 16S rRNA gene sequencing as *Oleomonas sagaranensis* AT18. It has also been investigated using different carbon and nitrogen sources. It showed that the strain was able to grow and reduce the surface tension of the culture supernatant to 25 mN/m. In all 5.30 g of biosurfactant yield was obtained after 54 h of cultivation by using molasses and NaNO<sub>3</sub> as carbon and nitrogen sources, respectively. The biosurfactant recovery by chloroform:methanol extraction showed a small critical micelle concentration value (8 mg/l), thermal and pH stability with respect to surface tension reduction. It also showed emulsification activity and a high level of salt concentration. The biosurfactant obtained was confirmed as a glycolipid by using a biochemical test, FT-IR and mass spectra. The crude biosurfactant showed a broad spectrum of antimicrobial activity and also had the ability to emulsify oil and enhance PAHs solubility.

**I. B. Ivshina<sup>1,2</sup>, E. V. Vikhareva<sup>3</sup>, M. I. Richkova<sup>1</sup>, A. N. Mukhutdinova<sup>1</sup>, Ju. N. Karpenko<sup>3</sup>. (1Institute of Ecology and Genetics of Microorganisms, Russian Academy of Sciences, 13 Golev Str., 614081 Perm, Russia, 2Perm State National Research University, 15 Bukirev Str., 614900 Perm, Russia, 3Perm State Pharmaceutical Academy, 2 Poleyaya Str., Perm, Russia. Email: ivshina@iegm.ru). Biodegradation of drotaverine hydrochloride by free and immobilized cells of *Rhodococcus rhodochrous* IEGM 608. World Journal of Microbiology and Biotechnology, Volume 28 (10) (2012): 2997-3006**

Drotaverine [1 - (3, 4-diethoxybenzylidene)-6, 7-diethoxy-1, 2, 3, 4 - tetrahydroisoquinoline] hydrochloride, an antispasmodic drug derived from benzylisoquinoline was evaluated for its biodegradability using a bacterial strain *Rhodococcus rhodochrous* IEGM 608. The experiments were performed under aerobic conditions with rhodococci cultures able to degrade drotaverine. In the presence of glucose, the removal efficiency of drotaverine by free *Rhodococcus* cells pre-grown with isoquinoline was above 80 % (200 mg/l, initial concentration) after 25 days. *Rhodococcus* immobilization on hydrophobized sawdust enhanced the biodegradation process, with the most marked drotaverine loss being observed during the first 5 days of fermentation. High metabolic activity of rhodococcal cells towards drotaverine was confirmed respirometrically. GC-MS analysis of transformation products resulting from drotaverine biodegradation revealed 3,4-diethoxybenzoic acid, 3,4-diethoxybenzaldehyde and 3,4-diethoxybenzoic acid ethyl ester which were detected in the culture medium until drotaverine completely disappeared. Based on these major and other minor metabolites, putative pathways for drotaverine biodegradation were proposed. The obtained data broadened the spectrum of organic xenobiotics oxidized by *Rhodococcus* bacteria and proved their potential in decontamination of natural ecosystems from pharma pollutants.

**Ghufrana Samin\*, Dick B. Janssen. (Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, the Netherlands. Email: d.b.janssen@rug.nl). Transformation and biodegradation of 1,2,3-trichloropropane (TCP). Environmental Science and Pollution Research, Volume 19(8) (2012): 3067-3078,**

1,2,3-Trichloropropane (TCP) is a persistent groundwater pollutant and a suspected human carcinogen. It is also an industrial chemical waste that has been formed in large amounts during epichlorohydrin manufacture. In view of the spread of TCP via groundwater and its toxicity, there is a need for cheap and efficient technologies for the cleanup of TCP-contaminated sites. In situ or on-site bioremediation of TCP is an option if biodegradation can be achieved and stimulated. This paper presents an overview of methods for the remediation of TCP-contaminated water with an emphasis on the possibilities of biodegradation. Although TCP is a xenobiotic chlorinated compound of high chemical stability, a number of abiotic and biotic conversions have been demonstrated, including abiotic oxidative conversion in the presence of a strong oxidant and reductive conversion by zero-valent zinc. Biotransformations that have been observed include reductive dechlorination, monooxygenase-mediated cometabolism, and enzymatic hydrolysis. No natural organisms are known that can use TCP as a carbon source for growth under aerobic conditions, but anaerobically TCP may serve as electron acceptor. The application of biodegradation is hindered by low degradation rates and incomplete mineralization. Protein engineering and genetic modification can be used to obtain microorganisms with enhanced TCP degradation potential.

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Polyvinyl alcohol (PVA) has been widely used as sizing agents in textile and manufacturing industry, and it is a refractory compound with low biodegradability. The objective of this paper was to treat the PVA-containing wastewater using gamma irradiation as a pretreatment strategy to improve its biodegradability and to determine the roles of different kinds of radical species played during pretreatment. Gamma radiation was carried out in a <sup>60</sup>Cobalt source station, PVA concentration was analyzed by using a visible spectrophotometer and specific oxygen uptake rate (SOUR, milligram of O<sub>2</sub> per gram of mixed liquor volatile suspended solids (MLVSS) per hour) was measured by a microrespirometer. The results showed that the biodegradability of PVA-containing wastewater with low initial concentration (e.g., 327.8 mg/l) could be improved greatly with increasing irradiation dose. However, PVA gel formation was observed at higher initial PVA concentration (e.g., 3,341.6 mg/l) and higher irradiation dose, which inhibited PVA degradation by aerobic microorganisms. However, the formed gel could be separated by microfiltration, which led to more than 90% total organic carbon (TOC) removal. Ionizing radiation could be used as a pretreatment technology for PVA-containing wastewater, and its combination with biological process is feasible.

**Nyuk-Min Chong\*, Chun-Shuo Chang, Shiu-Ching Tsai. (Department of Environmental Engineering, Da-Yeh University, No. 168, University Rd., Dacun, Changhua, Taiwan, 51591, Republic of China. Email: chong@mail.dyu.edu.tw). Evolutions of microbial degradation pathways for parent xenobiotic and for its metabolites follow different schemes. Environmental Science and Pollution Research, Volume 19(8) (2012): 3276-3281**

The pathways used by microorganisms for the metabolism of every xenobiotic substrate are specific. The catabolism of a xenobiotic goes through a series of intermediate steps and lower intermediates (metabolites) appear in sequence. The structure of the metabolites can be similar to the parents due to kinship. The purposes of this study were to examine if the degradation pathways that were developed for a parent xenobiotic are effective to degrade the parent's lower metabolites, and if the reverse is true. The xenobiotic substrates, 2,4-dichlorophenoxyacetic acid (2,4-D, the parent xenobiotic) and its metabolite 2,4-dichlorophenol (2,4-DCP), were independently subjected to acclimation and degradation tests by the biomasses of mixed-culture activated sludge and a pure culture of *Arthrobacter* sp. Activated sludge and *Arthrobacter* sp. that were acclimated to 2,4-D effectively degraded 2,4-D and the lower metabolites of 2,4-D, typically 2,4-DCP. During the degradation of 2,4-D, accumulations of the lower metabolites of 2,4-D were not found. The degradation pathways acquired from acclimation to 2,4-D are effective for all the metabolites of 2,4-D. However, pathways acquired from acclimation to 2,4-DCP are not effective in the degradation of the parent 2,4-D. Microorganisms acclimated to 2,4-D evolve their degradation pathways by a scheme that is different from the scheme the microorganisms employ when they are acclimated to the metabolites of 2,4-D.

**Ashis K. Mukherjee\*, Naba K. Bordoloi. (Microbial Biotechnology and Protein Research Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur, 784 028, Assam, India. Email: akm@tezu.ernet.in). Biodegradation of benzene, toluene, and xylene (BTX) in liquid culture and in soil by *Bacillus subtilis* and *Pseudomonas aeruginosa* strains and a formulated bacterial consortium. Environmental Science and Pollution Research, Volume 19 (8) (2012): 3380-3388**

The major aromatic constituents of petroleum products viz. benzene, toluene, and mixture of xylenes (BTX) are responsible for environmental pollution and inflict serious public concern. Therefore, BTX biodegradation potential of individual as well as formulated bacterial

consortium was evaluated. This study highlighted the role of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), nitrate, and phosphate in stimulating the biodegradation of BTX compounds under hypoxic condition. The individual bacterium viz. *Bacillus subtilis* DM-04 and *Pseudomonas aeruginosa* M and NM strains and a consortium comprising of the above bacteria were inoculated to BTX-containing liquid medium and in soil. The bioremediation experiment was carried out for 120 h in BTX-containing liquid culture and for 90 days in BTX-contaminated soil. The kinetics of BTX degradation either in presence or absence of  $\text{H}_2\text{O}_2$ , nitrate, and phosphate was analyzed using biochemical and gas chromatographic (GC) technique. Bacterial consortium was found to be superior in degrading BTX either in soil or in liquid medium as compared to degradation of same compounds by individual strains of the consortium. The rate of BTX biodegradation was further enhanced when the liquid medium/soil was exogenously supplemented with 0.01 % (v/v)  $\text{H}_2\text{O}_2$ , phosphate, and nitrate. The GC analysis of BTX biodegradation (90 days post-inoculation) in soil by bacterial consortium confirmed the preferential degradation of benzene compared to *m*-xylene and toluene. It may be concluded that the bacterial consortium in the present study can degrade BTX compounds at a significantly higher rate as compared to the degradation of the same compounds by individual members of the consortium. Further, addition of  $\text{H}_2\text{O}_2$  in the culture medium as an additional source of oxygen, and nitrate and phosphate as an alternative electron acceptor and macronutrient, respectively, significantly enhanced the rate of BTX biodegradation under oxygen-limited condition.

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This study investigated the acute effect of benzo[*a*]anthracene, a significant compound among polycyclic aromatic hydrocarbons, on the biodegradation of a synthetic organic substrate—a peptone/meat extract mixture—under aerobic conditions. A laboratory-scale sequencing batch reactor was sustained at steady state at a sludge age of 10 days with substrate feeding. Inhibition tests involved running a series of batch reactors initially seeded with the biomass obtained from the parent reactor. After the biomass seeding, the reactors were started with the peptone mixture and a range of initial benzo[*a*]anthracene concentrations between 0.5 and 88 mg/L. Experimental profiles of oxygen uptake rates and polyhydroxyalkanoates were evaluated by calibration of a selected model. Lower doses of benzo[*a*]anthracene had no effect on process kinetics. The noticeable acute impact was only observed with the addition of 88 mg/L of benzo[*a*]anthracene, but it was limited with the storage mechanism: the amount of organic substrate diverted to polyhydroxyalkanoates was significantly reduced with a corresponding decrease in the maximum storage rate,  $k_{\text{STO}}$ , from 2.7 down to 0.6 day<sup>-1</sup>. Similarly, the maximum growth rate from internally stored polyhydroxyalkanoates was lowered from 2.3 to 1.0 day<sup>-1</sup>. Among the mechanisms for direct substrate utilization, only the hydrolysis rate was slightly reduced, but otherwise, the overall COD removal efficiency was not affected.

**Abd El-Latif Hesham<sup>1,2,3\*</sup>, Sardar Khan<sup>4</sup>, Yu Tao<sup>1</sup>, Dong Li<sup>1</sup>, Yu Zhang<sup>1</sup>, Min Yang<sup>1</sup>.** (<sup>1</sup>State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing, 100085, China, <sup>2</sup>Genetics Department, Faculty of Agriculture, Assiut University, Assiut, Egypt, <sup>3</sup>Biological Science Department, Faculty of Science, King Khalid University, Abha, Saudi Arabia, <sup>4</sup>Department of Environmental Sciences, University of Peshawar, 25120 Peshawar, Pakistan. Email: yangmin@rcees.ac.cn). **Biodegradation of high molecular weight PAHs using isolated yeast mixtures: application of meta-genomic methods for community structure analyses. Environmental Science and Pollution Research, Volume 19 (8) (2012): 3568-3578**

Bioaugmentation for the removal of polycyclic aromatic hydrocarbons (PAHs) from wastewater using bacteria and yeasts is considered environment-friendly and a cost-effective technique. The effectiveness of this biodegradation system depends on the stability of inoculated microorganisms and the availability of nutrients. This study is aimed to investigate the removal of high molecular weight (HMW)-PAHs from biologically treated produced water using different biological systems. Three systems, inoculated with activated sludge (AS), the mixture of five yeast strains (MY), and the mixture of AS and the five yeast strains (SY), respectively, were constructed, and their performance for the removal of HMW-PAHs was compared over 10 weeks. The effluent of the biologically treated produced water from an oilfield was used as the influent after chrysene and benzo(a)pyrene were spiked as HMW-PAHs. Polymerase chain reaction-based denaturing gradient gel electrophoresis (PCR-DGGE) and fluorescent in situ hybridization (FISH) techniques were used to examine the changes in the structures and abundances of the bacterial and yeast communities in these three systems. Only SY and MY systems were capable to remove chrysene (90.7 % and 98.5 %, respectively) and benzo(a)pyrene (80.7 % and 95.2 %, respectively). PCR-DGGE analysis confirmed that all of the five yeast strains inoculated remained in the SY and MY systems, while FISH results showed that the relative abundance of yeast in the SY and MY systems (10.6 % to 21.9 %, respectively) were significantly higher than AS system (2.3 % to 7.8 %, respectively). The relative abundances of the catechol 2,3-dioxygenase (*C23O*) indicated that the copy number ratios of benzene ring cleavage gene *C23O* in the yeast amended systems were much higher than that in the AS system. In this study, all of the three systems were effective in removing the low molecular weight (LMW)-PAHs, while HMW-PAHs including chrysene and benzo(a)pyrene were efficiently removed by MY and SY systems, not by AS system. The high HMW-PAHs removal in the MY and SY bioaugmentation systems possibly attributed to the inoculation of the mixed yeast culture. By combining the PCR-DGGE results with the FISH analyses, it was found that yeast probably consisting mainly of the five inoculated strains inhabited in the two bioaugmentation systems as a dominant population. The relatively higher performance of the SY system might be attributed to the suspended growth type which permitted a more efficient contact between microbial cells and contaminants. The bioaugmentation systems (SY and MY) were successfully established by inoculating with five nonindigenous yeast strains and demonstrated high performance in removal of HMW-PAHs.

**Stefan Gartiser<sup>1\*</sup>, Christoph Hafner<sup>1</sup>, Kerstin Kronenberger-Schäfer<sup>1</sup>, Oliver Happel<sup>2</sup>, Christoph Trautwein<sup>3</sup>, Klaus Kümmerer<sup>4</sup>.** (<sup>1</sup>Hydrotox GmbH, Boetzingen Straße 29, 79111 Freiburg, Germany, <sup>2</sup>DVGW-Technologiezentrum Wasser (TZW), Karlsruher Straße 84, 76139 Karlsruhe, Germany, <sup>3</sup>Department of Environmental Health Sciences, Institute of Environmental Medicine and Hospital Epidemiology, Breisacher Str. 115 B,

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79106 Freiburg, Germany, <sup>4</sup>Institute of Sustainable and Environmental Chemistry, Leuphana Universität Lüneburg, Scharnhorststraße 1, C 13, 21335 Lüneburg, Germany. Email: gartiser@hydrotox.de). Approach for detecting mutagenicity of biodegraded and ozonated pharmaceuticals, metabolites and transformation products from a drinking water perspective. *Environmental Science and Pollution Research*, Volume 19 (8) (2012): 3597-3609

Many pharmaceuticals and related metabolites are not efficiently removed in sewage treatment plants and enter into surface water. There, they might be subject of drinking water abstraction and treatment by ozonation. In this study, a systematic approach for producing and effect-based testing of transformation products (TPs) during the drinking water ozonation process is proposed. For this, two pharmaceutical parent substances, three metabolites and one environmental degradation product were investigated with respect to their biodegradability and fate during drinking water ozonation. The Ames test (TA98, TA100) was used for the identification of mutagenic activity present in the solutions after testing inherent biodegradability and/or after ozonation of the samples. Suspicious results were complemented with the umu test. Due to the low substrate concentration required for ozonation, all ozonated samples were concentrated via solid phase extraction (SPE) before performing the Ames test. With the exception of piracetam, all substances were only incompletely biodegradable, suggesting the formation of stable TPs. Metformin, piracetam and guanyurea could not be removed completely by the ozonation process. We received some evidence that technical TPs are formed by ozonation of metformin and piracetam, whereas all tested metabolites were not detectable by analytical means after ozonation. In the case of guanyurea, one ozonation TP was identified by LC/MS. None of the experiments showed an increase of mutagenic effects in the Ames test. However, the SPE concentration procedure might lead to false-positive results due to the generation of mutagenic artefacts or might lead to false-negative results by missing adequate recovery efficiency. Thus, these investigations should always be accompanied by process blank controls that are carried out along the whole ozonation and SPE procedure. The study presented here is a first attempt to investigate the significance of transformation products by a systematic approach. However, the adequacy and sensitivity of the methodology need to be further investigated. The approach of combining biodegradation and ozonation with effect-based assays is a promising tool for the early detection of potential hazards from TPs as drinking water contaminants. It can support the strategy for the evaluation of substances and metabolites in drinking water. A multitude of possible factors which influence the results have to be carefully considered, among them the selectivity and sensibility of the mutagenicity test applied, the extraction method for concentrating the relevant compounds and the biocompatibility of the solvent. Therefore, the results have to be carefully interpreted and possible false-negative and false-positive results should be considered.

Qiansheng Huang, Chao Fang, Yajie Chen, Xinlong Wu, Ting Ye, Yi Lin, Sijun Dong. (Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, 361021, People's Republic of China. Email: sjdong@iue.ac.cn). Embryonic exposure to low concentration of bisphenol A affects the development of *Oryzias melastigma* larvae. *Environmental Science and Pollution Research*, Volume 19 (7) (2012): 2506-2514

The prevalence of bisphenol A (BPA) in the environment has attracted increasing attention because of the toxicity of this manmade pollutant. However, the toxicity related to cardiac development remains largely unknown. In the present paper, we investigated the cardiac toxicity of BPA using marine medaka (*Oryzias melastigma*) embryos. At 2 days postfertilization (dpf), the embryos were continuously exposed to a low concentration of BPA (200 µg/L) for the whole embryonic stage. Heart rate and sinus venosus (SV)–bulbus arteriosus (BA) distance were measured under microscopy. The mRNA expression levels of genes were quantified by SYBR real-time RT-PCR, and hematoxylin and eosin (H&E) staining was used to examine the histology of fish larvae hearts. Neither the heart rate nor the SV-BA distance of the embryos was affected by BPA exposure. However, the mRNA expression levels of Na<sup>+</sup>–K<sup>+</sup>–ATPase, BMP4, COX-1, FGF8, GATA4, and NKX2.5 were all downregulated at the critical developmental stages (6 and 10 dpf). Interestingly, the mRNA expression levels of COX-2 and LERP were significantly upregulated at 10 dpf. The mRNA expressions of inflammation-related genes (*TNFα*, *IL1β*, *SOD*, and *CCL11*) were all significantly upregulated after exposure. Moreover, we found that both the body length and the body width decreased in the larvae after embryonic exposure to BPA. The distributed foci of inflammation were observed in the juveniles after 2 weeks' depuration. Exposure to BPA at embryonic stages could alter the expression of heart development-related genes and inflammation-related genes of *O. melastigma*. The larvae hatched from exposed embryos showed the foci of inflammation in the heart ventricles and the decrease of the body length and width.

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Chlorinated ethanes and ethenes are among the most frequently detected organic pollutants of water. Their physicochemical properties are such that they can contaminate aquifers for decades. In favourable conditions, they can undergo degradation. In anaerobic conditions, chlorinated solvents can undergo reductive dechlorination. Abiotic dechlorination is usually slower than microbial but abiotic dechlorination is usually complete. In favourable conditions, abiotic reactions bring significant contribution to natural attenuation processes. Abiotic agents that may enhance the reductive dechlorination of chlorinated ethanes and ethenes are zero-valent metals, sulphide minerals or green rusts. At some sites, permanganate and Fenton's reagent can be used as remediation tool for oxidation of chlorinated ethanes and ethenes. Nanoscale iron or bimetallic particles, due to high efficiency in degradation of chlorinated ethanes and ethenes, have gained much interest. They allow for rapid degradation of chlorinated ethanes and ethenes in water phase, but they also give benefit of treating dense non-aqueous phase liquid.

**Prosun Tribedi, Subhasis Sarkar, Koushik Mukherjee, Alok K. Sil. (Department of Microbiology, University of Calcutta, 35 B. C. Road, Kolkata, 700019, India. Email: tribedi.prosun@gmail.com). Isolation of a novel *Pseudomonas* sp from soil that can efficiently degrade polyethylene succinate. Environmental Science and Pollution Research, Volume 19 (6) (2012): 2115-2124**

Polyethylene succinate (PES) is a biodegradable synthetic polymer and therefore widely used as a base material in plastic industry to circumvent the environmental problems related with the non-biodegradability of other polymers like polyethylene. Till date only few organisms have

been reported to have the ability to degrade PES. Therefore for better management of PES-related environmental waste, the present study is targeted towards isolating mesophilic organism(s) capable of more efficient degradation of PES. Strain AKS2 was isolated from soil based on survival on a selection plate wherein PES was used as sole carbon source. Ribotyping and biochemical tests revealed that AKS2 is a new strain of *Pseudomonas*. Scanning electron and atomic force microscopic analysis of the PES films obtained after incubation with AKS2 confirmed PES-degradation ability of AKS2, wherein an alteration in surface topology was observed. The kinetics of PES weight loss showed that AKS2 degrades PES maximally during its logarithmic growth phase at a rate of 1.65 mg/day. This degradation is mediated by esterase activity and may also involve cell-surface hydrophobicity. It has also been observed that AKS2 is able to degrade PES considerably even in the presence of glucose, which is likely to increase the bioremediation potential of this isolate. A new strain of *Pseudomonas* has been isolated from soil that is able to adhere to PES and degrade this polymer efficiently. This organism has the potential to be implemented as a useful tool for bioremediation of PES-derived materials.

**Thirumavalavan Munusamy, Ya-Lan Hu, Jiunn-Fwu Lee.** (Graduate Institute of Environmental Engineering, National Central University, Chung-Li, Taoyuan County, 320, Taiwan. Email: jflee@ncuen.ncu.edu.tw). **Adsorption and photodegradation of microcystin-LR onto sediments collected from reservoirs and rivers in Taiwan: a laboratory study to investigate the fate, transfer, and degradation of microcystin-LR.** *Environmental Science and Pollution Research*, Volume 19 (6) (2012): 2390-2399

This study demonstrated the adsorption capacity of microcystin-LR (MC-LR) onto sediment samples collected from different reservoirs (Emerald and Jade reservoirs) and rivers (Dongshan, Erhjen, and Wukai rivers) in Taiwan to investigate the fate, transport behavior, and photodegradation of MC-LR. Langmuir adsorption and photodegradation studies were carried out in the laboratory and tested the capability of sediments for MC-LR adsorption. These data suggested that sediments play a crucial role in microcystins degradation in aquatic systems. The results of batch experiments revealed that the adsorption of MC-LR varied significantly with texture, pH, and organic matter content of sediments. Silty and clay textures of the samples were associated with larger content of organic matter, and they displayed the enhanced MC-LR adsorption. Low pH sediment showed increased adsorption of MC-LR. The effective photodegradation of MC-LR (1.6 µg/mL) was achieved within 60 min under 254 nm light irradiation. A comparative study of adsorption capacity of all sediment samples was carried out and discussed with respect to different aspects. Among all, sediments collected from Jade reservoir showed enhanced MC-LR adsorption (11.86 µg/g) due to favored textural properties (BET surface area = 20.24 m<sup>2</sup>/g and pore volume = 80.70 nm). These data provide important information that may be applied to management strategies for improvement of water quality in reservoirs and rivers and other water bodies in Taiwan.

**Evangelos Karanasios<sup>1,2</sup>, Nikolaos G. Tsiropoulos<sup>2</sup>, Dimitrios G. Karpouzas<sup>3</sup>.** (<sup>1</sup>Department of Pesticide Control and Phytopharmacy, Benaki Phytopathological Institute, 8 St. Delta, Str., 145 61 Kifissia, Athens, Greece, <sup>2</sup>Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Fytokou Street, Nea Ionia-Volos, 38446, Greece, <sup>3</sup>Department of Biochemistry and Biotechnology, University of Thessaly, Ploutonos 26 and Aioulou Str., Larisa, 41221, Greece. Email: dkarpouzas@bio.uth.gr). **On-farm**

**biopurification systems for the depuration of pesticide wastewaters: recent biotechnological advances and future perspectives. *Biodegradation*, Volume 23 (6) (2012): 787-802**

Point source contamination of natural water resources by pesticides constitutes a serious problem and on-farm biopurification systems (BPS) were introduced to resolve it. This paper reviews the processes and parameters controlling BPS depuration efficiency and reports on recent biotechnological advances which have been used for enhancing BPS performance. Biomixture composition and water management are the two factors which either individually or through their interactions control the depuration performance of BPS. Which process (biodegradation or adsorption) will dominate pesticides dissipation in BPS depends on biomixture composition and the physicochemical properties of the pesticides. Biotechnological interventions such as augmentation with pesticide-degrading microbes or pesticide-primed matrices have resulted in enhanced biodegradation performance of BPS. Despite all these advancement in BPS research, there are still several issues which should be resolved to facilitate their full implementation. Safe handling and disposal of the spent biomixture is a key practical issue which needs further research. The use of BPS for the depuration of wastewaters from post-farm activities such as postharvest treatment of fruits should be a priority research issue considering the lack of alternative treatment systems. However, the key point hampering optimization of BPS is the lack of fundamental knowledge on BPS microbiology. The use of advanced molecular and biochemical methods in BPS would shed light into this issue in the future.

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The capacity of an anaerobic sediment to achieve the simultaneous biodegradation of phenol and carbon tetrachloride (CT) was evaluated, using humic acids (HA) as redox mediator. The presence of HA in sediment incubations increased the rate of biodegradation of phenol and the rate of dehalogenation (2.5-fold) of CT compared to controls lacking HA. Further experiments revealed that the electron-accepting capacity of HA derived from different organic-rich environments was not associated with their reducing capacity to achieve CT dechlorination. The collected kinetic data suggest that the reduction of CT by reduced HA was the rate-limiting step during the simultaneous biodegradation of phenol and CT. To our knowledge, the present study constitutes the first demonstration of the simultaneous biodegradation of two priority pollutants mediated by HA.

**Irina S. Moreira, Catarina L. Amorim, Maria F. Carvalho, Paula M. L. Castro. (Centro de Biotecnologia e Química Fina (CBQF), Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal. Email: plcastro@porto.ucp.pt). Degradation of difluorobenzenes by the wild strain *Labrys portucalensis*. *Biodegradation*, Volume 23 (5) (2012): 653-662**

This study focuses on the biodegradation of difluorobenzenes (DFBs), compounds commonly used as intermediates in the industrial synthesis of various pharmaceutical and agricultural

chemicals. A previously isolated microbial strain (strain F11), identified as *Labrys portucalensis*, able to degrade fluorobenzene (FB) as sole carbon and energy source, was tested for its capability to degrade 1,2-, 1,3- and 1,4-DFB in batch cultures. Strain F11 could use 1,3-DFB as a sole carbon and energy source, with quantitative release of fluoride, but 1,4-DFB was only degraded and defluorinated when FB was supplied simultaneously. Growth of strain F11 with 0.5 mM of 1,3-DFB led to stoichiometric release of fluoride ion. The same result was obtained in cultures fed with 1 mM of 1,3-DFB or 0.5 mM of 1,4-DFB, in the presence of 1 mM of FB. No growth occurred with 1,2-DFB as substrate, and degradation of FB was inhibited when supplied simultaneously with 1,2-DFB. To our knowledge, this is the first time biodegradation of 1,3-DFB as a sole carbon and energy source, and cometabolic degradation of 1,4-DFB, by a single bacterium, is reported.

**Paweł Cyplik<sup>1</sup>, Agnieszka Piotrowska-Cyplik<sup>2</sup>, Roman Marecik<sup>1</sup>, Jakub Czarny<sup>3</sup>, Agnieszka Drożdżyńska<sup>1</sup>, Łukasz Chrzanowski<sup>4</sup>.** (<sup>1</sup>Department of Biotechnology and Food Microbiology, Poznań University of Life Sciences, Wojska Polskiego 48, 60-627 Poznań, Poland, <sup>2</sup>Institute of Food Technology of Plant Origin, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland, <sup>3</sup>Institute of Forensic Genetics, Al. Mickiewicza 3/4, 85-071 Bydgoszcz, Poland, <sup>4</sup>Institute of Chemical Technology and Engineering, Poznań University of Technology, Pl. M. Skłodowskiej-Curie 2, 60-965 Poznań, Poland. Email: pcyplik@wp.pl). **Biological denitrification of brine: the effect of compatible solutes on enzyme activities and fatty acid degradation. Biodegradation, Volume 23(5) (2012): 663-672,**

The effect of the addition of compatible solutes (ectoine and trehalose) on the denitrification process of saline wastewater was studied. In saline wastewater, it was observed that the initial concentration of nitrates was 500 mg N l<sup>-1</sup>. A fatty substance isolated from oiled bleaching earth (waste of vegetable oil refining process) was used as a source of carbon. The consortium, which was responsible for the denitrification process originated from the wastewater of the vegetable oil industry. The consortium of microorganisms was identified by the use of restriction fragment length polymorphism of 16S rRNA gene amplicons and sequencing techniques. It was noted that ectoine affects significantly the activity of lipase and nitrate reductase, and resulted in faster denitrification compared to saline wastewater with the addition of trehalose or control saline wastewater (without compatible solutes). It was observed that relative enzyme activities of lipase and nitrate reductase increased by 32 and 35%, respectively, in the presence of 1 mM ectoine. This resulted in an increase in specific nitrate reduction rate in the presence of 1 mM ectoine to 5.7 mg N g<sup>-1</sup> VSS h<sup>-1</sup>, which was higher than in the absence of ectoine (3.2 mg N g<sup>-1</sup> VSS h<sup>-1</sup>). The addition of trehalose did not have an effect on nitrate removals. Moreover, it was found that trehalose was used up completely by bacteria as a source of carbon in the denitrification process. The fatty acids were biodegraded by 74% in the presence of 1 mM ectoine.

**Natesan Manickam<sup>1</sup>, Abhay Bajaj<sup>1</sup>, Harvinder S. Saini<sup>3</sup>, Rishi Shanker<sup>2</sup>.** (<sup>1</sup>Environmental Biotechnology, Indian Institute of Toxicology Research, (Council of Scientific & Industrial Research), Lucknow, 226 001, India, <sup>2</sup>Environmental Microbiology, Indian Institute of Toxicology Research, (Council of Scientific & Industrial Research), Lucknow, 226 001, India, <sup>3</sup>Department of Microbiology, Guru Nanak Dev University, Amritsar, 143005, Punjab, India. Email: nmanickam@iitr.res.in). **Surfactant mediated enhanced**

**biodegradation of hexachlorocyclohexane (HCH) isomers by *Sphingomonas* sp. NM05. Biodegradation, Volume 23 (5) (2012): 673-682**

Environmental biodegradation of several chlorinated pesticides is limited by their low solubility and sorption to soil surfaces. To mitigate this problem we quantified the effect of three biosurfactant viz., rhamnolipid, sophorolipid and trehalose-containing lipid on the dissolution, bioavailability, and biodegradation of HCH-isomers in liquid culture and in contaminated soil. The effect of biosurfactants was evaluated through the critical micelle concentration (CMC) value as determined for each isomer. The surfactant increased the solubilization of HCH isomers by 3–9folds with rhamnolipid and sophorolipid being more effective and showing maximum solubilization of HCH isomers at 40 µg/mL, compared to trehalose-containing lipid showing peak solubilization at 60 µg/mL. The degradation of HCH isomers by *Sphingomonas* sp. NM05 in surfactant-amended liquid mineral salts medium showed 30% enhancement in 2 days as compared to degradation in 10 days in the absence of surfactant. HCH-spiked soil slurry incubated with surfactant also showed around 30–50% enhanced degradation of HCH which was comparable to the corresponding batch culture experiments. Among the three surfactants, sophorolipid offered highest solubilization and enhanced degradation of HCH isomers both in liquid medium and soil culture. The results of this study suggest the effectiveness of surfactants in improving HCH degradation by increased bioaccessibility.

**L. Giacomucci<sup>1</sup>, F. Toja<sup>2</sup>, P. Sanmartín<sup>3</sup>, L. Toniolo<sup>2</sup>, B. Prieto<sup>3</sup>, F. Villa<sup>1</sup>, F. Cappitelli<sup>1</sup>. (<sup>1</sup>Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy, <sup>2</sup>Dipartimento di Chimica, Materiali e Ingegneria Chimica ‘Giulio Natta’, Politecnico di Milano, Via Mancinelli 7, 20131 Milan, Italy, <sup>3</sup>Departamento de Edafología y Química Agrícola, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain. Email: francesca.cappitelli@unimi.it). Degradation of nitrocellulose-based paint by *Desulfovibrio desulfuricans* ATCC 13541. Biodegradation, Volume 23(5) (2012):705-716**

Nitrocellulose is one of the most commonly used compounds in ammunition and paint industries and its recalcitrance to degradation has a negative impact on human health and the environment. In this study the capability of *Desulfovibrio desulfuricans* ATCC 13541 to degrade nitrocellulose as binder in paint was assayed for the first time. Nitrocellulose-based paint degradation was followed by monitoring the variation in nitrate, nitrite and ammonium content in the culture medium using Ultraviolet–Visible spectroscopy. At the same time cell counts and ATP assay were performed to estimate bacterial density and activity in all samples. Infrared spectroscopy and colorimetric measurements of paint samples were performed to assess chemical and colour changes due to the microbial action. Microscope observations of nitrocellulose-based paint samples demonstrated the capability of the bacterium to adhere to the paint surface and change the paint adhesive characteristics. Finally, preliminary studies of nitrocellulose degradation pathway were conducted by assaying nitrate- and nitrite reductases activity in *D. desulfuricans* grown in presence or in absence of paint. We found that *D. desulfuricans* ATCC 13541 is able to transform nitrocellulose as paint binder and we hypothesised ammonification as degradation pathway. The results suggest that *D. desulfuricans* ATCC 13541 is a good candidate as a nitrocellulose-degrading bacterium.

**Ergo Rikmann<sup>1</sup>, Ivar Zekker<sup>1</sup>, Martin Tomingas<sup>1</sup>, Taavo Tenno<sup>1</sup>, Anne Menert<sup>2</sup>, Liis Loorits<sup>2</sup>, Toomas Tenno<sup>1</sup>. (<sup>1</sup>Institute of Chemistry, University of Tartu, 14a Ravila Street, 50411 Tartu, Estonia, <sup>2</sup>Tallinn University of Technology, 5 Ehitajate Street, 19086 Tallinn,**

**Estonia. Email: ergo.rikmann@ut.ee). Sulfate-reducing anaerobic ammonium oxidation as a potential treatment method for high nitrogen-content wastewater. Biodegradation, Volume 23 (4) (2012): 509-524**

After sulfate-reducing ammonium oxidation (SRAO) was first assumed in 2001, several works have been published describing this process in laboratory-scale bioreactors or occurring in the nature. In this paper, the SRAO process was performed using reject water as a substrate for microorganisms and a source of  $\text{NH}_4^+$ , with  $\text{SO}_4^{2-}$  being added as an electron acceptor. At a moderate temperature of 20°C in a moving bed biofilm reactor (MBBR) sulfate reduction along with ammonium oxidation were established. In an upflow anaerobic sludge blanket reactor (UASBR) the SRAO process took place at 36°C. Average volumetric TN removal rates of 0.03 kg-N/m<sup>3</sup>/day in the MBBR and 0.04 kg-N/m<sup>3</sup>/day in the UASBR were achieved, with long-term moderate average removal efficiencies, respectively. *Uncultured bacteria clone P4* and *uncultured planctomycete clone Amx-PAN30* were detected from the biofilm of the MBBR, from sludge of the UASBR *uncultured Verrucomicrobiales bacterium clone De2102* and *Uncultured bacterium clone ATB-KS-1929* were found also. The stoichiometrical ratio of  $\text{NH}_4^+$  removal was significantly higher than could be expected from the extent of  $\text{SO}_4^{2-}$  reduction. This phenomenon can primarily be attributed to complex interactions between nitrogen and sulfur compounds and organic matter present in the wastewater. The high  $\text{NH}_4^+$  removal ratio can be attributed to sulfur-utilizing denitrification/denitrification providing the evidence that SRAO is occurring independently and is not a result of sulfate reduction and anammox.  $\text{HCO}_3^-$  concentrations exceeding 1,000 mg/l were found to have an inhibiting effect on the SRAO process. Small amounts of hydrazine were naturally present in the reaction medium, indicating occurrence of the anammox process. Injections of anammox intermediates, hydrazine and hydroxylamine, had a positive effect on SRAO process performance, particularly in the case of the UASBR.

**Yongming Zhang<sup>1</sup>, Xuejing Pu<sup>1</sup>, Miaomiao Fang<sup>1</sup>, Jun Zhu, Lujun Chen<sup>2</sup>, Bruce E. Rittmann<sup>3</sup>. (<sup>1</sup>Department of Environmental Engineering, College of Life and Environmental Science, Shanghai Normal University, 200234 Shanghai, People's Republic of China, <sup>2</sup>School of Environment, Tsinghua University, 100084 Beijing, People's Republic of China, <sup>3</sup>Swette Center for Environmental Biotechnology, Biodesign Institute, Arizona State University, Tempe, AZ 85287-5701, USA. Corresponding author, Email: zhym@shnu.edu.cn). 2,4,6-trichlorophenol (TCP) photobiodegradation and its effect on community structure. Biodegradation, Volume 23 (4) (2012): 575-583**

The mechanisms occurring in a photolytic circulating-bed biofilm reactor (PCBBR) treating 2,4,6-trichlorophenol (TCP) were investigated using batch experiments following three protocols: photodegradation alone (P), biodegradation alone (B), and intimately coupled photodegradation and biodegradation (P&B). Initially, the ceramic particles used as biofilm carriers rapidly adsorbed TCP, particularly in the B experiments. During the first 10 min, the TCP removal rate for P&B was equal to the sum of the rates for P and B, and P&B continued to have the greatest TCP removal, with the TCP concentration approaching zero only in the P&B experiments. When phenol, an easily biodegradable compound, was added along with TCP in order to promote TCP mineralization by means of secondary utilization, P&B was superior to P and B in terms of mineralization of TCP, giving 95% removal of chemical oxygen demand

(COD). The microbial communities, examined by clone libraries, changed dramatically during the P&B experiments. Whereas *Burkholderia xenovorans*, a known degrader of chlorinated aromatics, was the dominant strain in the TCP-acclimated inoculum, it was replaced in the P&B biofilm by strains noted for biofilm formation and biodegrading non-chlorinated aromatics.

**P. V. Suresh, P. K. Anil Kumar. (Meat, Fish and Poultry Technology Department, Central Food Technological Research Institute (Council of Scientific and Industrial Research), Mysore, 570 020, India. Corresponding author, Email: drsureshpv@cftri.res.in). Enhanced degradation of  $\alpha$ -chitin materials prepared from shrimp processing byproduct and production of *N*-acetyl-D-glucosamine by thermoactive chitinases from soil mesophilic fungi. Biodegradation, Volume 23 (4) (2012): 597-607**

Soil isolates of mesophilic *Penicillium monoverticillium* CFR 2, *Aspergillus flavus* CFR 10 and *Fusarium oxysporum* CFR 8 were cultivated in solid state fermentation (SSF) using wheat bran solid medium supplemented with  $\alpha$ -chitin in order to produce chitinolytic enzyme. Under SSF cultivation, maximum enzymes (U/g IDS) production was 41.0 (endo-chitinase) and 195.4 ( $\beta$ -*N*-acetylhexosaminidase) by *P. monoverticillium*, 26.8 (endo-chitinase) and 222.1 ( $\beta$ -*N*-acetylhexosaminidase) by *A. flavus* and 13.3 (endo-chitinase) and 168.3 ( $\beta$ -*N*-acetylhexosaminidase) by *F. oxysporum* after 166 h of incubation. The crude endo-chitinase and  $\beta$ -*N*-acetylhexosaminidase derived from *A. flavus* and *F. oxysporum* revealed optimum temperature at  $62 \pm 1^\circ\text{C}$ , but the enzymes from *P. monoverticillium* showed optimum temperature at  $52 \pm 1^\circ\text{C}$  for maximum activity. Several fold increase in endo-chitinase and  $\beta$ -*N*-acetylhexosaminidase activities in the crude enzymes preparation was achieved after concentrating with polyethylene glycol. The concentrated crude chitinases from *P. monoverticillium*, *A. flavus* and *F. oxysporum*, respectively yielded 95.6, 96.6 and 96.1 mmol/l of *N*-acetyl-D-glucosamine (GlcNAc) in 48 h of reaction from colloidal chitin. While, the crude enzyme preparations of *P. monoverticillium*, *A. flavus* and *F. oxysporum* produced 10.11, 6.85 and 10.7 mmol/l of GlcNAc respectively, in 48 h of reaction from crystalline  $\alpha$ -chitin. HPLC analysis of colloidal chitin hydrolysates prepared with crude chitinases derived from *P. monoverticillium*, *A. flavus* and *F. oxysporum* revealed that the major reaction product was monomeric GlcNAc (~80%) and a small amount of (GlcNAc)<sub>4</sub> (~20%), indicating the potential of these enzymes for efficient production of GlcNAc from  $\alpha$ -chitin.

**Sangeeta Yadav, Ram Chandra. (Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow, 226025, Uttar Pradesh, India. Email: rc\_microitrc@yahoo.co.in). Biodegradation of organic compounds of molasses melanoidin (MM) from biomethanated distillery spent wash (BMDS) during the decolourisation by a potential bacterial consortium. Biodegradation, Volume 23 (4) (2012): 609-620**

Molasses melanoidin (MM) is a major pollutant in biomethanated distillery spent wash (BMDS) due to its recalcitrant properties. The 75% colour and 71% COD of MM (1,000 ppm) were reduced with developed bacterial consortium comprising *Proteus mirabilis* (IITRM5; FJ581028), *Bacillus* sp. (IITRM7; FJ581030), *Raoultella planticola* (IITRM15; GU329705) and *Enterobacter sakazakii* (IITRM16, FJ581031) in the ratio of 4:3:2:1 within 10 days at optimized nutrient. Bacterial consortium showed manganese peroxidase and laccase activity during MM decolourisation. The dominant growth of *R. planticola* and *E. sakazakii* was noted in consortium during MM decolourisation. The comparative GC-MS analysis of extracted compounds of control and degraded samples showed that most of the compounds present in

control were completely utilized by bacterial consortium along with production of some metabolites. The developed bacterial consortium could be a tool for the decolourisation and degradation of melanoidin containing BMDS.

### Biosensor

**S. Munzi<sup>a,b</sup>, L. Paoli<sup>b,c</sup>, E. Fiorini<sup>b</sup>, S. Loppi<sup>b</sup>.** (<sup>a</sup> Center for Environmental Biology, University of Lisbon, Campo Grande, Bloco C2, 1749-016 Lisbon, Portugal, <sup>b</sup> Department of Environmental Science, University of Siena, via P.A. Mattioli 4, 53100 Siena, Italy, <sup>c</sup> Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, 84523 Bratislava, Slovakia). **Physiological response of the epiphytic lichen *Evernia prunastri*(L.) Ach. to ecologically relevant nitrogen concentrations. *Environmental Pollution*, Volume 171 (2012) : 25–29**

This study investigated the physiological response of the epiphytic lichen *Evernia prunastri* to ecologically relevant concentrations of nitrogen compounds. Lichen samples were sprayed for 4 weeks either with water or 50, 150 and 500  $\mu\text{M}$   $\text{NH}_4\text{Cl}$ . The integrity of cell membranes and chlorophyll *a* fluorescence emission ( $F_V/F_M$  and  $\text{PI}_{\text{ABS}}$ ) were analyzed. No membrane damage occurred after the exposure period.  $F_V/F_M$ , a classical fluorescence indicator, decreased during the second week of treatment with 500  $\mu\text{M}$   $\text{NH}_4\text{Cl}$  and the third week with 50 and 150  $\mu\text{M}$   $\text{NH}_4\text{Cl}$ .  $\text{PI}_{\text{ABS}}$ , an overall index of the photosynthetic performance, was more sensitive and decreased already during the first week with 500  $\mu\text{M}$   $\text{NH}_4\text{Cl}$  and the second week with 150  $\mu\text{M}$   $\text{NH}_4\text{Cl}$ . Since *E. prunastri* has been exposed to ammonium loads corresponding to real environmental conditions, these findings open the way to an effective use of this species as early indicators of environmental nitrogen excess.

**Keywords:** Ammonium; Biomonitoring; Cell membrane damage;  $F_V/F_M$ ; Performance index

**Baojing Gu<sup>a,b</sup>, Xiaoli Dong<sup>b</sup>, Changhui Peng<sup>c,d</sup>, Weidong Luo<sup>a,e</sup>, Jie Chang<sup>b,e</sup>, Ying Ge<sup>b,e</sup>.** (<sup>a</sup> College of Economics, Zhejiang University, Hangzhou 310027, PR China, <sup>b</sup> Department of Biology Science, College of Life Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, PR China, <sup>c</sup> Department of Biology Science, Institute of Environment Sciences, University of Quebec at Montreal, Montreal H3C3P8, Canada, <sup>d</sup> Laboratory for Ecological Forecasting and Global Change, College of Forestry, Northwest Agriculture and Forest University, Yangling 712100, China, <sup>e</sup> Research Center for Sustainable Development, Zhejiang University, Hangzhou 310058, PR China). **The long-term impact of urbanization on nitrogen patterns and dynamics in Shanghai, China. *Environmental Pollution*, Volume 171(2012): 30–37**

Urbanization is an important process that alters the regional and global nitrogen biogeochemistry. In this study, we test how long-term urbanization (1952–2004) affects the nitrogen flows, emissions and drivers in the Greater Shanghai Area (GSA) based on the coupled human and natural systems (CHANS) approach. Results show that: (1) total nitrogen input to the GSA increased from 57.7 to 587.9 Gg N  $\text{yr}^{-1}$  during the period 1952–2004, mainly attributing to fossil fuel combustion (43%), Haber–Bosch nitrogen fixation (31%), and food/feed import (26%); (2) per capita nitrogen input increased from 13.5 to 45.7 kg N  $\text{yr}^{-1}$ , while per gross

domestic product (GDP) nitrogen input reduced from 22.2 to 0.9 g N per Chinese Yuan, decoupling of nitrogen with GDP; (3) emissions of reactive nitrogen to the environment transformed from agriculture dominated to industry and human living dominated, especially for air pollution. This study provides decision-makers a novel view of nitrogen management.

**Keywords:** Agriculture; Biogeochemistry; Decoupling; Fossil fuel; Nitrogen pollution; Policy; Removal capacity

**Dasong Lin<sup>a, b</sup>, Qixing Zhou<sup>b</sup>, Yingming Xu<sup>a</sup>, Chun Chen<sup>a, b</sup>, Ye Li<sup>b</sup>. (Key Laboratory of Original Agro-environmental Quality, Ministry of Agriculture/Tianjin Key Laboratory of Agro-environment and Agro-product Safety, Institute of Agro-environmental Protection, Ministry of Agriculture, Tianjin 300191, China, <sup>b</sup> Key Laboratory of Pollution Processes and Environmental Criteria, Ministry of Education, College of Environmental Sciences and Engineering, Nankai University, Tianjin 300071, China). Physiological and molecular responses of the earthworm (*Eisenia fetida*) to soil chlortetracycline contamination. Environmental Pollution, Volume 171(2012): 46–51**

This study aims to evaluate toxic effects of exposure to chlortetracycline (CTC) in soil on reproductive endpoints (juvenile counts and cocoon counts), biochemical responses, and genotoxic potentials of the earthworm *Eisenia fetida*. Results showed that juvenile counts and cocoon counts of the tested earthworms were reduced after exposure to CTC. The effective concentrations (EC<sub>50</sub> values) for juvenile and cocoon counts were 96.1 and 120.3 mg/kg, respectively. Treatment of earthworms with CTC significantly changed the activity of catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase (GST). An increase in malondialdehyde (MDA) indicated that CTC could cause cellular lipid peroxidation in the tested earthworms. The percentage of DNA in the tail of single-cell gel electrophoresis of coelomocytes as an indication of DNA damage increased after treatment with different doses of CTC, and a dose-dependent DNA damage of coelomocytes was found. In conclusion, CTC induces physiological responses and genotoxicity on earthworms.

**Keywords:** Soil pollution; Chlortetracycline; Oxidative stress; Genotoxicity; Biomarker; *Eisenia fetida*

**Kyung-Seok Ko<sup>1</sup>, Pyeong-Koo Lee<sup>1</sup>, In Chul Kong<sup>2</sup>. (<sup>1</sup>Geologic Environment Division, Korea Institute of Geoscience and Mineral Resources (KIGAM), Daejeon, 305-350, Republic of Korea, <sup>2</sup>Department of Environmental Engineering, Yeungnam University, Kyungbuk, 712-749, Republic of Korea. Email: kyungsok@kigam.re.kr). Evaluation of the toxic effects of arsenite, chromate, cadmium, and copper using a battery of four bioassays. Applied Microbiology and Biotechnology, Volume 95 (5) (2012): 1343-1350**

The sensitivities of four different kinds of bioassays to the toxicities of arsenite, chromate, cadmium, and copper were compared. The different bioassays exhibited different sensitivities, i.e., they responded to different levels of toxicity of each of the different metals. However, with the exception of the  $\alpha$ -glucosidase enzyme activity, arsenite was the most toxic compound towards all the tested organisms, exhibiting the highest toxic effect on the seeds of *Lactuca*, with an EC<sub>50</sub> value of 0.63 mg/L. The sensitivities of *Lactuca* and *Raphanus* were greater than the sensitivities of two other kinds of seeds tested. Therefore, these were the seeds appropriate for use in a seed germination assay. A high revertant mutagenic ratio (5:1) of *Salmonella typhimurium* was observed with an arsenite concentration of 0.1  $\mu$ g/plate, indicative of a high

possibility of mutagenicity. These different results suggested that a battery of bioassays, rather than one bioassay alone, is needed as a more accurate and better tool for the bioassessment of environmental pollutants.

**Marcel Gutiérrez-Correa<sup>1</sup>, Yvette Ludeña<sup>1</sup>, Gordon Ramage<sup>2</sup>, Gretty K. Villena<sup>1</sup>.** (<sup>1</sup>Laboratorio de Micología y Biotecnología, Universidad Nacional Agraria La Molina, Av. La Molina s/n, Lima 12, Peru, <sup>2</sup>School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK. Email: mgclmb@lamolina.edu.pe). **Recent Advances on Filamentous Fungal Biofilms for Industrial Uses. Applied Biochemistry and Biotechnology, Volume 167(5) (2012): 1235-1253**

Industrial enzymes are produced by submerged fermentation (SF) and by solid-state fermentation (SSF) to a lesser extent. Although SSF has several advantages, its scale-up is difficult. The role of physiological and genetic properties of microorganisms growing attached to surfaces could explain the advantages of SSF. Filamentous fungi are naturally adapted to growth on surfaces and in these conditions they show a particular physiological behavior which is different from that in SF; thus, they also form biofilms. Fermentation by filamentous fungal biofilms (FFB) is a homogeneous production system within a liquid environment based on the infrastructure of the SF process with the productive efficiency of the SSF. Enzyme production levels of FFB are much higher than those obtained in SF and they are also amenable of mixed fungal cultivation. Transcriptomic and proteomic tools are used to uncover the fundamental biological issues behind FFB. Several genes encoding cellulolytic enzymes are either differentially expressed or overexpressed in FFB. Moreover, our proteomic studies of *Aspergillus niger* biofilms compared to SF indicate that many intracellular proteins are either differentially expressed or overexpressed. Clinically important fungi like *A. fumigatus* also form biofilms when they infect lungs and recent studies demonstrate same gene expression features. These results support our hypothesis of cell adhesion and its role in the new schemes for improved fermentative production of industrial enzymes.

**Kajal Jindal<sup>a</sup>, Monika Tomar<sup>b</sup>, Vinay Gupta<sup>a</sup>.** (<sup>a</sup> Department of Physics and Astrophysics, University of Delhi, Delhi 110007, India, <sup>b</sup> Department of Physics, Miranda House, University of Delhi, Delhi 110007, India). **CuO thin film based uric acid biosensor with enhanced response characteristics. Biosensors and Bioelectronics, Volume 38(1) (2012): 11–18**

An efficient reagentless uric acid biosensor has been realized using a copper oxide (CuO) thin film matrix grown onto platinum (Pt) coated corning glass substrates by pulsed laser deposition (PLD) technique. The p-type CuO matrix successfully introduces redox property in the electrode and provides enhanced electron communication features. Sensing response obtained by the bioelectrocatalytic oxidation of uric acid by uricase/CuO/Pt/glass electrode was studied without any external mediator using cyclic voltammetry (CV) and photometric assay. The studies reveal that the uricase/CuO/Pt/glass bio-electrode exhibits good linearity over a wide range of 0.05 mM to 1.0 mM uric acid concentration with enhanced response of 2.7 mA/mM and high shelf life (>14 weeks). A low Michaelis–Menten constant ( $K_m$ ) of 0.12 mM, indicate that the immobilized enzyme (uricase) has enhanced affinity towards its analyte (uric acid). The results confirm promising application of the p-type CuO thin film matrix for the realization of a reagentless integrated implantable biosensor.

**Keywords:** Uric acid; Copper oxide; Thin film; Pulsed laser deposition; Biosensor

**Chang Liu<sup>a</sup>, Vittoria Balsamo<sup>b</sup>, Dali Sun<sup>a</sup>, Melodie Naja<sup>d</sup>, Xuemei Wang<sup>e</sup>, Barry Rosen<sup>c</sup>, Chen-Zhong Li<sup>a</sup>.** (<sup>a</sup> Nanobioengineering/Nanobioelectronics Laboratory, Department of Biomedical Engineering, Florida International University, 10555 W Flagler Street, Miami, FL 33174, USA, <sup>b</sup> Departamento de Ciencia de los Materiales, Universidad Simon Bolivar, Apartado 89000, Caracas1080 A, Venezuela, <sup>c</sup> Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine, Florida International University, 11200 S.W. 8th Street, AHC 2, Miami, FL 33199, USA, <sup>d</sup> Everglades Foundation, 18001 Old Cutler Road, Palmetto Bay, FL 33157, USA, <sup>e</sup> State Key Lab of Bioelectronics (Chien-Shiung Wu Laboratory), Southeast University, No. 2 Sipailou, Nanjing 210096, China). **A 3D localized surface plasmon resonance biosensor for the study of trivalent arsenic binding to the ArsA ATPase. Biosensors and Bioelectronics, Volume 38(1) (2012): 19–26**

A self-assembled 3D hydrogel–nanoparticle composite integrated surface plasmon resonance (SPR) sensor is reported here. The novel assembled substrate was developed by means of a surface mediated radical co-polymerization process to obtain a highly sensitive hydrogel-based thin film that possesses specific binding sites for target analytes. Initially, amino group modified gold nanoparticles (AuNPs) were covalently linked to acrylic acid monomer. Following this, N-isopropylacrylamide (NIPAAm) and AuNPs linked acrylic acid (AAc) monomers were randomly co-polymerized by the “grafting from” method in the presence of initiator and crosslinker onto the sensing surface. Surface characterization techniques were utilized to evaluate the thickness and composition of the hydrogel-nanoparticle film. The sensing platform was employed to study the binding kinetics and conformational changes of the ArsA ATPase as a consequence of binding trivalent arsenicals under a variety of conditions. ArsA, the catalytic subunit of the ArsAB arsenite (As(III)) translocating ATPase, is one of the five proteins encoded by the arsenical resistance (*ars*) operon of plasmid R773 in cells of *Escherichia coli*, that confers resistance to trivalent and pentavalent salts of the metalloid arsenic. SPR measurements indicate that the 3D hydrogel-nanoparticle coated sensors exhibited a higher sensitivity than that of the 2D AuNPs decorated sensors. Binding of As(III) to ArsA is greatly facilitated by the presence of magnesium ion and ATP.

**Keywords:** Localized surface plasmon resonance; Optical biosensor; Arsenic; Binding kinetics; Nanoparticle; 3-D polymer

**Jingming Gong, Xiaoqing Wang, Xue Li, Kewei Wang.** (Key Laboratory of Pesticide and Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, PR China). **Highly sensitive visible light activated photoelectrochemical biosensing of organophosphate pesticide using biofunctional crossed bismuth oxyiodide flake arrays. Biosensors and Bioelectronics, Volume 38(1) (2012): 43–49**

A new, highly sensitive and selective biosensor for the photoelectrochemical (PEC) detection of organophosphate pesticides (OPs) has been developed, whereby newly synthesized crossed bismuth oxyiodide (BiOI) nanoflake arrays (BiOINFs) are fabricated as a photoactive electrode via a successive ionic layer adsorption and reaction (SILAR) approach. The smart integration of BiOINFs with biomolecules acetylcholinesterase (AChE) yields a novel AChE–BiOINFs hybrid, constructing a three-dimensional (3D) porous network biosensing platform. The composition and surface structure of the sensor were carefully characterized by X-ray diffraction (XRD),

scanning electron microscopy (SEM), and various electrochemical techniques. Such interlaced network architectures, providing better mass transport and allowing more AChE loading per unit area, as well as the intrinsically strong visible light-harvesting effect from BiOI, greatly facilitate the PEC responses. On the basis of the effect of OPs on the photocurrent of AChE–BiOINFs/ITO, a highly sensitive visible light-activated photoelectrochemical biosensor was developed for biosensing OPs. The conditions for OPs detection were optimized by using methyl parathion (MP) as a model OP compound. Under the optimized experimental conditions, our results show that such a newly designed AChE–BiOINFs/ITO photoactive electrode provides remarkably improved sensitivity and selectivity for the biosensing of OPs. The detection limit was found to be as low as about  $0.04 \text{ ng mL}^{-1}$  ( $S/N=3$ ). Toward the goal for practical applications, the resulting sensor was further evaluated by monitoring MP in spiked vegetable samples, showing fine applicability for the detection of MP in real samples.

**Keywords:** Organophosphate pesticides; BiOI flake arrays; Photoelectrochemical detection; Biosensor; Acetylcholinesterase

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A new electrochemical DNA biosensor for bovine papillomavirus (BPV) detection that was based on screen-printed electrodes was comprehensively studied by electrochemical methods of cyclic voltammetry (CV) and differential pulse voltammetry (DPV). A BPV probe was immobilised on a working electrode (gold) modified with a polymeric film of poly-L-lysine (PLL) and chitosan. The experimental design was carried out to evaluate the influence of polymers, probe concentration (BPV probe) and immobilisation time on the electrochemical reduction of methylene blue (MB). The polymer poly-L-lysine (PLL), a probe concentration of  $1 \mu\text{M}$  and an immobilisation time of 60 min showed the best result for the BPV probe immobilisation. With the hybridisation of a complementary target sequence (BPV target), the electrochemical signal decreased compared to a BPV probe immobilised on the modified PLL-gold electrode. Viral DNA that was extracted from cattle with papillomatosis also showed a decrease in the MB electrochemical reduction, which suggested that the decreased electrochemical signal corresponded to a bovine papillomavirus infection. The hybridisation specificity experiments further indicated that the biosensor could discriminate the complementary sequence from the non-complementary sequence. Thus, the results showed that the development of analytical devices, such as a biosensor, could assist in the rapid and efficient detection of bovine papillomavirus DNA and help in the prevention and treatment of papillomatosis in cattle.

**Keywords:** Bovine papillomavirus; Electrochemical DNA biosensor; Screen-printed electrode

**Liang Yuan, Wei Wei, Songqin Liu. (State Key Laboratory of Bioelectronics, School of Chemistry and Chemical Engineering, Southeast University, Jiangning District, Nanjing 211189, PR China). Label-free electrochemical immunosensors based on surface-initiated atom radical polymerization. *Biosensors and Bioelectronics*, Volume 38(1) (2012): 79–85**

A novel label-free immunosensing strategy for sensitive detection of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) via surface-initiated atom transfer radical polymerization (SI-ATRP) was proposed. In this strategy, the Au electrode was first modified by consecutive SI-ATRP of ferrocenylmethyl methacrylate (FMMA) and glycidyl methacrylate (GMA), and TNF- $\alpha$  antibody was coupled to the copolymer segment of GMA (PGMA) by aqueous carbodiimide coupling reaction. Subsequently, the target TNF- $\alpha$  antigen was captured onto the Au electrode surface through immunoreaction. The whole process was confirmed by scanning electron microscopy (SEM) and surface plasmon resonance (SPR) measurements. With introduction of redox polymer segment of FMMA (PFMMA) as electron-transfer mediator, the antigen-coupled Au electrode exhibited well electrochemical behavior, as revealed by cyclic voltammetry measurement. This provided a sensing platform for sensitive detection of TNF- $\alpha$  with a low detection limit of 3.9 pg mL<sup>-1</sup>. Furthermore, the “living” characteristics of the ATRP process can not only be readily controlled but also allow further surface functionalization of the electrodes, thus the proposed method presented a way for label-free and flexible detection of biomolecules.

**Keywords:** Surface-initiated atom radical polymerization; Glycidyl methacrylate; Ferrocenylmethyl methacrylate; Immunosensing; Tumor necrosis factor- $\alpha$

**Wentao Shi<sup>a</sup>, Chunxiu Liu<sup>a</sup>, Yilin Song<sup>a</sup>, Nansen Lin<sup>a,b</sup>, Shuai Zhou<sup>a,b</sup>, Xinxia Cai<sup>a,b</sup>. (<sup>a</sup> State Key Laboratory of Transducer, Institute of Electronics, Chinese Academy of Science, Beijing 100190, PR China, <sup>b</sup> Graduate School of Chinese Academy of Science, Beijing 100190, PR China). An ascorbic acid amperometric sensor using over-oxidized polypyrrole and palladium nanoparticles composites. *Biosensors and Bioelectronics*, Volume 38(1) (2012): 100–106**

We constructed a highly responsive ascorbic acid (AA) sensor utilizing over-oxidized polypyrrole (OPPy) and Palladium nanoparticles (PdNPs) composites (OPPy-PdNPs). In the presence of PdNPs, polypyrrole (PPy) was coated on a gold (Au) electrode through cyclic voltammetry (CV) and over-oxidized at a fixed potential in NaOH solution. The PdNPs were characterized using ultraviolet–visible (UV–vis) spectrum and transmission electron microscopy (TEM). The surface of OPpy-PdNPs on the Au electrode was investigated using field-emission scanning electron microscopy (FE-SEM). Results revealed that the OPpy-PdNPs-modified Au electrode (OPpy-PdNPs/Au) has the capacity to catalyze the oxidation of AA by lowering its oxidation potential to 0 V. The OPpy-PdNPs/Au electrode exhibited 2 different linear concentration ranges. In the low concentration range (1–520  $\mu$ M), OPpy-PdNPs/Au exhibited a direct linear relation with current responses and had high sensitivity (570  $\mu$ A mM<sup>-1</sup> cm<sup>-2</sup>) and a high correlation coefficient (0.995). In contrast, in the higher concentration range (120–1600  $\mu$ M), the relationship between current responses and concentration of AA can be represented by a two-parameter sigmoidal equation. In addition, the sensor exhibited a short response time (less than 2 s) and a very low limit of detection of 1  $\mu$ M. The electrochemical AA sensor constructed in this study was simple, inexpensive, reproducible, sensitive, and resistant to

interference. Thus, the proposed sensor has great potential for detecting AA in complex biosystems and can be applied in various fields, particularly neuroscience.

**Keywords:** Ascorbic acid; Palladium nanoparticles; Over-oxidized polypyrrole; Detection

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A novel glucose biosensor was developed based on the adsorption of glucose oxidase at a TiO<sub>2</sub>-Graphene (GR) nanocomposite electrode. A TiO<sub>2</sub>-GR composite was synthesized from a colloidal mixture of TiO<sub>2</sub>nanoparticles and graphene oxide (GO) nanosheets by an aerosol assisted self-assembly (AASA). The particle morphology of all TiO<sub>2</sub>-GR composites was spherical in shape. It was observed that micron-sized TiO<sub>2</sub> particles were encapsulated by GR nanosheets and that the degree of encapsulation was proportional to the ratio of GO/TiO<sub>2</sub>. The amperometric response of the glucose biosensor fabricated by the TiO<sub>2</sub>-GR composite was linear against a concentration of glucose ranging from 0 to 8 mM at -0.6 V. The highest sensitivity was noted at about 6.2  $\mu\text{A}/\text{mM cm}^2$ . The as prepared glucose biosensor based on the TiO<sub>2</sub>-GR composite showed higher catalytic performance for glucose redox than a pure TiO<sub>2</sub> and GR biosensor.

**Keywords:** Glucose biosensor; TiO<sub>2</sub>-GR composite; Aerosol assisted self-assembly; Cyclic voltammetry

**Yifeng Zhang, Irimi Angelidaki. (Department of Environmental Engineering, Building 113, Technical University of Denmark, DK-2800 Lyngby, Denmark). A simple and rapid method for monitoring dissolved oxygen in water with a submersible microbial fuel cell (SBMFC). Biosensors and Bioelectronics, Volume 38(1)(2012): 189-194**

A submersible microbial fuel cell (SBMFC) was developed as a biosensor for *in situ* and real time monitoring of dissolved oxygen (DO) in environmental waters. Domestic wastewater was utilized as a sole fuel for powering the sensor. The sensor performance was firstly examined with tap water at varying DO levels. With an external resistance of 1000  $\Omega$ , the current density produced by the sensor ( $5.6 \pm 0.5$ – $462.2 \pm 0.5$  mA/m<sup>2</sup>) increased linearly with DO level up to  $8.8 \pm 0.3$  mg/L (regression coefficient,  $R^2=0.9912$ ), while the maximum response time for each measurement was less than 4 min. The current density showed different response to DO levels when different external resistances were applied, but a linear relationship was always observed. Investigation of the sensor performance at different substrate concentrations indicates that the organic matter contained in the domestic wastewater was sufficient to power the sensing activities. The sensor ability was further explored under different environmental conditions (e.g. pH, temperature, conductivity, and alternative electron acceptor), and the results indicated that a calibration would be required before field application. Lastly, the sensor was tested with different environmental waters and the results showed no significant difference ( $p>0.05$ ) with

that measured by DO meter. The simple, compact SBMFC sensor showed promising potential for direct, inexpensive and rapid DO monitoring in various environmental waters.

**Keywords:** Dissolved oxygen; Biosensor; Microbial fuel cell; Submersible; Electrical current; Environmental waters

**Byung-Wook Park<sup>a</sup>, Rui Zheng<sup>b</sup>, Kyoung-A Ko<sup>c</sup>, Brent D. Cameron<sup>b</sup>, Do-Young Yoon<sup>d</sup>, Dong-Shik Kim<sup>a</sup>.** (<sup>a</sup> Department of Chemical and Environmental Engineering, University of Toledo, Toledo, OH 43606, United States, <sup>b</sup> Department of Bioengineering, University of Toledo, Toledo, OH 43606, United States, <sup>c</sup> Department of Pharmacology, University of Toledo, Toledo, OH 43606, United States, <sup>d</sup> Department of Chemical Engineering, Kwangwoon University, Seoul 139-701, Republic of Korea). **A novel glucose biosensor using bi-enzyme incorporated with peptide nanotubes. Biosensors and Bioelectronics, Volume 38(1) (2012): 295–301**

A novel amperometric glucose biosensor was developed using the bio-inspired peptide nanotube (PNT) as an encapsulation template for enzymes. Horseradish peroxidase (HRP) was encapsulated by the PNT and glucose oxidase (GOx) was co-immobilized with the PNT on a gold nanoparticle (AuNP)-modified electrode. A binary SAM of 3-mercaptopropionic acid (MPA) and 1-tetradecanethiol (TDT) was formed on the surface of the electrode to immobilize the PNT and GOx. The resulting electrode appeared to provide the enzymes with a biocompatible nanoenvironment as it sustained the enhanced enzyme activity for an extended time and promoted possible direct electron transfer through the PNT to the electrode. Performance of the biosensor was evaluated in terms of its detection limit, sensitivity, pH, response time, selectivity, reproducibility, and stability in a lab setting. In addition the sensor was tested for real samples. The composite of AuNP-SAM-PNT/HRP-GOx to fabricate a sensor electrode in this study exhibited a linear response with glucose in the concentration range of 0.5–2.4 mM with a  $R^2$ -value of 0.994. A maximum sensitivity of 0.3 mA M<sup>-1</sup> and reproducibility (RSD) of 1.95% were demonstrated. The PNT-encapsulated enzyme showed its retention of >85% of the initial current response after one month of storage.

**Keywords:** Glucose biosensor; Bi-enzyme; Peptide nanotube (PNT); Binary self-assembled monolayer (SAM); Encapsulation; Amperometric sensor

**Xiao-Jing Xing, Xue-Guo Liu, Yue-He, Qing-Ying Luo, Hong-Wu Tang, Dai-Wen Pang.** (Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Research Center for Nanobiology and Nanomedicine (MOE 985 Innovative Platform), Wuhan Institute of Biotechnology, and State Key Laboratory of Virology, Wuhan University, Wuhan 430072, China). **Graphene oxide based fluorescent aptasensor for adenosine deaminase detection using adenosine as the substrate. Biosensors and Bioelectronics, Volume 37 (1) (2012): 61–67**

We present a novel fluorescent aptasensor for simple and accurate detection of adenosine deaminase (ADA) activity and inhibition on the basis of graphene oxide (GO) using adenosine (AD) as the substrate. This aptasensor consists of a dye-labeled single-stranded AD specific aptamer, GO and AD. The fluorescence intensity of the dye-labeled AD specific aptamer is quenched very efficiently by GO as a result of strong  $\pi$ - $\pi$  stacking interaction and excellent electronic transference of GO. In the presence of AD, the fluorescence of the GO-based probe is

recovered since the competitive binding of AD and GO with the dye-labeled aptamer prevents the adsorption of dye-labeled aptamer on GO. When ADA was introduced to this GO-based probe solution, the fluorescence of the probe was quenched owing to ADA can convert AD into inosine which has no affinity to the dye-labeled aptamer, thus allowing quantitative investigation of ADA activity. The as-proposed sensor is highly selective and sensitive for the assay of ADA activity with a detection limit of 0.0129 U/mL in clean buffer, which is more than one order of magnitude lower than the previous reports. Meanwhile, a good linear relationship with the correlation coefficient of  $R=0.9922$  was obtained by testing 5% human serum containing a series of concentrations of ADA. Additionally, the inhibition effect of erythro-9-(2-hydroxy-3-nonyl) adenine on ADA activity was investigated in this design. The GO-based fluorescence aptasensor not only provides a simple, cost-effective and sensitive platform for the detection of ADA and its inhibitor but also shows great potential in the diagnosis of ADA-relevant diseases and drug development.

**Keywords:** Graphene oxide; Aptamer; Adenosine; Adenosine deaminase; Inhibition

**Merlin Raud<sup>1</sup>, Marti Tutt<sup>1</sup>, Eerik Jõgi<sup>2</sup>, Timo Kikas<sup>3</sup>.** (<sup>1</sup>Institute of Chemistry, University of Tartu, Ravila 14a, 50411 Tartu, Estonia, <sup>2</sup>Institute of Technology, University of Tartu, Nooruse St 1, 50411 Tartu, Estonia, <sup>3</sup>Institute of Technology, Estonian University of Life Sciences, Kreutzwaldi 56, Tartu, 51014, Estonia. Email: merlin.raud@ut.ee). **BOD biosensors for pulp and paper industry wastewater analysis. Environmental Science and Pollution Research, Volume 19(7) (2012): 3039-3045**

Two semi-specific microbial biosensors were constructed for the analysis of biochemical oxygen demand (BOD) in high-cellulose-content pulp and paper industry wastewaters. The biosensors were based on living cells of *Bacillus subtilis* and *Paenibacillus* sp. immobilized in an agarose gel matrix. Semi-specific microorganisms were isolated from various samples (decaying sawdust and rabbit manure) and were chosen based on their ability to assimilate cellulose.

The biosensors were calibrated with the Organization for Economic Cooperation and Development synthetic wastewater, and measurements with different wastewaters were conducted. The response time of biosensors using the steady-state method was 20–25 min, and the service life of immobilized microorganisms was 96 days. Detection limit was 5 mg/l of BOD<sub>7</sub> while linear ranges extended up to 55 and 50 mg/l of the BOD<sub>7</sub> for *B. subtilis*- and *Paenibacillus* sp.-based biosensors, respectively. Repeatability and reproducibility of both biosensors were within the limits set by APHA—less than 15.4%. In comparison, both biosensors overestimated the BOD<sub>7</sub> values in paper mill wastewaters and underestimated the BOD<sub>7</sub> in aspen pulp mill wastewater. The semi-specific biosensors are suitable for the estimation of organic pollution derived from cellulose, while the detection of pollution derived from tannins and lignins was minor. Better results in terms of accuracy and repeatability were gained with *Paenibacillus* sp. biosensor.

## **Bioengineering**

**Gang Luo, Irimi Angelidaki\*.** (Department of Environmental Engineering, Technical University of Denmark, DK-2800, Kgs Lyngby, Denmark; Email: Irimi Angelidaki (iria@env.dtu.dk). \*Department of Environmental Engineering, Technical University of

**Denmark, DK-2800, Kgs Lyngby, Denmark). Integrated biogas upgrading and hydrogen utilization in an anaerobic reactor containing enriched hydrogenotrophic methanogenic culture. *Biotechnology and Bioengineering*, Volume 109 (11) (2012): 2729–2736**

Biogas produced by anaerobic digestion, is mainly used in a gas motor for heat and electricity production. However, after removal of CO<sub>2</sub>, biogas can be upgraded to natural gas quality, giving more utilization possibilities, such as utilization as autogas, or distant utilization by using the existing natural gas grid. The current study presents a new biological method for biogas upgrading in a separate biogas reactor, containing enriched hydrogenotrophic methanogens and fed with biogas and hydrogen. Both mesophilic- and thermophilic anaerobic cultures were enriched to convert CO<sub>2</sub> to CH<sub>4</sub> by addition of H<sub>2</sub>. Enrichment at thermophilic temperature (55°C) resulted in CO<sub>2</sub> and H<sub>2</sub>bioconversion rate of 320 mL CH<sub>4</sub>/(gVSS h), which was more than 60% higher than that under mesophilic temperature (37°C). Different dominant species were found at mesophilic- and thermophilic-enriched cultures, as revealed by PCR–DGGE. Nonetheless, they all belonged to the order *Methanobacteriales*, which can mediate hydrogenotrophic methanogenesis. Biogas upgrading was then tested in a thermophilic anaerobic reactor under various operation conditions. By continuous addition of hydrogen in the biogas reactor, high degree of biogas upgrading was achieved. The produced biogas had a CH<sub>4</sub> content, around 95% at steady-state, at gas (mixture of biogas and hydrogen) injection rate of 6 L/(L day). The increase of gas injection rate to 12 L/(L day) resulted in the decrease of CH<sub>4</sub> content to around 90%. Further study showed that by decreasing the gas–liquid mass transfer by increasing the stirring speed of the mixture the CH<sub>4</sub> content was increased to around 95%. Finally, the CH<sub>4</sub> content around 90% was achieved in this study with the gas injection rate as high as 24 L/(L day).

**Keywords:** anaerobic digestion; biogas upgrading; H<sub>2</sub>; CO<sub>2</sub>; CH<sub>4</sub>

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In this study, we perform mass transfer characterization (*kLa*) on a novel mechanically driven/stirred Process Scouting Device, PSD, (SuperSpinner D 1000®, SSD) and demonstrate that this novel device can be viewed as disposable bioreactor. Using patch-based optical sensors, we were able to monitor critical cell culture environmental conditions such as dissolved oxygen (DO) and pH in SSD for comparison to a 1 L standard spinner (SS) flask. We also coupled

these mass transfer studies with mixing time studies where we observed relative high mixing times (5.2 min) that are typically observed in production scale bioreactors. Decreasing the mixing time 3.5-fold resulted in 30% increase in  $kLa$  (from 2.3 to 3.0  $\text{h}^{-1}$ ) and minimum DO level increased from 0% to 20% for our model hybridoma cell line. Finally, maximum viable cell density and protein titer stayed within  $\pm 20\%$  of historical data, from our standard 5 L stirred bioreactor (Biostat®) operated under active DO control.

**Keywords:** disposable bioreactors; optical sensors; spinner flasks; mass transfer; mixing; seed train

**Ajay Singh<sup>1</sup>, Brajesh Singh<sup>2</sup>, Owen Ward<sup>1</sup>.** (<sup>1</sup>Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, Canada, <sup>2</sup>Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW, 2751, Australia. Corresponding author, Email: [ajasingh@uwaterloo.ca](mailto:ajasingh@uwaterloo.ca)). **Potential applications of bioprocess technology in petroleum industry. Biodegradation, Volume 23 (6) (2012): 865-880**

Petroleum refining is traditionally based on the use of physicochemical processes such as distillation and chemical catalysis that operate under high temperatures and pressures conditions, which are energy intensive and costly. Biotechnology has become an important tool for providing new approaches in petroleum industry during oil production, refining and processing as well as managing environmentally safe pollutant remediation and disposal practices. Earlier biotechnology applications in the petroleum industry were limited to microbial enhanced oil recovery, applications of bioremediation to contaminated marine shorelines, soils and sludges. The potential role of bioprocess technology in this industry has now expanded further into the areas of biorefining and upgrading of fuels, production of fine chemicals, control of souring during production and air VOC biofiltration. In this paper we provide an overview of the major applications of bioprocesses and technology development in the petroleum industry both in upstream and downstream areas and highlight future challenges and opportunities.

### **Pollen Biotechnology**

**R. Balasubramanian<sup>1</sup>, P. Nainar<sup>2</sup>, A. Rajasekar<sup>1</sup>.** (<sup>1</sup>Department of Civil and Environmental Engineering, Faculty of Engineering, National University of Singapore, Block EA, 9 Engineering Drive 1, Singapore, 117576, Singapore, <sup>2</sup>Department of Microbiology, Tirunelveli Medical College, Tirunelveli, 627011, India. Corresponding author, Email: [eserbala@nus.edu.sg](mailto:eserbala@nus.edu.sg)). **Airborne bacteria, fungi, and endotoxin levels in residential microenvironments: a case study. Aerobiologia, Volume 28 (3) (2012): 375-390**

Limited data are currently available on the concentrations of airborne bacteria, fungi, and endotoxins in indoor environments. The levels of aerial bacteria and fungi were measured at several microenvironments within a well-ventilated residential apartment in Singapore including the living room, kitchen, bedroom, toilet, and at a workplace environment by sampling indoor air onto culture medium plates using the 6-stage Andersen sampler. Total microbial counts were determined by collecting the air samples in water with the Andersen sampler, staining the

resultant extracts with a fluorescent dye, acridine orange, and counting the microbes using a fluorescent microscope. The levels of airborne endotoxins were also determined by sampling the airborne microorganisms onto 0.4 µm polycarbonate membrane filter using the MiniVol sampler at 5 l/min for 20 h with a PM<sub>2.5</sub> cut-off device. The aerial bacterial and fungal concentrations were found to be in the ranges of 117–2,873 CFU/m<sup>3</sup> and 160–1,897 CFU/m<sup>3</sup>, respectively. The total microbial levels ranged from 49,000 to 218,000 microbes/m<sup>3</sup>. The predominant fungi occurring in the apartment were *Aspergillus* and *Penicillium* while the predominant bacterial strains appeared to be *Staphylococcus* and *Micrococcus*. The average indoor endotoxin level was detectable in the range of 6–39 EU/m<sup>3</sup>. The amount of ventilation and the types of human activities carried out in the indoor environment appeared to be important factors affecting the level of these airborne biological contaminants.

### **Biotechnology Policy Issue**

**Martin Petrick, Patrick Zier. (Leibniz-Institute of Agricultural Development in Central and Eastern Europe (IAMO), Halle (Saale), Germany). Common Agricultural Policy effects on dynamic labour use in agriculture. Food Policy, Volume 37 (6) (2012): 671–678**

The aim of this study is to investigate the effects of direct payments and rural development measures of the EU's Common Agricultural Policy (CAP) on employment in agriculture. We work with a dynamic labour demand equation augmented by the full set of policy instruments of the CAP, which is estimated on a panel dataset of 69 East German regions. We present results for four estimators which differ in how they eliminate the fixed effects and how they address the endogeneity of the lagged dependent variable. The results suggest that there were few desirable effects on job maintenance in agriculture. While there is some indication that investment subsidies have halted labour shedding on farms, a rise in the general wage level reduced labour use in agriculture. Changes in direct payments had no employment effects. Generally, labour adjustment exhibits a strong path dependency.

**Keywords:** Agricultural employment; Dynamic panel data models; Common Agricultural Policy; East Germany

### **Agricultural Biotechnology**

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We study levels and trends in agricultural pesticide use for a large cross-section of countries using FAO data for the period 1990–2009. Our analysis shows that a 1% increase in crop output per hectare is associated with a 1.8% increase in pesticide use per hectare but that the growth in intensity of pesticide use levels off as countries reach a higher level of economic development. However, very few high income countries have managed to significantly reduce the level of intensity of their pesticide use, because decreases in insecticide use at higher income levels are

largely offset by increases in herbicide and fungicide use. The results also show very rapid growth in the intensity of pesticide use for several middle income countries such as Brazil, Mexico, Uruguay, Cameroon, Malaysia and Thailand. Complementing our analysis with data from the Rotterdam Convention on Prior Informed Consent (PIC), we show that hazardous pesticides covered in the PIC procedure are more weakly regulated in lower than in higher income countries. We discuss the policy challenges facing developing countries with a rapid growth in pesticide use and recommend a four-pronged strategy, including an environmental tax on pesticides with revenues allocated to long-term investments in awareness building, the development of integrated crop management methods and the setting of food safety standards. The interactions between these measures should help contribute to the effectiveness of the overall strategy package.

**Keywords:** Agricultural development; Crop protection policy; Cross-country comparison; Environmental Kuznets curve; Pesticide regulation

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This paper examines the treatment effects of the agricultural cooperative and publicly funded extension services on individual household economy, using data collected by the author from watermelon-producing farm households in rural Nanjing. The cooperative addressed in this study restricts the participation of small-scale farmers, implying that selection into the program must be taken into consideration for an accurate assessment of the treatment effect. The econometric analysis revealed that government extension services have a modest effect on farm income. In contrast, the treatment effect of the agricultural cooperative is robust and substantially large, accounting for nearly 70% of the pre-matching difference. This suggests that the agricultural cooperative system is an important avenue for farmers to improve their economic status. The treatment effect of the agricultural cooperative is highly heterogeneous; the economic benefits arising from the cooperative are significant only for small-scale farms. We can argue that a coexistence of smallholder exclusion and the treatment effect in favor of small-scale farms poses serious challenges to pro-poor agricultural growth in China.

**Keywords:** Agricultural cooperatives; Contract farming; Treatment effect; China

## Bioenergy

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*Clostridium* spp. produce *n*-butanol in the acetone/butanol/ethanol process. For sustainable industrial scale butanol production, a number of obstacles need to be addressed including choice of feedstock, the low product yield, toxicity to production strain, multiple-end products and downstream processing of alcohol mixtures. This review describes the use of lignocellulosic feedstocks, bioprocess and metabolic engineering, downstream processing and catalytic refining of *n*-butanol.

**Ryan M. Bright, Francesco Cherubini, Anders H. Strømman. (Industrial Ecology Program, Department of Energy and Process Engineering, Norwegian University of Science and Technology, Høgskoleringen 5, E-1, 7491 Trondheim, Norway). Climate impacts of bioenergy: Inclusion of carbon cycle and albedo dynamics in life cycle impact assessment. *Environmental Impact Assessment Review*, Volume 37 (2012): 2–11**

Life cycle assessment (LCA) can be an invaluable tool for the structured environmental impact assessment of bioenergy product systems. However, the methodology's static temporal and spatial scope combined with its restriction to emission-based metrics in life cycle impact assessment (LCIA) inhibits its effectiveness at assessing climate change impacts that stem from dynamic land surface–atmosphere interactions inherent to all biomass-based product systems. In this paper, we focus on two dynamic issues related to anthropogenic land use that can significantly influence the climate impacts of bioenergy systems: i) temporary changes to the terrestrial carbon cycle; and ii) temporary changes in land surface albedo—and illustrate how they can be integrated within the LCA framework.

In the context of active land use management for bioenergy, we discuss these dynamics and their relevancy and outline the methodological steps that would be required to derive case-specific biogenic CO<sub>2</sub> and albedo change characterization factors for inclusion in LCIA. We demonstrate our concepts and metrics with application to a case study of transportation biofuel sourced from managed boreal forest biomass in northern Europe. We derive GWP indices for three land management cases of varying site productivities to illustrate the importance and need to consider case- or region-specific characterization factors for bioenergy product systems. Uncertainties and limitations of the proposed metrics are discussed.

**Keywords:** Forest management; Biomass; Land use; Climate change; GWP; LCA

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As biofuel usage has boomed over the past decade, so has research and regulatory interest in its carbon accounting. This paper examines one aspect of that carbon accounting: the baseline, i.e. the reference case against which other conditions or changes can be compared. A literature search and analysis identified four baseline types: no baseline; reference point; marginal fossil

fuel; and biomass opportunity cost. The fourth one, biomass opportunity cost, is defined in more detail, because this is not done elsewhere in the literature. The four baselines are then applied to the carbon footprint of a wood-fired power plant.

The footprint of the resulting wood-fired electricity varies dramatically, according to the type of baseline. Baseline type is also found to be the footprint's most significant sensitivity. Other significant sensitivities are: efficiency of the power plant; the growth (or re-growth) rate of the forest that supplies the wood; and the residue fraction of the wood. Length of the policy horizon is also an important factor in determining the footprint.

The paper concludes that because of their significance and variability, baseline choices should be made very explicit in biofuel carbon footprints.

**Keywords:** Biogenic carbon; Carbon footprint; Biomass power; Biofuel; Baseline

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Due to their large biomass forests assume an important role in the global carbon cycle by moderating the greenhouse effect of atmospheric pollution. The Kyoto Protocol recognises this contribution by allocating carbon credits to countries which are able to create new forest areas. Sequestered carbon provides an environmental benefit thus must be taken into account in cost-benefit analysis of afforestation projects. Furthermore, like timber output carbon credits are now tradable assets in the carbon exchange.

By using British data, this paper looks at the issue of identifying optimum felling age by considering carbon sequestration benefits simultaneously with timber yields. The results of this analysis show that the inclusion of carbon benefits prolongs the optimum cutting age by requiring trees to stand longer in order to soak up more CO<sub>2</sub>. Consequently this finding must be considered in any carbon accounting calculations.

**Keywords:** Carbon sequestration; Environmental impact of forestry; Optimum cutting age

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Modification and loss of forests due to natural and anthropogenic disturbance contribute an estimated 20% of annual greenhouse gas (GHG) emissions worldwide. Although forest carbon pool modeling rarely suggests a 'carbon neutral' flux profile, the life cycle assessment community and associated product carbon footprint protocols have struggled to account for the GHG emissions associated with forestry, specifically, and land use generally. Principally, this is

due to underdeveloped linkages between life cycle inventory (LCI) modeling for wood and forest carbon modeling for a full range of forest types and harvest practices, as well as a lack of transparency in globalized forest supply chains. In this paper, through a comparative study of U.S. and Chinese coated freesheet paper, we develop the initial foundations for a methodology that rescales IPCC methods from the national to the product level, with reference to the approaches in three international product carbon footprint protocols. Due to differences in geographic origin of the wood fiber, the results for two scenarios are highly divergent. This suggests that both wood LCI models and the protocols need further development to capture the range of spatial and temporal dimensions for supply chains (and the associated land use change and modification) for specific product systems. The paper concludes by outlining opportunities to measure and reduce uncertainty in accounting for net emissions of biogenic carbon from forestland, where timber is harvested for consumer products.

**Keywords:** Forest carbon; Land use change; Land use modification; Product carbon footprint protocols; Biogenic carbon; Wood life cycle inventories

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Multi-walled carbon nanotubes (MWNTs) were functionalized with pyrogallol and used in a composite with silica as a support for a Cu–Co based catalyst. The catalysts were characterized using X-ray diffraction, transmission electron microscopy, and H<sub>2</sub> temperature programmed reduction. The effects of pyrogallol and the weight ratio of silica to MWNTs on the performance of the catalyst were studied in a fixed bed reactor. The increase of the amount of MWNTs in the catalyst support was found to favor decreased methanol production and increased production of C<sub>2</sub> + alcohols. Using pyrogallol in catalyst preparation was also found to increase the production of C<sub>2</sub> + alcohols. It was concluded that pyrogallol improves the distribution and uniformity of metal particles on the support, decreases the size of metal particles and increasing the rate of catalytic reduction.

**Keywords:** Carbon nanotube; Pyrogallol; Syngas; Ethanol

**Bei Wang, Christopher Q. Lan, Mark Horsman.** (Department of Chemical and Biological Engineering, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5). **Closed photobioreactors for production of microalgal biomasses. *Biotechnology Advances*, Volume 30 (4) (2012): 904–912**

Microalgal biomasses have been produced industrially for a long history for application in a variety of different fields. Most recently, microalgae are established as the most promising species for biofuel production and CO<sub>2</sub> bio-sequestration owing to their high photosynthesis efficiency. Nevertheless, design of photobioreactors that maximize solar energy capture and conversion has been one of the major challenges in commercial microalga biomass production. In this review, we systematically survey the recent developments in this field.

**Keywords:** Microalgae; Biomass; Photobioreactor; Design; Biofuel

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The combined anaerobic–aerobic conditions in air-cathode single-chamber MFCs were used to completely mineralize pentachlorophenol (PCP; 5mg/L), in the presence of acetate or glucose. Degradation rates of  $0.140 \pm 0.011$  mg/L-h (acetate) and  $0.117 \pm 0.009$  mg/L-h (glucose) were obtained with maximum power densities of  $7.7 \pm 1.1$  W/m<sup>3</sup> ( $264 \pm 39$  W/m<sup>2</sup>, acetate) and  $5.1 \pm 0.1$  W/m<sup>3</sup> ( $175 \pm 5$  W/m<sup>2</sup>, glucose). At a higher PCP concentration of 15mg/L, PCP degradation rates increased to  $0.171 \pm 0.01$  mg/L-h (acetate) and  $0.159 \pm 0.01$  mg/L-h (glucose). However, power was inversely proportional to initial PCP concentration, with decreases of 0.255W/mg PCP (acetate) and 0.184W/mg PCP (glucose). High pH (9.0, acetate; 8.0, glucose) was beneficial to exoelectrogenic activities and power generation, whereas an acidic pH=5.0 decreased power but increased PCP degradation rates ( $0.195 \pm 0.002$  mg/L-h, acetate;  $0.173 \pm 0.005$  mg/L-h, glucose). Increasing temperature from 22 to 35°C enhanced power production by 37% (glucose) to 70% (acetate), and PCP degradation rates ( $0.188 \pm 0.01$  mg/L-h, acetate;  $0.172 \pm 0.009$  mg/L-h, glucose). Dominant exoelectrogens of *Pseudomonas* (acetate) and *Klebsiella* (glucose) were identified in the biofilms. These results demonstrate that PCP degradation using air-cathode single-chamber MFCs may be a promising process for remediation of water contaminated with PCP as well as for power generation.

**Keywords:** microbial fuel cell; PCP degradation rate; power production; mineralization

**S. Marx\*, J. Brandling and P van der Gryp.** (Energy systems, School of Chemical and Minerals Engineering, North-West University (Potchefstroom Campus), Potchefstroom, South Africa. \*Corresponding author. E-mail: sanette.marx@nwu.ac.za. Tel: +27 18 299 1995). Ethanol production from tropical sugar beet juice. *African Journal of Biotechnology*, Volume 11(54) (2012): 11709-11720

Starch and sugar resources have been extensively researched to find a suitable renewable source of energy to supplement the world's ever increasing demand for energy while also abating global warming by stemming the addition of earthbound carbon dioxide into the atmosphere. Sugar beet has been used as a source for sugar production for some time, but its development as a large

scale agricultural crop in South Africa has been limited by the large production of sugarcane in tropical areas. Recent trials in the Eastern Cape region have shown some promise for cultivating sugar beets on a large scale. In this study, the influence of process variables such as initial sugar concentration (dilution), pH, yeast concentration and nitrogen source addition were investigated to assess the influence of these variables on the bioethanol production potential of tropical sugar beet. High ethanol yields were obtained without dilution (approximately  $0.47 \text{ g.g sugar}^{-1}$ ) while a pH of 4 and a concentration of  $5 \text{ g.L}^{-1}$  yeast (*Saccharomyces cerevisiae*) produced the largest amount of ethanol in the shortest fermentation time. The addition of a nitrogen source such as ammonium sulphate significantly increased the ethanol yield. It was concluded from the results of this research that bioethanol can be produced economically from tropical sugar beet cultivars grown in South Africa.

**Keywords:** Tropical sugar beet, fermentation, dilution, pH, bioethanol yield.

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Global energy crisis and limited supply of petroleum fuels have rekindled the worldwide focus towards development of a sustainable technology for alternative fuel production. Utilization of abundant renewable biomass offers an excellent opportunity for the development of an economical biofuel production process at a scale sufficiently large to have an impact on sustainability and security objectives. Additionally, several environmental benefits have also been linked with the utilization of renewable biomass. Butanol is considered to be superior to ethanol due to its higher energy content and less hygroscopy. This has led to an increased research interest in butanol production from renewable biomass in recent years. In this paper, we review the various aspects of utilizing renewable biomass for clostridial butanol production. Focus is given on various alternative substrates that have been used for butanol production and on fermentation strategies recently reported to improve butanol production.

**Keywords:** Biomass; Butanol; *Clostridium*; Fermentation; Recovery

**Leticia Regueiro<sup>a</sup>, Marta Carballa<sup>a</sup>, Juan A. Álvarez<sup>b</sup>, Juan M. Lema<sup>a</sup>.** (<sup>a</sup> Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Rúa Lope Gómez de Marzoa s/n, 15782 Santiago de Compostela, Spain, <sup>b</sup> AIMEN Technological Center, C/Relva, 27 A – Torneiros, 36410 Porriño Pontevedra, Spain). **Enhanced methane production from pig manure anaerobic digestion using fish and biodiesel wastes as co-substrates. Bioresource Technology, Volume 123 (2012): 507–513**

Co-digestion of pig manure (PM<sup>1</sup>) with fish (FW<sup>2</sup>) and biodiesel waste (BW<sup>3</sup>) was evaluated and compared with sole PM digestion. Results indicated that co-digestion of PM with FW and/or BW is possible as long as ammonium and volatile fatty acids remained under inhibitory levels by adjusting the operating conditions, such as feed composition, organic loading rate (OLR) and hydraulic retention time (HRT). PM and FW co-digestion (90:10 and 95:5, w/w<sup>4</sup>) was possible at OLR of 1–1.5 g COD/L d, resulting in biogas production rates of 0.4–0.6 L/L d and COD

removal efficiencies of 65–70%. Regarding BW, good results (biogas production of 0.9 L/L d and COD elimination of 85%) were achieved with less than 5% feeding rate. Overall, operating at the same OLR, the biogas production and methane content in the co-digester was higher than in the only PM digester.

**Keywords:** Agroindustrial wastes; Ammonia; Co-digestion; *Methanosarcina*; Volatile fatty acids

**Tsukasa Ito<sup>a</sup>, Kazumi Yoshiguchi<sup>b</sup>, Herto Dwi Ariesyady<sup>b, c</sup>, Satoshi Okabe<sup>b</sup>.** (<sup>a</sup> Department of Civil and Environmental Engineering, Graduate School of Engineering, Gunma University, Kiryu, Gunma 376-8515, Japan, <sup>b</sup> Division of Environmental Engineering, Faculty of Engineering, Hokkaido University, Sapporo, Hokkaido 060-8628, Japan, <sup>c</sup> Faculty of Civil and Environmental Engineering, Institute of Technology Bandung, Bandung 40132, Indonesia). **Identification and quantification of key microbial trophic groups of methanogenic glucose degradation in an anaerobic digester sludge. *Bioresource Technology*, Volume 123 (2012): 599–607**

We investigated the major phylogenetic groups and population size of glucose-, propionate-, and acetate-degrading bacteria in the glucose-degrading anaerobic digester sludge by stable-isotope probing analysis of 16S rRNA (RNA-SIP) with [<sup>13</sup>C<sub>6</sub>]glucose followed by time course analysis of microautoradiography combined with fluorescent in situ hybridization (MAR-FISH) with [U-<sup>14</sup>C]glucose. The results indicated that glucose was predominately degraded to CH<sub>4</sub> and CO<sub>2</sub> by glucose-degrading *Propionibacterium* and *Olsenella* that are belonging to the phylum *Actinobacteria*, propionate-degrading *Smithella* and *Syntrophobacter*, and acetate-degrading *Methanosaeta* and *Synergistes* group 4 in this anaerobic sludge. The population size of propionate degraders was the smallest among three trophic groups and the specific degradation rate of propionate was also low. The specific degradation rate of acetate was low even though their population size was comparable to the glucose degraders. These results could explain why the degradation of propionate and acetate was the rate-limiting step in methanogenic glucose degradation.

**Keywords:** Glucose degraders; Propionate degraders; Acetate degraders; RNA-SIP; MAR-FISH

**Ru Liu<sup>a</sup>, Chongyang Gao<sup>b</sup>, Yang-Guo Zhao<sup>a, c</sup>, Aijie Wang<sup>b</sup>, Shanshan Lu<sup>a</sup>, Min Wang<sup>a</sup>, Farhana Maqbool<sup>a</sup>, Qing Huang<sup>a</sup>.** (<sup>a</sup> College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China, <sup>b</sup> School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China, <sup>c</sup> Key Laboratory of Marine Environment and Ecology, Ocean University of China, Ministry of Education, Qingdao 266100, China). **Biological treatment of steroidal drug industrial effluent and electricity generation in the microbial fuel cells. *Bioresource Technology*, Volume 123 (2012): 86–91**

The single chamber microbial fuel cells (MFCs) were used to treat steroidal drug production wastewater (SPW) and generate electricity simultaneously. The results indicated that the maximum COD removal efficiency reached 82%, total nitrogen and sulfate removal rate approached 62.47% and 26.46%, respectively. The maximum power density and the Coulombic efficiency reached to 22.3 W m<sup>-3</sup> and 30%, respectively. The scanning electron microscope

showed that the dominant microbial populations were remarkably different in morphology on the surface of SPW and acetate-fed anodes. PCR-denaturing gradient gel electrophoresis profiles revealed that the microbial community structure fed with different concentrations of SPW presented a gradual succession and unique bacterial sequences were detected on the SPW and acetate-fed anodes. This research demonstrates that MFCs fed with SPW achieved a high efficiency of power density and simultaneous nutrient removal, and the dominant microorganisms on the anode were related to the types and the concentrations of substrates.

**Keywords:** Microbial fuel cell (MFC); Steroidal drug production wastewater (SPW); Bacterial community; Denaturing gradient gel electrophoresis (DGGE)

**Shuangxiu Wu<sup>a</sup>, Xiaoxu Li<sup>a,b</sup>, Jun Yu<sup>a</sup>, Quanxi Wang<sup>b</sup>.** (<sup>a</sup> The CAS Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, No.7 Beitucheng West Road, Chaoyang District, Beijing 100029, PR China, <sup>b</sup> College of Life and Environmental Science, Shanghai Normal University, No. 100 Guilin Road, Xuhui District, Shanghai 200234, PR China). **Increased hydrogen production in co-culture of *Chlamydomonas reinhardtii* and *Bradyrhizobium japonicum*. *Bioresource Technology*, Volume 123 (2012): 184–188**

Co-cultivation of *Bradyrhizobium japonicum* with *Chlamydomonas reinhardtii* strain cc849 or the transgenic strain lba, which was hetero-expressed the gene of the soybean leghemoglobin apoprotein Lba in chloroplasts of the strain cc849, in Tris–acetate-phosphate (TAP) or TAP-sulfur free media, improved H<sub>2</sub> yield. H<sub>2</sub> production was 14 times and growth was 26% higher when strain lba and *B. japonicum* were co-cultured, as compared with cultivation of the algal strain alone under the same conditions. The increase in respiration rate or fast O<sub>2</sub> consumption by about 8 times in the co-cultures was the major reason for the improvement.

**Keywords:** Hydrogen production; Co-culture; *Chlamydomonas reinhardtii*; *Bradyrhizobium japonicum*

**Dipankar Ghosh, Irma Flore Sobro, Patrick C. Hallenbeck.** (Département de Microbiologie et Immunologie, Université de Montréal, CP 6128 Succursale Centre-ville, Montréal, Québec, Canada H3C 3J7). **Optimization of the hydrogen yield from single-stage photofermentation of glucose by *Rhodobacter capsulatus* JP91 using response surface methodology. *Bioresource Technology*, Volume 123 (2012): 199–206**

Hydrogen production from glucose via single-stage photofermentation was examined with the photosynthetic bacterium *Rhodobacter capsulatus* JP91 (hup-). Response surface methodology with Box–Behnken design was used to optimize the independent experimental variables of glucose concentration, glutamate concentration and light intensity, as well as examining their interactive effects for maximization of molar hydrogen yield. Under optimal condition with a light intensity of 175 W/m<sup>2</sup>, 35 mM glucose, and 4.5 mM glutamate, a maximum hydrogen yield of 5.5 (±0.15) mol H<sub>2</sub>/mol glucose, and a maximum nitrogenase activity of 246 (±3.5) nmol C<sub>2</sub>H<sub>4</sub>/ml/min were obtained. Densitometric analysis of nitrogenase Fe-protein expression under different conditions showed significant variation in Fe-protein expression with a maximum at the optimized central point. Even under optimum conditions for hydrogen production, a significant fraction of the Fe-protein was found in the ADP-ribosylated state, suggesting that further improvement in yields might be possible.

**Keywords:** Biohydrogen; Photofermentation; Photosynthetic bacteria; H<sub>2</sub> yields

**Jean Nepomuscene Ntihuga<sup>a,b</sup>, Thomas Senn<sup>a</sup>, Peter Gschwind<sup>b</sup>, Reinhard Kohlus<sup>b</sup>.** (<sup>a</sup> Department of Fermentation Technology, Institute of Food Science and Biotechnology, Hohenheim Universität, Garbenstrasse 23, 70599 Stuttgart, Germany, <sup>b</sup> Department of Food Process Engineering, Institute of Food Science and Biotechnology, Hohenheim Universität, Garbenstrasse 25, 70599 Stuttgart, Germany). **Efficiency of Blenke cascade system for continuous bio-ethanol fermentation. Bioresource Technology, Volume 123 (2012): 221–229**

A gas lift-system with inserts (so-called Blenke cascade system) for continuous bio-ethanol fermentation was constructed. Gas introduced at the bottom of the column created toroidal vortices in the fluid cells between inserts, enhancing mixing and improving residence time behavior without stirring equipment being necessary. The parameters mash type, start-up strategy, yeast-recycle model and yeast separation were studied concerning the efficiency of the ethanol production. The best results obtained were for a filtered mash, a double saccharification principle (DSP), a batch start-up strategy, an activation-recycle model and a lamella settler connected in series with a small conventional gravitational settler for yeast cells separation. Using this system, the fermentation residence time was  $\tau = 4\text{--}5.5$  h, depending on substrate type. Eighty five percent of the yeast cells could be separated. High volumetric ethanol productivity ( $Q_p = 20.43$  g/L h) and yield  $E_y = 98\%$  were achieved. Continuous fermentation, yeast recycling and sedimentation were contamination-free processes.

**Keywords:** Bio-ethanol; Blenke cascade system; Continuous process; Start-up strategy; Yeast recycle model

**Alison Reiche, Kathlyn M. Kirkwood.** (Department of Chemical and Biological Engineering, Centre for Catalysis Research and Innovation, University of Ottawa, 161 Louis Pasteur Private, Ottawa, Ontario, Canada K1N 6N5). **Comparison of *Escherichia coli* and anaerobic consortia derived from compost as anodic biocatalysts in a glycerol-oxidizing microbial fuel cell. Bioresource Technology, Volume 123 (2012): 318–323**

Using glycerol from biodiesel production as a fuel in a microbial fuel cell (MFC) will generate electricity and value-added by-products from what is currently considered waste. This research screened *Escherichia coli* W3110 (ATCC 27325) and a mixed culture enriched from compost (AR2) as anodic biocatalysts in a mediatorless glycerol-oxidizing MFC. In an H-type MFC, the mixed culture AR2 biocatalyst produced a maximum power density of  $11.7$  mW m<sup>-2</sup> compared to  $9.8$  mW m<sup>-2</sup> using *E. coli* W3110 as the anodic catalyst. In batch operation of the fuel cell, the mixed culture AR2 was able to anaerobically consume  $29$  g/L of glycerol compared to only  $3.3$  g/L using the *E. coli* strain. The mixed culture was also shown to concurrently produce 1,3-propanediol, a value-added product, and electricity from a pure glycerol feedstock in an MFC.

**Keywords:** Microbial fuel cell; Glycerol; Anodic biocatalyst; Biodiesel waste; *Escherichia coli*

**Jinyong Yan, Aitao Li, Yi Xu, Thao P.N. Ngo, Szechao Phua, Zhi Li.** (Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore). **Efficient production of biodiesel from waste grease:**

**One-pot esterification and transesterification with tandem lipases. *Bioresource Technology*, Volume 123 (2012) : 332–337**

A novel concept and efficient method for producing biodiesel (FAME) from grease (15–40 wt% free fatty acid, FFA) were developed by using tandem lipases for one-pot esterification of FFA and transesterification of triglyceride with methanol in a solvent-free system. Combining immobilized *Candida antarctica* lipase B (CALB) (Novozyme 435) favoring the esterification and immobilized *Thermomyces lanuginosus* lipase (TLL) (Lipozyme TLIM) preferring the transesterification at 2:8 (wt/wt) gave FAME in 80% yield, being better than that with Novozyme 435 or Lipozyme TLIM. Recombinant *Escherichia coli* (Calb/Tll) co-expressing CALB and TLL was engineered as a more efficient tandem-lipases system. Using wet or dry cells (4 wt%) gave FAME in 87% or 95% yield, which is much better than that with *E. coli* cells expressing either CALB or TLL alone. Cells of *E. coli* (Calb/Tll) were recycled for five times and retained 75% productivity, thus being practical for producing biodiesel from grease.

**Keywords:** Biodiesel; Grease; Free fatty acid; Whole-cells catalysis; Tandem enzymes

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The myxomycetes are a group of primitive phagotrophic eukaryotes characterized by a distinctive plasmodial stage that is well known for its rapid growth rate. In the present study, biomass and lipid production of several different species of myxomycetes were investigated. *Physarum polycephalum* was found to produce the highest amounts of both dry biomass (1.30 g), and lipid (0.143 g) per 20 mL medium (equal to 65.0 g biomass and 7.15 g lipid per one liter of medium). Analysis of *P. polycephalum* lipids by thin layer chromatography (TLC) and fatty acid methyl esters (FAMES) by gas chromatography–mass spectrometry (GC–MS) techniques showed that the major lipid type is triglyceride (95.5%), followed by phospholipids (2.6%); diglyceride (0.92%) and monoglyceride (0.92%). Myxomycete lipids consist of three dominant fatty acids: oleic (20%), linoleic (33%), and palmitoleic (17%). These results suggest that *P. polycephalum* has considerable potential as a source of lipids for biodiesel production.

**Keywords:** Biodiesel; Lipids; Myxomycetes; *Physarum polycephalum*; Plasmodium

**Shengyang Hu<sup>1</sup>, Libai Wen<sup>1</sup>, Yun Wang, Xinsheng Zheng, Heyou Han.** (State Key Laboratory of Agricultural Microbiology, College of Science, Huazhong Agricultural University, Wuhan 430070, China). **Gas–liquid countercurrent integration process for continuous biodiesel production using a microporous solid base KF/CaO as catalyst. *Bioresource Technology*, Volume 123 (2012): 413–418**

A continuous-flow integration process was developed for biodiesel production using rapeseed oil as feedstock, based on the countercurrent contact reaction between gas and liquid, separation of glycerol on-line and cyclic utilization of methanol. Orthogonal experimental design and response

surface methodology were adopted to optimize technological parameters. A second-order polynomial model for the biodiesel yield was established and validated experimentally. The high determination coefficient ( $R^2 = 98.98\%$ ) and the low probability value ( $Pr < 0.0001$ ) proved that the model matched the experimental data, and had a high predictive ability. The optimal technological parameters were: 81.5 °C reaction temperature, 51.7 cm fill height of catalyst KF/CaO and 105.98 kPa system pressure. Under these conditions, the average yield of triplicate experiments was 93.7%, indicating the continuous-flow process has good potential in the manufacture of biodiesel.

**Keywords:** Biodiesel; Gas–liquid countercurrent integration process; Microporous catalyst; Transesterification

**M.E. Alzate<sup>a</sup>, R. Muñoz<sup>a</sup>, F. Rogalla<sup>b</sup>, F. Fdz-Polanco<sup>a</sup>, S.I. Pérez-Elvira<sup>a</sup>.** (<sup>a</sup> Department of Chemical Engineering and Environmental Technology, University of Valladolid, Dr. Mergelina s/n, Valladolid, Spain, <sup>b</sup> AQUALIA Gestión Integral del Agua, S.A, Spain). **Biochemical methane potential of microalgae: Influence of substrate to inoculum ratio, biomass concentration and pretreatment. Bioresource Technology, Volume 123 (2012): 488–494**

The anaerobic digestion of three microalgae mixtures was evaluated at different substrate to inoculum (S/I) ratios (0.5, 1 and 3), biomass concentrations (3, 10 and 20 gTS/kg) and pretreatments (thermal hydrolysis, ultrasound and biological treatment). An S/I ratio of 0.5 and 10 gTS/kg resulted in the highest final methane productivities regardless of the microalgae tested (ranging from 188 to 395 mL CH<sub>4</sub>/gVS<sub>added</sub>). The biological pretreatment supported negligible enhancements on CH<sub>4</sub> productivity, while the highest increase (46–62%) was achieved for the thermal hydrolysis. The optimum temperature of this pretreatment depended on the microalgae species. The ultrasound pretreatment brought about increases in CH<sub>4</sub> productivity ranging from 6% to 24% at 10,000 kJ/kgTS, without further increases at higher energy inputs. The results here obtained confirmed the lack of correlation between the solubilization degree and the methane enhancement potential and pointed out that anaerobic digestion of algae after thermal pretreatment is a promising technology for renewable energy production.

**Keywords:** BPM assays; Biodegradability; Microalgae digestion; Pretreatments; Solubilization degree

**Xun Hu, Richard Gunawan, Daniel Mourant, Yi Wang, Caroline Lievens, Weerawut Chaiwat, Liping Wu, Chun-Zhu Li.** (Fuels and Energy Technology Institute, Curtin University of Technology, GPO Box U1987, Perth, WA 6845, Australia). **Esterification of bio-oil from mallee (*Eucalyptus loxophleba* ssp. *gratae*) leaves with a solid acid catalyst: Conversion of the cyclic ether and terpenoids into hydrocarbons. Bioresource Technology, Volume 123 (2012): 249–255**

Bio-oil from pyrolysis of mallee (*Eucalyptus loxophleba* ssp. *gratae*) leaves differs from that obtained with wood by its content of cyclic ethers, terpenoids and N-containing organic compounds. Upgrading of the leaf bio-oil in methanol with a solid acid catalyst was investigated and it was found that the N-containing organics in the bio-oil lead to deactivation of the catalyst in the initial stage of exposure and have to be removed via employing high catalyst loading to

allow the occurrence of other acid-catalysed reactions. Eucalyptol, the main cyclic ether in the bio-oil, could be converted into the aromatic hydrocarbon, *p*-cymene, through a series of intermediates including  $\alpha$ -terpineol, terpinolene, and  $\alpha$ -terpinene. Various steps such as ring-opening, dehydration, isomerisation, and aromatization were involved in the conversion of eucalyptol. The terpenoids in bio-oil could also be converted into aromatic hydrocarbons that can serve as starting materials for the synthesis of fine chemicals, via the similar processes.

**Keywords:** Mallee leaves; Bio-oil; Eucalyptol; Terpenoids; Acid-catalysed reactions

**A. Ranga Rao<sup>1</sup>, G.A. Ravishankar, R. Sarada. (Plant Cell Biotechnology Department, CSIR-Central Food Technological Research Institute, Mysore 570 020, India). Cultivation of green alga *Botryococcus braunii* in raceway, circular ponds under outdoor conditions and its growth, hydrocarbon production. Bioresource Technology, Volume 123 (2012): 528–533**

The present study focused on cultivation, seasonal variation in growth, hydrocarbon production, fatty acids profiles of *Botryococcus braunii* (LB-572 and N-836) in raceway & circular ponds under outdoor conditions. After 18 days of cultivation the biomass yield and hydrocarbon contents were increased in both raceway and circular ponds. The fat content was found to be around 24% (w/w) with palmitic and oleic acids as prominent fatty acids. Hydrocarbons of C<sub>20</sub>–C<sub>30</sub> carbon chain length were higher in raceway and circular ponds. Maximum biomass yield (2 g L<sup>-1</sup>) and hydrocarbon content (28%) were observed in Nov–Dec. In case of *B. braunii* (N-836) after 25 days of cultivation the biomass yield was 1 g L<sup>-1</sup> and hydrocarbon content was 27%. Supplementation of 0.1% NaHCO<sub>3</sub> in the medium resulted in biomass yield of 1.5 g L<sup>-1</sup> and hydrocarbon content of 30% compared to control.

**Keywords:** Microalgae; *Botryococcus braunii*; Outdoor cultivation; Hydrocarbon; Fatty acids

**S. Venkata Mohan', M. Prathima Devi. (Bioengineering and Environmental Centre (BEEC), CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad 500607, India). Fatty acid rich effluent from acidogenic biohydrogen reactor as substrate for lipid accumulation in heterotrophic microalgae with simultaneous treatment. Bioresource Technology, Volume 123 (2012): 627–635**

Acid-rich effluent generated from acidogenic biohydrogen production process was evaluated as substrate for lipid synthesis by integrating with heterotrophic cultivation of mixed microalgae. Experiments were performed both with synthetic volatile fatty acids (SVFA) and fermented fatty acids (FFA) from biohydrogen producing reactor. Fatty acid based platform evidenced significant influence on algal growth as well as lipid accumulation by the formation of triglycerides through fatty acid synthesis. Comparatively FFA documented higher biomass and lipid productivity (1.42 mg/ml (wet weight); 26.4%) than SVFAs ((HAc + HBU + HPr), 0.60 mg/ml; 23.1%). Lipid profiles varied with substrates and depicted 18 types of saturated and unsaturated fatty acids with wide fuel and food characteristics. The observed higher concentrations of Chl *b* over Chl *a* supports the biosynthesis of triacylglycerides. Microalgae diversity visualized the presence of lipid accumulating species viz., *Scenedesmus* sp. and *Chlorella* sp. Integration of microalgae cultivation with biohydrogen production showed lipid productivity for biodiesel production along with additional treatment.

**Keywords:** Dark-fermentation; Wastewater treatment; Biodiesel; Volatile fatty acids; *Chlorella vulgaris*

**M. Venkateswar Reddy, G.N. Nikhil, S. Venkata Mohan, Y.V. Swamy, P.N. Sarma.** (Bioengineering and Environmental Centre (BEEC), CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad 500 607, India). *Pseudomonas otitidis* as a potential biocatalyst for polyhydroxyalkanoates (PHA) synthesis using synthetic wastewater and acidogenic effluents. *Bioresource Technology*, Volume 123 (2012): 471–479

Polyhydroxyalkanoates (PHA) production using *Pseudomonas otitidis*, a newly isolated strain from PHA producing bioreactor was investigated using synthetic acids (SA) and acidogenic effluents (AE) from biohydrogen reactor at different organic loading rates (OLRs). *P. otitidis* showed ability to grow and accumulate PHA, with simultaneous waste remediation. AE showed less PHA production (54%, OLR3), than SA (58%, OLR2). PHA composition showed co-polymer, poly-3(hydroxy butyrate-co-hydroxy valerate), P3(HB-co-HV). Bioprocess evaluation and enzymatic activities showed good correlation with PHA production. Kinetic studies on the growth of bacteria using different models at varying OLR were substantiated with PHA production. High substrate removal was registered at OLR1 (SA, 87%; AE, 82%). AE could be used as an alternative for pure substrates keeping in view of their high cost.

**Keywords:** Bioplastics; Wastewater treatment; P3(HB-co-HV); Dehydrogenase; Biohydrogen

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Thermal pretreatment were tested to increase the recovery of hydrocarbons from *Botryococcus braunii*/water mixtures via extraction with hexane. The effectiveness of treatment temperature was dependent on the *B. braunii* strain and the lowest temperatures which recovered over 90% of hydrocarbons were 60, 85 and 75 °C for the Yamanaka, Showa and Kawaguchi-1 strains respectively. The holding times of thermal pretreatment had less of an influence on hydrocarbon recovery than treatment temperature and, depending on the strain, recoveries of between 74.9% and 94.9% were achieved after thermal pretreatments at 80–90 °C for only 2.5 min. These results suggest thermal pretreatment could reduce the energy consumption of oil recovery process from wet *B. braunii* cells.

**Keywords:** *Botryococcus braunii*; Oil extraction; Biofuel; Hydrocarbon; Thermal pretreatment

**Pravakar Mohanty<sup>a, b</sup>, Madhumita Patel<sup>a</sup>, Kamal K. Pant<sup>a, 1</sup>.** (<sup>a</sup> Department of Chemical Engineering, Indian Institute of Technology, New Delhi 110016, India, <sup>b</sup> Department of Chemical Engineering, Catalysis and Chemical Reaction Engineering Laboratories, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A9). Hydrogen

**production from steam reforming of acetic acid over Cu–Zn supported calcium aluminate. *Bioresource Technology*, Volume 123 (2012): 558–565**

Hydrogen can be produced by catalytic steam reforming (CSR) of biomass-derived oil. Typically bio oil contains 12–14% acetic acid; therefore, this acid was chosen as model compound for reforming of biooil with the help of a Cu–Zn/Ca–Al catalyst for high yield of H<sub>2</sub> with low CH<sub>4</sub> and CO content. Calcium aluminate support was prepared by solid–solid reaction at 1350 °C. X-ray diffraction indicates 12CaO·7Al<sub>2</sub>O<sub>3</sub> as major, CaA<sub>14</sub>O<sub>7</sub> and Ca<sub>5</sub>A<sub>16</sub>O<sub>14</sub> as minor phases. Cu and Zn were loaded onto the support by wet-impregnation at 10 and 1 wt.%, respectively. The catalysts were characterized by Brunauer–Emmett–Teller (BET), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy TEM and the surface area for both support and Cu–Zn were 10.5 and 5.8 m<sup>2</sup>/g, respectively. CSR was carried out in a tubular fixed bed reactor (I.D. = 19 mm) at temperatures between 600 and 800 °C with 3-g loadings and (H<sub>2</sub>O/acetic acid) wt. ratio of 9:1. Significantly high (80%) yield of hydrogen was obtained over Cu–Zn/Ca–Al catalyst, as incorporation of Zn enhanced the H<sub>2</sub> yield by reducing deactivation of the catalyst. The coke formation on the support (Ca-12/Al-7) surface was negligible due to the presence of excess oxygen in the 12CaO·7Al<sub>2</sub>O<sub>3</sub> phase.

**Keywords:** H<sub>2</sub> production; Acetic acid; Steam reforming; Ca–Al/Cu–Zn catalyst

**S. Oncel, M. Sabankay. (Department of Bioengineering, Faculty of Engineering, University of Ege, 35100 Bornova, Izmir, Turkey). Microalgal biohydrogen production considering light energy and mixing time as the two key features for scale-up. *Bioresource Technology*, Volume 121 (2012): 228–234**

This study focuses on a scale-up procedure considering two vital parameters light energy and mixing for microalgae cultivation, taking *Chlamydomonas reinhardtii* as the model microorganism. Applying two stage hydrogen production protocol to 1 L flat type and 2.5 L tank type photobioreactors hydrogen production was investigated with constant light energy and mixing time. The conditions that provide the shortest transfer time to anaerobic culture (light energy; 2.96 kJ s<sup>-1</sup> m<sup>-3</sup> and mixing time; 1 min) and highest hydrogen production rate (light energy; 1.22 kJ s<sup>-1</sup> m<sup>-3</sup> and mixing time; 2.5 min) are applied to 5 L photobioreactor. The final hydrogen production for 5 L system after 192 h was measured as 195 ± 10 mL that is comparable with the other systems is a good validation for the scale-up procedure.

**Keywords:** Biofuels; Biohydrogen; Photobioreactor; Scale-up; *Chlamydomonas reinhardtii*

**Xuya Yu<sup>a, b</sup>, Peng Zhao<sup>a, b</sup>, Cian He<sup>b</sup>, Junjun Li<sup>a</sup>, Xianhua Tang<sup>a</sup>, Junpei Zhou<sup>a</sup>, Zunxi Huang<sup>a, c</sup>. (<sup>a</sup> Engineering Research Center of Sustainable Development and Utilization of Biomass Energy, Ministry of Education, Kunming 650500, China, <sup>b</sup> College of Life Sciences and Biotechnology, Kunming University of Science and Technology, Kunming 650500, China, <sup>c</sup> Key Laboratory of Yunnan for Biomass Energy and Biotechnology of Environment, Kunming 650500, China). Isolation of a novel strain of *Monoraphidium* sp. and characterization of its potential application as biodiesel feedstock. *Bioresource Technology*, Volume 121 (2012): 256–262**

A novel green microalgae strain from Lake Fuxian has been isolated and identified as a potential feedstock for biodiesel production. The novel strain was named *Monoraphidium* sp. FXY-10 based on its morphological and genomic characterization. The lipid productivities, fatty acid

profiles, and microalgae recovery efficiency ( $\eta_a$ ) of FXY-10 were investigated and compared under autotrophic and heterotrophic conditions. FXY-10 under autotrophic conditions exhibited a higher cellular lipid content (56.8%) than those under heterotrophic conditions (37.56%). However, FXY-10 growing under heterotrophic conditions exhibited more than 20-fold increase in lipid productivity compared with that under autotrophic conditions ( $148.74 \text{ mg L}^{-1} \text{ d}^{-1}$  versus  $6.88 \text{ mg L}^{-1} \text{ d}^{-1}$ ). Moreover, higher saturated and monounsaturated fatty acids (77.5%) of FXY-10 was obtained under heterotrophic culture conditions, suggesting its potential as a biodiesel feedstock. Gravity sedimentation was proposed as the harvesting biomass method based on the 97.9% microalgae recovery efficiency of heterotrophic cells after settling for 24 h.

**Keywords:** Microalgae; FXY-10; *Monoraphidium* sp.; Biodiesel production

Anja Günther<sup>a</sup>, Torsten Jakob<sup>a</sup>, Reimund Goss<sup>a</sup>, Swetlana König<sup>b</sup>, Daniel Spindler<sup>b</sup>, Norbert Rübiger<sup>c</sup>, Saskia John<sup>c</sup>, Susanne Heithoff<sup>c</sup>, Mark Fresewinkel<sup>d</sup>, Clemens Posten<sup>d</sup>, Christian Wilhelm<sup>a</sup>. (<sup>a</sup>University of Leipzig, Institute of Biology, Department of Plant Physiology, Johannisallee 23, D-04103 Leipzig, Germany, <sup>b</sup> Saxon Institute of Applied Biotechnology, Permoserstr. 15, D-04318 Leipzig, Germany, <sup>c</sup> University of Bremen, Institute for Environmental Process Engineering, Leobener Str. 33, D-28359 Bremen, Germany, <sup>d</sup> Karlsruhe Institute of Technology, Institute of Life Science Engineering, Bioprocess Engineering, Fritz-Haber-Weg 2, D-76131 Karlsruhe, Germany). Methane production from glycolate excreting algae as a new concept in the production of biofuels. *Bioresource Technology*, Volume 121 (2012): 454–457

It is the aim of the present work to introduce a new concept for methane production by the interaction of a glycolate-excreting alga (*Chlamydomonas reinhardtii*) and methanogenic microbes operating in separate compartments within one photobioreactor. This approach requires a minimum number of metabolic steps to convert light energy to methane thereby reducing the energetic and financial costs of biomass formation, harvest and refinement. In this feasibility study it is shown that the physiological limitations for sustained glycolate production can be circumvented by the use of *C. reinhardtii* mutants whose carbon concentrating mechanisms or glycolate dehydrogenase are suppressed. The results also demonstrate that methanogenic microbes are able to thrive on glycolate as single carbon source for a long time period, delivering biogas composed of CO<sub>2</sub>/methane with only very minor contamination.

**Keywords:** Biofuel; Biomethane; Bioreactor; *Chlamydomonas*

Ruengwit Sawangkeaw<sup>a</sup>, Sunsanee Teeravitud<sup>a</sup>, Pornpote Piumsomboon<sup>a, b</sup>, Somkiat Ngamprasertsith<sup>a, b</sup>. (<sup>a</sup> Fuels Research Center, Department of Chemical Technology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand, <sup>b</sup> Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand). Biofuel production from crude palm oil with supercritical alcohols: Comparative LCA studies. *Bioresource Technology*, Volume 120 (2012): 6–12

A recent life cycle assessment (LCA) reported that biodiesel production in supercritical alcohols (SCA) produces a higher environmental load than the homogeneous catalytic process because an enormous amount of energy is required to recover excess alcohol. However, the excess alcohol

could be dramatically reduced by increasing the operating temperature to 400 °C; although the product would have to be considered as an alternative biofuel instead of biodiesel. A comparative LCA of the biodiesel production in two SCA at 300 °C (C-SCA) and novel biofuel production in the same two SCA at 400 °C (N-SCA) is presented. It was clear that the N-SCA process produces a dramatically reduced environmental load over that of the C-SCA process due to a lower amount of excess alcohol being used. The N-SCA process could be improved in terms of its environmental impact by changing from fossil fuel to biomass-based fuels for the steam generation.

**Keywords:** Biofuel; Biodiesel; Life cycle assessment; Supercritical alcohol; Vegetable oil

**Xue Xia, Yanmei Sun, Peng Liang, Xia Huang. (State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, PR China). Long-term effect of set potential on biocathodes in microbial fuel cells: Electrochemical and phylogenetic characterization. Bioresource Technology, Volume 120 (2012): 26–33**

The long-term effect of set potential on oxygen reducing biocathodes was investigated in terms of electrochemical and biological characteristics. Three biocathodes were poised at 200, 60 and –100 mV vs. saturated calomel electrode (SCE) for 110 days, including the first 17 days for startup. Electrochemical analyses showed that 60 mV was the optimum potential during long-term operation. The performance of all the biocathodes kept increasing after startup, suggesting a period longer than startup time needed to make potential regulation more effective. The inherent characteristics without oxygen transfer limitation were studied. Different from short-term regulation, the amounts of biomass were similar while the specific electrochemical activity was significantly influenced by potential. Moreover, potential showed a strong selection for cathode bacteria. Clones 98% similar with an uncultured Bacteroidetes bacterium clone CG84 accounted for 75% to 80% of the sequences on the biocathodes that showed higher electrochemical activity (60 and –100 mV).

**Keywords:** Microbial fuel cell; Biocathode; Set potential; Long-term effect

**Isik Coban<sup>a</sup>, Sayit Sargin<sup>a</sup>, Melih Soner Celiktas<sup>b</sup>, Ozlem Yesil-Celiktas<sup>a</sup>. (<sup>a</sup> Department of Bioengineering, Faculty of Engineering, Ege University, 35100 Bornova-Izmir, Turkey). Bioethanol production from raffinate phase of supercritical CO<sub>2</sub> extracted *Stevia rebaudiana* leaves. Bioresource Technology, Volume 120 (2012): 52–59**

The extracts of *Stevia rebaudiana* are marketed as dietary supplements and utilized as natural sweetening agent in food products. Subsequent to extraction on industrial scale, large quantities of solid wastes are produced. The aim of this study was to investigate the bioconversion efficiency of supercritical CO<sub>2</sub> extracted *S. rebaudiana* residues. Therefore, leaves were extracted with supercritical CO<sub>2</sub> and ethanol mixture in order to obtain glycosides, then the raffinate phase was hydrolyzed by both dilute acid and various concentrations of cellulase and β-glucosidase cocktail. The maximum yield of reducing sugars reached 25.67 g/L under the optimal conditions of enzyme pretreatment, whereas 32.00 g/L was reached by consecutive enzymatic and acid hydrolyses. Bioethanol yield (20 g/L, 2.0% inoculum, 2 days) based on the sugar consumed was 45.55% corresponding to a productivity of 0.19 kg/m<sup>3</sup>h which demonstrates challenges to be utilized as a potential feedstock for the production of bioethanol.

Keywords: Biofuel; Bioethanol; Stevia; Supercritical fluid extraction; Cellulase

**Wen Wang<sup>a</sup>, Li Xie<sup>a</sup>, Gang Luo<sup>a</sup>, Qi Zhou<sup>a</sup>, Qin Lu<sup>a, b</sup>. ( <sup>a</sup> State Key Laboratory of Pollution Control and Resources Reuse, Tongji University, 1239 Siping Road, Shanghai 200092, PR China, <sup>b</sup> Ovivo Water Technologies, Zhangjiang Hi-Tech Park, Shanghai 201203, PR China). Optimization of biohydrogen and methane recovery within a cassava ethanol wastewater/waste integrated management system. *Bioresource Technology*, Volume 120 (2012): 165–172**

Thermophilic co-fermentation of cassava stillage (CS) and cassava excess sludge (CES) were investigated for hydrogen and methane production. The highest hydrogen yield (37.1 ml/g-total-VS added) was obtained at  $VS_{CS}/VS_{CES}$  of 7:1, 17% higher than that with CS digestion alone. The CES recycle enhanced the substrate utilization and improved the buffer capacity. Further increase the CES fraction led to changed VFA distribution and more hydrogen consumption. FISH analysis revealed that both hydrogen producing bacteria and hydrogen consuming bacteria were enriched after CES recycled, and the acetobacteria percentage increased to 12.4% at  $VS_{CS}/VS_{CES}$  of 6:2. Relatively high efficient and stable hydrogen production was observed at  $VS_{CS}/VS_{CES}$  of 5:3 without pH adjusted and any pretreatment. The highest total energy yield, the highest COD and VS degradation were obtained at  $VS_{CS}/VS_{CES}$  of 7:1. GFC analysis indicated that the hydrolysis behavior was significantly improved by CES recycle at both hydrogen and methane production phase.

**Keywords:** Thermophilic co-digestion; Hydrogen; Methane; Integrated management

**K.P. Gregoire, J.G. Becker. (Department of Civil & Environmental Engineering, University of Maryland, College Park, College Park, MD 20742, USA , Department of Environmental Science & Technology, University of Maryland, College Park, MD 20742, USA). Design and characterization of a microbial fuel cell for the conversion of a lignocellulosic crop residue to electricity. *Bioresource Technology*, Volume 119 (2012) : 208–215**

Agricultural crop residues contain high amounts of biochemical energy as cellulose and lignin. A portion of this biomass could be sustainably harvested for conversion to bioenergy to help offset fossil fuel consumption. In this study, the potential for converting lignocellulosic biomass directly to electricity in a microbial fuel cell (MFC) was explored. Design elements of tubular air cathode MFCs and leach-bed bioreactors were integrated to develop a new solid-substrate MFC in which cellulose hydrolysis, fermentation, and anode respiration occurred in a single chamber. Electricity was produced continuously from untreated corncob pellets for >60 d. Addition of rumen fluid increased power production, presumably by providing growth factors to anode-respiring bacteria. Periodic exposure to oxygen also increased power production, presumably by limiting the diversion of electrons to methanogenesis. In the absence of methanogenesis, bioaugmentation with *Geobacter metallireducens* further improved MFC performance. Under these conditions, the maximum power density was 230 mW/m<sup>3</sup>.

Keywords: Microbial fuel cell; Bioenergy; Lignocellulose; Crop residues

**K. Srilatha<sup>a</sup>, B.L.A. Prabhavathi Devi<sup>b</sup>, N. Lingaiah<sup>a</sup>, R.B.N. Prasad<sup>b</sup>, P.S. Sai Prasad<sup>a</sup>. (<sup>a</sup>Catalysis Laboratory, I&PC Division, Indian Institute of Chemical Technology, Hyderabad 500607, India, <sup>b</sup> Centre for Lipid Research, Indian Institute of Chemical Technology, Hyderabad 500607, India). Biodiesel production from used cooking oil by two-step heterogeneous catalyzed process. *Bioresource Technology*, Volume 119 (2012): 306–311**

The present study demonstrates the production of biodiesel from used cooking oil containing high free fatty acid by a two-step heterogeneously catalyzed process. The free fatty acids were first esterified with methanol using a 25 wt.% TPA/Nb<sub>2</sub>O<sub>5</sub> catalyst followed by transesterification of the oil with methanol over ZnO/Na-Y zeolite catalyst. The catalysts were characterized by XRD, FT-IR, BET surface area and CO<sub>2</sub>-TPD. In the case of transesterification the effect of reaction parameters, such as catalyst concentration, methanol to oil molar ratio and reaction temperature, on the yield of ester were investigated. The catalyst with 20 wt.% ZnO loading on Na-Y exhibited the highest activity among the others. Both the solid acid and base catalysts were found to be reusable for several times indicating their efficacy in the two-step process.

**Keywords:** Biodiesel; Transesterification; Two-step method; Solid catalyst; Zinc oxide

**Mark D. Redwood, Rafael L. Orozco, Artur J. Majewski, Lynne E. Macaskie. (School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK). An integrated biohydrogen refinery: Synergy of photofermentation, extractive fermentation and hydrothermal hydrolysis of food wastes. *Bioresource Technology*, Volume 119 (2012): 384–392**

An *Integrated Biohydrogen Refinery* (IBHR) and experimental net energy analysis are reported. The IBHR converts biomass to electricity using hydrothermal hydrolysis, extractive biohydrogen fermentation and photobiological hydrogen fermentation for electricity generation in a fuel cell. An extractive fermentation, developed previously, is applied to waste-derived substrates following hydrothermal pre-treatment, achieving 83-99% biowaste destruction. The selective separation of organic acids from waste-fed fermentations provided suitable substrate for photofermentative hydrogen production, which enhanced the gross energy generation up to 11-fold. Therefore, electrodialysis provides the key link in an IBHR for 'waste to energy'. The IBHR compares favourably to 'renewables' (photovoltaics, on-shore wind, crop-derived biofuels) and also emerging biotechnological options (microbial electrolysis) and anaerobic digestion.

**Keywords:** Integrated biohydrogen refinery; Waste to energy; Electro-fermentation; Photofermentation; Hydrothermal hydrolysis

**Xufeng Yuan<sup>1</sup>, Yanzhuan Cao<sup>1</sup>, Jiajia Li, Boting Wen, Wanbin Zhu, Xiaofen Wang, Zongjun Cui. (College of Agronomy and Biotechnology/Center of Biomass Engineering, China Agricultural University, Beijing 100193, China). Effect of pretreatment by a microbial consortium on methane production of waste paper and cardboard. *Bioresource Technology*, Volume 118 (2012): 281–288**

A microbial consortium MC1 was used to pretreat filter paper, office paper, newspaper, and cardboard to enhance methane production. The results of pretreatment indicated that sCOD of

hydrolysates of the four substrates increased significantly in the early stage, and peaked on day 7. During pretreatment, ethanol, acetic acid, propionic acid, butyric acid, and glycerol were the predominant volatile organic products in hydrolysates. MC1 had strong degradation ability on the four substrates, and the weight loss of filter paper, office paper, newspaper, and cardboard reached 78.3%, 80.5%, 39.7%, and 49.7%, respectively. The results of anaerobic digestion showed that methane production yields and rates of the four substrates significantly increased after pretreatment. This study is the first attempt to explore the microbial pretreatment method for anaerobic digestion of waste paper and cardboard. Microbial consortium pretreatment could be an effective method for enhancing methane production of waste paper and cardboard into bioenergy.

**Keywords:** Microbial consortium; Methane production; Pretreatment; Waste paper and cardboard; Hydrolysate

**Booki Min<sup>a, b</sup>, Finn Willy Poulsen<sup>c</sup>, Anders Thygesen<sup>d</sup>, Irimi Angelidaki<sup>b</sup>.** (<sup>a</sup> Department of Environmental Science and Engineering, Kyung Hee University, 1 Seocheon-dong, Yongin-si, Gyeonggi-do 446-701, Republic of Korea, <sup>b</sup> Department of Environmental Engineering, Technical University of Denmark, DK-2800 Lyngby, Denmark, <sup>c</sup> Fuel Cell and Solid State Chemistry Department, National Laboratory for Sustainable Energy, Technical University of Denmark P.O. Box 49, DK-4000 Roskilde, Denmark). **Electric power generation by a submersible microbial fuel cell equipped with a membrane electrode assembly. Bioresource Technology, Volume 118 (2012): 412–417**

Membrane electrode assemblies (MEAs) were incorporated into the cathode chamber of a submersible microbial fuel cell (SMFC). A close contact of the electrodes could produce high power output from SMFC in which anode and cathode electrodes were connected in parallel. In polarization test, the maximum power density was 631 mW/m<sup>2</sup> at current density of 1772 mA/m<sup>2</sup> at 82 Ω. With 180-Ω external resistance, one set of the electrodes on the same side could generate more power density of 832 ± 4 mW/m<sup>2</sup> with current generation of 1923 ± 4 mA/m<sup>2</sup>. The anode, inclusive a biofilm behaved ohmic, whereas a Tafel type behavior was observed for the oxygen reduction. The various impedance contributions from electrodes, electrolyte and membrane were analyzed and identified by electrochemical impedance spectroscopy. Air flow rate to the cathode chamber affected microbial voltage generation, and higher power generation was obtained at relatively low air flow less than 2 mL/min.

**Keywords:** Microbial fuel cell; Membrane electrode assembly; Submersible microbial fuel cell; Voltage generation; Electrochemical impedance spectroscopy

**Chao Ma<sup>a</sup>, Xueqing Wang<sup>a</sup>, Liejin Guo<sup>a</sup>, Xiaomin Wu<sup>a</sup>, Honghui Yang<sup>a, b</sup>.** (<sup>a</sup> International Research Center for Renewable Energy, State Key Laboratory of Multiphase Flow in Power Engineering, Xi'an Jiaotong University, Xi'an 710049, PR China, <sup>b</sup> Department of Environmental Engineering, Xi'an Jiaotong University, Xi'an 710049, PR China). **Enhanced photo-fermentative hydrogen production by *Rhodobacter capsulatus* with pigment content manipulation. Bioresource Technology, Volume 118 (2012); 490–495**

High content of pigment in purple nonsulfur photosynthetic bacteria hinders its photo-hydrogen production rate under intense light irradiation. In order to alleviate the light shielding effect and

improve its photo-fermentative hydrogen production performance, *pufQ*, which is the regulatory gene of bacteriochlorophyll biosynthesis in *Rhodobacter capsulatus*, was cloned and relocated in the genome under *cbb3* promoter by homologous recombination. The UV-vis spectra indicated that the light absorption of the mutant between 300 and 900 nm was reduced. Photo-hydrogen production experiments by the recombinant and wild type strain were carried out in 350 mL photo bioreactors using acetic and butyric acid as substrate. The results showed that the hydrogen production of recombinant with reduced pigment was 27% higher than that of its parental strain, indicating that it is effective on enhancing photo-fermentative hydrogen production by manipulating pigment biosynthesis in purple nonsulfur photosynthetic bacteria.

**Keywords:** Biohydrogen; Photo-fermentative hydrogen production; Reduced pigment; Bacteriochlorophyll biosynthesis

**G. Mendow, N.S. Veizaga, B.S. Sánchez, C.A. Querini. (Instituto de Investigaciones en Catálisis y Petroquímica-INCAPE-(FIQ-UNL, CONICET), Santiago del Estero 2654, 3000 Santa Fe, Argentina). Biodiesel production by two-stage transesterification with ethanol by washing with neutral water and water saturated with carbon dioxide. Bioresource Technology, Volume 118 (2012): 598–602**

Industrial production of ethyl esters is impeded by difficulties in purifying the product due to high amounts of soap formed during transesterification. A simple biodiesel wash process was developed that allows successful purification of samples containing high amounts of soap. The key step was a first washing with neutral water, which removed the soaps without increasing the acidity or affecting the process yield. Afterward, the biodiesel was washed with water saturated with CO<sub>2</sub>, a mild acid that neutralized the remaining soaps and extracted impurities. The acidity, free-glycerine, methanol and soaps concentrations were reduced to very low levels with high efficiency, and using non-corrosive acids. Independently of the initial acidity, it was possible to obtain biodiesel within EN14214 specifications. The process included the recovery of soaps by hydrolysis and esterification, making it possible to obtain the theoretical maximum amount of biodiesel.

**Keywords:** Biodiesel; Ethanol; Transesterification; Purification; Production process

**Ashik Sathish, Ronald C. Sims. (Department of Biological Engineering, Utah State University, 4105 Old Main Hill, Logan, UT 84322-4105, United States). Biodiesel from mixed culture algae via a wet lipid extraction procedure. Bioresource Technology, Volume 118 (2012): 643–647**

Microalgae are a source of renewable oil for liquid fuels. However, costs for dewatering/drying, extraction, and processing have limited commercial scale production of biodiesel from algal biomass. A wet lipid extraction procedure was developed that was capable of extracting 79% of transesterifiable lipids from wet algal biomass (84% moisture) via acid and base hydrolysis (90 °C and ambient pressures), and 76% of those extracted lipids were isolated, by further processing, and converted to FAMES. Furthermore, the procedure was capable of removing chlorophyll contamination of the algal lipid extract through precipitation. In addition, the procedure generated side streams that serve as feedstocks for microbial conversion to additional bioproducts. The capability of the procedure to extract lipids from wet algal biomass, to reduce/remove chlorophyll contamination, to potentially reduce organic solvent demand, and to

generate feedstocks for high-value bioproducts presents opportunities to reduce costs of scaling up algal lipid extraction for biodiesel production.

**Keywords:** Biodiesel; Bioproducts; Chlorophyll; Microalgae; Wet lipid extraction

**L. Lemée, D. Kpogbemabou, L. Pinard, R. Beauchet, J. Laduranty. (Université de Poitiers, CNRS, UMR 7285, IC2MP, 4 rue Michel Brunet, Poitiers 86022, France). Biological pretreatment for production of lignocellulosic biofuel. Bioresource Technology, Volume 117 (2012): 234–241**

Lignocellulosic biomass was submitted to a biological pretreatment prior to a catalytic hydroliquefaction in order to produce biofuel. The biodegradation process was conducted over 3 months in a reactor under controlled conditions. During the biodegradation process the organic matter was characterised and its evolution was correlated with physico-chemical parameters. In parallel with the analysis of the lipidic fraction, analytical pyrolysis was used to monitor bacterial activity. The alterations of branched to linear fatty acids ratio and of mono- to diacids ratio were compared when determined by thermochemolysis and observed in the directly extractable lipids. The evolution of the phytol to the corresponding isoprenic ketone ratio was observed to be dependent on the desorption technique since it decreases using headspace while it increases using pyrolysis. “Humic”/“fulvic acids” ratio, infrared spectroscopy and thermodifferential analysis were used to determine the degree of OM complexification.

**Keywords:** Lignocellulosic biomass; Biological pretreatment; Catalytic hydroliquefaction; Biofuel; Analytical pyrolysis

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Biodiesel synthesis from rice bran fatty acids (RBFA) was carried out using cesium exchanged 12-tungstophosphoric acid (TPA) catalysts. The physico-chemical properties of the catalysts were derived from X-ray diffraction (XRD), Fourier transform infrared (FTIR), temperature programmed desorption (TPD) of NH<sub>3</sub> and scanning electron microscopy (SEM). The characterization techniques revealed that the Keggin structure of TPA remained intact as Cs replaced protons. The partial exchange of Cs for protons resulted in an increase in acidity and the catalysts with one Cs<sup>+</sup> (Cs<sub>1</sub>H<sub>2</sub>PW<sub>12</sub>O<sub>40</sub>) showed highest acidity. Under optimized conditions about 92% conversion of RBFA was obtained. The catalyst was reused for five times and retained of its original activity. Pseudo-first order model was applied to correlate the experimental kinetic data. Modified tungstophosphoric acids are efficient solid acid catalysts for the synthesis of biodiesel from the oils containing high FFA.

**Keywords:** Esterification; Rice bran fatty acids; Biodiesel; 12-Tungstophosphoric acid; Cesium salts

**Huan Wang, Dongmei Liu, Lu Lu, Zhiwei Zhao, Yongpeng Xu, Fuyi Cui. (State Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China). Degradation of algal organic matter using microbial fuel cells and its association with trihalomethane precursor removal. *Bioresource Technology*, Volume 116 (2012) : 80–85**

In order to provide an alternative for removal of algal organic matter (AOM) produced during algal blooms in aquatic environment, microbial fuel cell (MFC) was used to study AOM degradation and its association with THM precursor removal. The chemical oxygen demand (COD) removals in MFCs were  $81 \pm 6\%$  and  $73 \pm 3\%$  for AOM from *Microcystis aeruginosa* (AOM<sub>M</sub>) and *Chlorella vulgaris* (AOM<sub>C</sub>), respectively. THM precursor was also effectively degraded (AOM<sub>M</sub>  $85 \pm 2\%$ , AOM<sub>C</sub>  $72 \pm 4\%$ ). The major AOM components (proteins, lipids, and carbohydrates) were obviously removed in MFCs. The contribution of each component to the THM formation potential (THMFP) was obtained based on calculation. The THMFP produced from soluble microbial products was very low. If the energy input during operation process was not considered, MFCs treatment could recover electrical energy of  $0.29 \pm 0.02$  kWh/kg COD (AOM<sub>M</sub>) and  $0.35 \pm 0.06$  kWh/kg COD (AOM<sub>C</sub>).

**Keywords:** Algal organic matter; Microbial fuel cells; AOM composition; THM precursor

**Chao Li, Lili Ding, Hao Cui, Libin Zhang, Ke Xu, Hongqiang Ren. (State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210046, Jiangsu, PR China). Application of conductive polymers in biocathode of microbial fuel cells and microbial community. *Bioresource Technology*, Volume 116 (2012): 459–465**

Four kinds of conductive polymers, polyaniline (PANI) and its co-polymers poly (aniline-co-aminophenol) (PANOA), poly (aniline-co-2, 4-diaminophenol) (PANDAP) and poly (aniline-1, 8-diaminonaphthalene) (PANDAN) were applied to modify carbon felts as the aerobic abiotic cathodes and biocathodes in microbial fuel cells (MFC). Compare to unmodified, all the four polymers can significantly improve the power densities for both abiotic cathodes (increased by 300%) and biocathodes (increased by 180%). The co-polymers with different functional groups introduction had further special advantages in MFC performance: PANOA and PANDAP with –OH showed less sensitivity to DO and pH change in cathode; PANDAP and PANDAN with –NH<sub>3</sub> provided better attachment condition for biofilm which endowed them higher power output. With the help of conductive polymer coats, the cathode biofilm became thicker, and according to biodiversity analysis, the predominated phyla changed from  $\beta$ -*Proteobacteria* (unmodified) to  $\alpha$ ,  $\gamma$ -*Proteobacteria* (modified), which may be responsible for the superiority of the modified MFCs.

**Keywords:** Microbial fuel cell; Conductive polymer; Biocathode; Biodiversity; Functional groups

**Jing Gan<sup>a</sup>, Wenqiao Yuan<sup>b</sup>, Loretta Johnson<sup>c</sup>, Donghai Wang<sup>a</sup>, Richard Nelson<sup>d</sup>, Ke Zhang<sup>a</sup>. (<sup>a</sup>Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS 66506, United States, <sup>b</sup> Department of Biological and Agricultural Engineering, North Carolina State University, Raleigh, NC 27695, United States, <sup>c</sup> Department of Biology, Kansas State University, Manhattan, KS 66506, United States, <sup>d</sup> Department of Chemical Engineering, Kansas State University, Manhattan, KS 66506,**

**United States). Hydrothermal conversion of big bluestem for bio-oil production: The effect of ecotype and planting location. Bioresource Technology, Volume 116 (2012): 413–420**

Three ecotypes (CKS, EKS, IL) and one cultivar (KAW) of big bluestem (*Andropogon gerardii*) that were planted in three locations (Hays, KS; Manhattan, KS; and Carbondale, IL) were converted to bio-oil via hydrothermal conversion. Significant differences were found in the yield and elemental composition of bio-oils produced from big bluestem of different ecotypes and/or planting locations. Generally, the IL ecotype and the Carbondale, IL and Manhattan, KS planting locations gave higher bio-oil yield, which can be attributed to the higher total cellulose and hemicellulose content and/or the higher carbon but lower oxygen contents in these feedstocks. Bio-oil from the IL ecotype also had the highest carbon and lowest oxygen contents, which were not affected by the planting location. Bio-oils from big bluestem had yield, elemental composition, and chemical compounds similar to bio-oils from switchgrass and corncobs, although mass percentages of some of the compounds were slightly different.

**Keywords:** Bio-oil; Big bluestem; Hydrothermal conversion; Liquefaction; Planting location

**Asir Obadiah<sup>a</sup>, Gnanadurai Ajji Swaroopa<sup>b</sup>, Samuel Vasanth Kumar<sup>a</sup>, Kenthorai Raman Jeganathan<sup>c</sup>, Alagunambi Ramasubbu<sup>d</sup>.** (<sup>a</sup> Department of Chemistry, Karunya University, Coimbatore 641114, Tamilnadu, India, <sup>b</sup> Department of Biotechnology, Karunya University, Coimbatore 641114, Tamilnadu, India, <sup>c</sup> Novozymes South Asia Pvt. Ltd., Bangalore 560066, India, <sup>d</sup> Post Graduate and Research Department of Chemistry, Govt. Arts College (Autonomous), Coimbatore, 641018, Tamilnadu, India). **Biodiesel production from Palm oil using calcined waste animal bone as catalyst. Bioresource Technology, Volume 116 (2012): 512–516**

Waste animal bones was employed as a cost effective catalyst for the transesterification of palm oil. The catalyst was calcined at different temperatures to transform the calcium phosphate in the bones to hydroxyapatite and 800 °C was found to give the best yield of biodiesel. The catalyst was characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), energy dispersive spectrometry (EDS) and Fourier transform infrared spectrometry (FT-IR). Under the optimal reaction conditions of 20 wt.% of catalyst, 1:18 oil to methanol molar ratio, 200 rpm of stirring of reactants and at a temperature of 65 °C, the methyl ester conversion was 96.78% and it was achieved in 4 h. The catalyst performed equally well as the laboratory-grade CaO. Animal bone is therefore a useful raw material for the production of a cheap catalyst for transesterification.

**Keywords:** Animal bone; Heterogeneous catalyst; Hydroxyapatite; Palm oil; Transesterification

**Ying Ying Tye<sup>a</sup>, Keat Teong Lee<sup>b</sup>, Wan Nadiah Wan Abdullah<sup>a</sup>, Cheu Peng Leh<sup>a</sup>.** (<sup>a</sup> School of Industrial Technology, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia, <sup>b</sup> School of Chemical Engineering, Universiti Sains Malaysia, Engineering Campus, Seri Ampang, 14300 Nibong Tebal, Penang, Malaysia). **Potential of *Ceiba pentandra* (L.) Gaertn. (kapok fiber) as a resource for second generation bioethanol: Effect of various simple pretreatment methods on sugar production. Bioresource Technology, Volume 116 (2012): 536–539**

The importance of bioethanol currently has increased tremendously as it can reduce the total dependency on fossil-fuels, especially gasoline, in the transportation sector. In this study, *Ceiba pentandra* (kapok fiber) was introduced as a new resource for bioethanol production. The results of chemical composition analysis showed that the cellulose (alpha- and beta-) contents were 50.7%. The glucose composition of the fiber was 59.8%. The high glucose content indicated that kapok fiber is a potential substrate for bioethanol production. However, without a pretreatment, the kapok fiber only yielded 0.8% of reducing sugar by enzymatic hydrolysis. Thus, it is necessary to pre-treat the kapok fiber prior to hydrolysis. Taking into account environmentally friendliness, only simple pretreatments with minimum chemical or energy consumption was considered. It was interesting to see that by adopting merely water, acid and alkaline pretreatments, the yield of reducing sugar was increased to 39.1%, 85.2% and >100%, respectively.

**Keywords:** Enzymatic hydrolysis; Kapok fiber; Pretreatment; Reducing sugar; Second generation bioethanol

**J. R. Miranda, P. C. Passarinho, L. Gouveia. (Laboratório Nacional de Energia e Geologia (LNEG), Unidade de Bioenergia, Estrada do Paço do Lumiar, 1649-038 Lisbon, Portugal. L. Gouveia. Email: luisa.gouveia@lneg.pt). Bioethanol production from *Scenedesmus obliquus* sugars: the influence of photobioreactors and culture conditions on biomass production. Applied Microbiology and Biotechnology, Volume 96(2) (2012): 555-564**

A closed-loop vertical tubular photobioreactor (PBR), specially designed to operate under conditions of scarce flat land availability and irregular solar irradiance conditions, was used to study the potential of *Scenedesmus obliquus* biomass/sugar production. The results obtained were compared to those from an open-raceway pond and a closed-bubble column. The influence of the type of light source and the regime (natural vs artificial and continuous vs light/dark cycles) on the growth of the microalga and the extent of the sugar accumulation was studied in both PBRs. The best type of reactor studied was a closed-loop PBR illuminated with natural light/dark cycles. In all the cases, the relationship between the nitrate depletion and the sugar accumulation was observed. The microalga *Scenedesmus* was cultivated for 53 days in a raceway pond (4,500 L) and accumulated a maximum sugar content of 29 % g/g. It was pre-treated for carrying out ethanol fermentation assays, and the highest ethanol concentration obtained in the hydrolysate fermented by *Kluyveromyces marxianus* was 11.7 g/L.

**Jingnan Lu<sup>1</sup>, Christopher J. Brigham<sup>2</sup>, Claudia S. Gai<sup>2</sup>, Anthony J. Sinskey<sup>2,3,4</sup>. (<sup>1</sup>Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA, <sup>2</sup>Department of Biology, Massachusetts Institute of Technology, Bldg. 68-370, 77 Massachusetts Avenue, Cambridge, MA 02139, USA, <sup>3</sup>Division of Health Sciences and Technology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA, <sup>4</sup>Engineering Systems Division, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA. Email: asinskey@mit.edu). Studies on the production of branched-chain alcohols in engineered *Ralstonia eutropha*. Applied Microbiology and Biotechnology, Volume 96(1) (2012): 283-297**

Wild-type *Ralstonia eutropha* H16 produces polyhydroxybutyrate (PHB) as an intracellular carbon storage material during nutrient stress in the presence of excess carbon. In this study, the excess carbon was redirected in engineered strains from PHB storage to the production of

isobutanol and 3-methyl-1-butanol (branched-chain higher alcohols). These branched-chain higher alcohols can directly substitute for fossil-based fuels and be employed within the current infrastructure. Various mutant strains of *R. eutropha* with isobutyraldehyde dehydrogenase activity, in combination with the overexpression of plasmid-borne, native branched-chain amino acid biosynthesis pathway genes and the overexpression of heterologous ketoisovalerate decarboxylase gene, were employed for the biosynthesis of isobutanol and 3-methyl-1-butanol. Production of these branched-chain alcohols was initiated during nitrogen or phosphorus limitation in the engineered *R. eutropha*. One mutant strain not only produced over 180 mg/L branched-chain alcohols in flask culture, but also was significantly more tolerant of isobutanol toxicity than wild-type *R. eutropha*. After the elimination of genes encoding three potential carbon sinks (*ilvE*, *bkdAB*, and *aceE*), the production titer improved to 270 mg/L isobutanol and 40 mg/L 3-methyl-1-butanol. Semicontinuous flask cultivation was utilized to minimize the toxicity caused by isobutanol while supplying cells with sufficient nutrients. Under this semicontinuous flask cultivation, the *R. eutropha* mutant grew and produced more than 14 g/L branched-chain alcohols over the duration of 50 days. These results demonstrate that *R. eutropha* carbon flux can be redirected from PHB to branched-chain alcohols and that engineered *R. eutropha* can be cultivated over prolonged periods of time for product biosynthesis.

**Nan Hu<sup>1,2</sup>, Bo Yuan<sup>2</sup>, Juan Sun<sup>1</sup>, Shi-An Wang<sup>2</sup>, Fu-Li Li<sup>2</sup>.** (<sup>1</sup>College of Animal Science and Technology, Qingdao Agricultural University, Qingdao, Shandong, 266109, China, <sup>2</sup>Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, Shandong, 266101, China. **Juan Sun (Corresponding author) Email: sunjuan7603@sohu.com).** **Thermotolerant *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* strains representing potentials for bioethanol production from Jerusalem artichoke by consolidated bioprocessing. *Applied Microbiology and Biotechnology*, Volume 95 (5) (2012): 1359-1368**

Thermotolerant inulin-utilizing yeast strains are desirable for ethanol production from Jerusalem artichoke tubers by consolidated bioprocessing (CBP). To obtain such strains, 21 naturally occurring yeast strains isolated by using an enrichment method and 65 previously isolated *Saccharomyces cerevisiae* strains were investigated in inulin utilization, extracellular inulinase activity, and ethanol fermentation from inulin and Jerusalem artichoke tuber flour at 40 °C. The strains *Kluyveromyces marxianus* PT-1 (CGMCC AS2.4515) and *S. cerevisiae* JZ1C (CGMCC AS2.3878) presented the highest extracellular inulinase activity and ethanol yield in this study. The highest ethanol concentration in Jerusalem artichoke tuber flour fermentation (200 g□L<sup>-1</sup>) at 40 °C achieved by *K. marxianus* PT-1 and *S. cerevisiae* JZ1C was 73.6 and 65.2 g□L<sup>-1</sup>, which corresponded to the theoretical ethanol yield of 90.0 and 79.7 %, respectively. In the range of 30 to 40 °C, temperature did not have a significant effect on ethanol production for both strains. This study displayed the distinctive superiority of *K. marxianus* PT-1 and *S. cerevisiae* JZ1C in the thermotolerance and utilization of inulin-type oligosaccharides reserved in Jerusalem artichoke tubers. It is proposed that both *K. marxianus* and *S. cerevisiae* have considerable potential in ethanol production from Jerusalem artichoke tubers by a high temperature CBP.

**Meredith C. Edwards, Joy Doran-Peterson.** (<sup>1</sup>Department of Microbiology, University of Georgia, 1000 Cedar Street, 550, Biological Sciences, Athens, GA 30602, USA.

**Email: jpeterso@uga.edu). Pectin-rich biomass as feedstock for fuel ethanol production. Applied Microbiology and Biotechnology, Volume 95 (3) (2012): 565-575,**

The USA has proposed that 30 % of liquid transportation fuel be produced from renewable resources by 2030 (Perlack and Stokes 2011). It will be impossible to reach this goal using corn kernel-based ethanol alone. Pectin-rich biomass, an under-utilized waste product of the sugar and juice industry, can augment US ethanol supplies by capitalizing on this already established feedstock. Currently, pectin-rich biomass is sold (at low value) as animal feed. This review focuses on the three most studied types of pectin-rich biomass: sugar beet pulp, citrus waste and apple pomace. Fermentations of these materials have been conducted with a variety of ethanologens, including yeasts and bacteria. *Escherichia coli* can ferment a wide range of sugars including galacturonic acid, the primary component of pectin. However, the mixed acid metabolism of *E. coli* can produce unwanted side products. *Saccharomyces cerevisiae* cannot naturally ferment galacturonic acid nor pentose sugars but has a homoethanol pathway. *Erwinia chrysanthemi* is capable of degrading many of the cell wall components of pectin-rich materials, including pectin. *Klebsiella oxytoca* can metabolize a diverse array of sugars including cellobiose, one degradation product of cellulose. However, both *E. chrysanthemi* and *K. oxytoca* produce side products during fermentation, similar to *E. coli*. Using pectin-rich residues from industrial processes is beneficial because the material is already collected and partially pretreated to facilitate enzymatic deconstruction of the plant cell walls. Using biomass already produced for other purposes is an attractive practice because fewer greenhouse gases (GHG) will be anticipated from land-use changes.

**Ioannis Dogaris, Olga Gkounta, Diomi Mamma, Dimitris Kekos. (Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 9 Iroon Polytechniou Str., Zografou Campus, 157 80 Zografou, Greece. mail: kekos@chemeng.ntua.gr). Bioconversion of dilute-acid pretreated sorghum bagasse to ethanol by *Neurospora crassa*. Applied Microbiology and Biotechnology, Volume 95 (2) (2012): 541-550**

Bioethanol production from sweet sorghum bagasse (SB), the lignocellulosic solid residue obtained after extraction of sugars from sorghum stalks, can further improve the energy yield of the crop. The aim of the present work was to evaluate a cost-efficient bioconversion of SB to ethanol at high solids loadings (16 % at pretreatment and 8 % at fermentation), low cellulase activities (1–7 FPU/g SB) and co-fermentation of hexoses and pentoses. The fungus *Neurospora crassa* DSM 1129 was used, which exhibits both depolymerase and co-fermentative ability, as well as mixed cultures with *Saccharomyces cerevisiae* 2541. A dilute-acid pretreatment (sulfuric acid 2 g/100 g SB; 210 °C; 10 min) was implemented, with high hemicellulose decomposition and low inhibitor formation. The bioconversion efficiency of *N. crassa* was superior to *S. cerevisiae*, while their mixed cultures had negative effect on ethanol production. Supplementing the in situ produced *N. crassa* cellulolytic system (1.0 FPU/g SB) with commercial cellulase and  $\beta$ -glucosidase mixture at low activity (6.0 FPU/g SB) increased ethanol production to 27.6 g/l or 84.7 % of theoretical yield (based on SB cellulose and hemicellulose sugar content). The combined dilute-acid pretreatment and bioconversion led to maximum cellulose and hemicellulose hydrolysis 73.3 % and 89.6 %, respectively.

**Jikun Huang<sup>a</sup>, Jun Yang<sup>a</sup>, Siwa Msangi<sup>b</sup>, Scott Rozelle<sup>c, d</sup>, Alfons Weersink<sup>e</sup>. (<sup>a</sup> Center for Chinese Agricultural Policy, Chinese Academy of Sciences, China, <sup>b</sup> International Food Policy Research Institute, United States, <sup>c</sup>Stanford University, United States, <sup>d</sup> University**

of Waikato, New Zealand, <sup>e</sup> University of Guelph, Canada). **Biofuels and the poor: Global impact pathways of biofuels on agricultural markets. Food Policy, Volume 37 (4) (2012): 439–451**

This study seeks to assess the future impacts of biofuel production on regional agricultural and related sectors over the next decade with a specific focus on the vulnerable regions of developing nations. Using a modification of the GTAP modeling platform to account for the global interactions of regional biofuel and food markets, the analysis shows that biofuel production levels depend on the assumption about the future price of energy and the nature of the substitutability between biofuels and petroleum-based transport fuels. Low energy prices reduce the demand for biofuels and thus require greater government support to meet the desired production targets. At the other extreme, when prices are high and there is scope for substituting biofuels for petroleum-based fuels, the volume of biofuels produced will exceed the mandates. Even when biofuels are being mainly produced in developed countries, our results indicate that there are impact pathways that extend far beyond the borders of the US, Brazil and the EU. Prices of feedstock and non-feedstock commodities rise in developing countries. There is also a rise in value added from the agricultural sector—a gain that is enjoyed by the owners of land and labor, including unskilled. Hence, to the extent that agriculture is a key sector in getting growth started and addressing poverty needs, the emergence of biofuels can (in this way at least) be a positive force.

**Keywords:** Biofuels; Global markets; Developing countries; Prices

**Qiong Shang<sup>1,2</sup>, Jiao Lei<sup>1</sup>, Wei Jiang<sup>1</sup>, Houfang Lu<sup>1</sup>, Bin Liang<sup>1</sup>.** (<sup>1</sup>College of Chemical Engineering, Sichuan University, Chengdu, 610065, People's Republic of China, <sup>2</sup>*Present address:* College of Chemistry and Environmental Science, Lanzhou City University, Lanzhou, 730070, People's Republic of China. Email: luhouf@163.com). **Production of Tung Oil Biodiesel and Variation of Fuel Properties During Storage. Applied Biochemistry and Biotechnology, Volume 168 (1) (2012): 106-115**

The crude Tung oil with 4.72 mg KOH/g of acid value (AV) was converted by direct transesterification, and the reaction mixture was quantified. The phase distribution data showed that 38.24% of excess methanol, 11.76% of KOH, 10.13% of soap and 4.36% of glycerol were in the biodiesel phase; 0.35% of biodiesel dissolved in the glycerol phase. Tung oil biodiesel as well as its blends with 0<sup>#</sup> diesel was investigated under different storage conditions. The results indicated that higher temperature greatly influenced the storage stability, especially when the volume fraction of Tung oil biodiesel is increased in the blends.

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Mixed fungal cultures used for making tempe, a fermented soy bean food, were screened for biomass conversion. Thirty-two zygomycetes strains from two tempe cultures were isolated and identified as *Rhizopus*, *Mucor*, *Rhizomucor*, and *Absidia* species based upon morphology. The dry weight biomass of these strains contained 49% to 63% protein and 10–24% chitosan. The strains with the best growth performance were selected and registered at Culture Collection of Gothenburg University as *Rhizomucor* CCUG 61146 and *Rhizomucor* CCUG 61147. These strains were able to grow both aerobically and micro-aerobically. Their ethanol yields were 0.38–0.47, 0.19–0.22, and 0.31–0.38 g/g on glucose, xylose, and a mix sugars consisting of cellobiose, glucose, xylose, arabinose, galactose, and mannose, respectively. The biomass yield of the strains varied between 65 and 140 mg dry weight/g glucose.

**Pankaj K. Rai, S. P. Singh, R. K. Asthana. (Centre of Advanced study in Botany, Banaras Hindu University, Varanasi, 221005, India. Email: spsinghbhu@rediffmail.com). Biohydrogen Production from Cheese Whey Wastewater in a Two-Step Anaerobic Process. Applied Biochemistry and Biotechnology, Volume 167 (6) (2012): 1540-1549**

Cheese whey-based biohydrogen production was seen in batch experiments via dark fermentation by free and immobilized *Enterobacter aerogenes* MTCC 2822 followed by photofermentation of VFAs (mainly acetic and butyric acid) in the spent medium by *Rhodopseudomonas* BHU 01 strain. *E. aerogenes* free cells grown on cheese whey diluted to 10 g lactose/L, had maximum lactose consumption (79%), high production of acetic acid (1,900 mg/L), butyric acid (537.2 mg/L) and H<sub>2</sub> yield (2.04 mol/mol lactose; rate, 1.09 mmol/L/h). The immobilized cells improved lactose consumption (84%), production of acetic acid (2,100 mg/L), butyric acid (718 mg/L) and also H<sub>2</sub> yield (3.50 mol/mol lactose; rate, 1.91 mmol/L/h). *E. aerogenes* spent medium (10 g lactose/L) when subjected to photofermentation by free *Rhodopseudomonas* BHU 01 cells, the H<sub>2</sub> yield reached 1.63 mol/mol acetic acid (rate, 0.49 mmol/L/h). By contrast, immobilized *Rhodopseudomonas* cells improved H<sub>2</sub> yield to 2.69 mol/mol acetic acid (rate, 1.87 mmol/L/h). The cumulative H<sub>2</sub> yield for free and immobilized bacterial cells was 3.40 and 5.88 mol/mol lactose, respectively. Bacterial cells entrapped in alginate, had a sluggish start of H<sub>2</sub> production but outperformed the free cells subsequently. Also, the concomitant COD reduction for free cells (29.5%) could be raised to 36.08% by immobilized cells. The data suggest that two-step fermentative H<sub>2</sub> production from cheese whey involving immobilized bacterial cells, offers greater substrate to- hydrogen conversion efficiency, and the effective removal of organic load from the wastewater in the long-term.

**Amanda J. Palumbo<sup>1</sup>, Sean C. Taylor<sup>1</sup>, Sarah L. Addison<sup>1</sup>, Alison H. Slade<sup>1</sup>, Chris N. Glover<sup>2</sup>. (<sup>1</sup>Scion, 49 Sala street, Rotorua, 3010, New Zealand, <sup>2</sup>School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, 8140, New Zealand. Email: amandajpalumbo@gmail.com). Metal biosorption in lignocellulosic biofuel biorefinery effluent: an initial step towards sustainability of water resources. Journal of Industrial Microbiology & Biotechnology, Volume 39 (9) (2012): 1345-1356**

Biosorption of metals by microorganisms is a promising technology to remove accumulated non-process elements in highly recycled biorefinery process water. Removal of these elements would enable greater water reuse and reduce the environmental impact of effluent discharge. A model lignocellulosic ethanol biorefinery wastewater was created based on pulp mill effluent. This generated a wastewater with an environmentally realistic high loading of dissolved natural organic matter (900 mg/l), a potentially important factor influencing metal biosorption. Analysis

of feedstock and pulp mill effluent indicated that Mn and Zn are likely to be problematic in highly recycled lignocellulosic ethanol biorefinery process water. Therefore, the growth of several bacteria and fungi from existing collections, and some isolated from pulp mill effluent were tested in the model wastewater spiked with Mn and Zn (0.2 mM). Wastewater isolates grew the best in the wastewater. Metal uptake varied by species and was much greater for Zn than Mn. A bacterium, *Novosphingobium nitrogenifigens* Y88<sup>T</sup>, removed the most metal per unit biomass, 35 and 17 mg Mn/g. No other organism tested decreased the Mn concentration. A yeast, *Candida tropicalis*, produced the most biomass and removed the most total metal (38 % of Zn), while uptake per unit biomass was 24 mg Zn/g. These results indicate that microorganisms can remove significant amounts of metals in wastewater with high concentrations of dissolved natural organic matter. Metal sorption by autochthonous microorganisms in an anaerobic bioreactor may be able to extend water reuse and therefore lower the water consumption of future biorefineries.

**Nikolaos Kopsahelis<sup>a</sup>, Loulouda Bosnea<sup>a</sup>, Argyro Bekatorou<sup>a</sup>, Constantina Tzia<sup>b</sup>, Maria Kanellaki<sup>a</sup>.** (<sup>a</sup> Food Biotechnology Group, Section of Analytical, Environmental and Applied Chemistry, Department of Chemistry, University of Patras, GR-26500 Patras, Greece, <sup>b</sup> Laboratory of Food Chemistry and Technology, School of Chemical Engineering, National Technical University of Athens, 5 Iroon Polytechniou St., 15780 Athens, Greece). **Alcohol production from sterilized and non-sterilized molasses by *Saccharomyces cerevisiae* immobilized on brewer's spent grains in two types of continuous bioreactor systems. Biomass and Bioenergy, Volume 45 (2012): 87–94**

In this work an integrated cost effective system for continuous alcoholic fermentation of a cheap raw material (molasses) is described, involving yeast immobilized by a simple method on brewer's spent grains, able to ferment in the temperature range 30–40 °C, and two types of bioreactors, a Multistage Fixed Bed Tower (MFBT) and a Packed Bed reactor (PB). The MFBT bioreactor gave better results regarding ethanol concentration, productivity and conversion. Furthermore, the use of sterilized and non-sterilized molasses, fed in two similar MFBT bioreactors, showed that ethanol concentration ( $\text{kg m}^{-3}$ ) was significantly ( $p < 0.05$ ) affected by the sterilization process as well as temperature alteration. Higher ethanol concentrations were obtained in the case of non-sterilized molasses (47.4–50.6  $\text{kg m}^{-3}$  at 35 °C and 44.2–48.2  $\text{kg m}^{-3}$  at 40 °C), compared to sterilized molasses, where ethanol concentration ranged from 35.6 to 46.6  $\text{kg m}^{-3}$  at 35 °C and 30.8–44.2  $\text{kg m}^{-3}$  at 40 °C. During 32 days of continuous operation using non-sterilized molasses no contamination was observed. Industrialization of the proposed system seems to have a potential, mainly due to its high fermentation efficiency and the obtained high operational stability.

**Keywords:** Continuous alcoholic fermentation; Non-sterilized molasses; Immobilized yeast cells; Ethanol; Brewer's spent grains

**Ravi Naidu<sup>1,2,4</sup>, Subhas Nandy, MallavarapuMegharaj<sup>1,2</sup>, R. P. Kumar<sup>1,2</sup>, Sreenivasulu Chadalavada<sup>1,2</sup>, Zuliang Chen<sup>1,2</sup>, Mark Bowman<sup>3</sup>.** (<sup>1</sup>Centre for Environmental Risk Assessment and Remediation (CERAR), University of South Australia, Mawson Lakes Campus, SA, 5095, Australia, <sup>2</sup>Cooperative Research Centre for Contamination Assessment and Remediation of Environment (CRC CARE), PO Box 169, Port Adelaide, SA, 5015, Australia, <sup>3</sup>Department of Defence, Canberra, ACT, 2600, Australia, <sup>4</sup>CRC

**CARE Pty Ltd, University of South Australia, Building X, Mawson Lakes, SA, 5095, Australia). Monitored natural attenuation of a long-term petroleum hydrocarbon contaminated sites: a case study. Biodegradation, Volume 23 (6) (2012): 881-895**

This study evaluated the potential of monitored natural attenuation (MNA) as a remedial option for groundwater at a long-term petroleum hydrocarbon contaminated site in Australia. Source characterization revealed that total petroleum hydrocarbons (TPH) as the major contaminant of concern in the smear zone and groundwater. Multiple lines of evidence involving the geochemical parameters, microbiological analysis, data modelling and compound-specific stable carbon isotope analysis all demonstrated natural attenuation of hydrocarbons occurring in the groundwater via intrinsic biodegradation. Groundwater monitoring data by Mann–Kendall trend analysis using properly designed and installed groundwater monitoring wells shows the plume is stable and neither expanding nor shrinking. The reason for stable plume is due to the presence of both active source and natural attenuation on the edge of the plume. Assuming no retardation and no degradation the contaminated plume would have travelled a distance of 1,096 m (best case) to 11,496 m (worst case) in 30 years. However, the plume was extended only up to about 170 m from its source. The results of these investigations provide strong scientific evidence for natural attenuation of TPH in this contaminated aquifer. Therefore, MNA can be applied as a defensible management option for this site following significant reduction of TPH in the source zone.

### **Nano Biotechnology**

**Gregor Hommes<sup>a</sup>, Christoph A. Gasser<sup>a</sup>, Chaim B.C. Howald<sup>a</sup>, Roland Goers<sup>a</sup>, Dietmar Schlosser<sup>c</sup>, Patrick Shahgaldian<sup>b</sup>, Philippe F.-X. Corvini<sup>a,d</sup>. (<sup>a</sup> Institute for Ecopreneurship, School of Life Sciences, University of Applied Sciences Northwestern Switzerland, Gründenstrasse 40, Muttenz CH-4132, Switzerland, <sup>b</sup> Institute for Chemistry and Bioanalytics, School of Life Sciences, University of Applied Sciences Northwestern Switzerland, Gründenstrasse 40, Muttenz CH-4132, Switzerland, <sup>c</sup> Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, Permoserstrasse 15, Leipzig D-04318, Germany, <sup>d</sup> School of the Environment, Nanjing University, Hankou Road 22, 210093 Nanjing, China). Production of a robust nanobiocatalyst for municipal wastewater treatment. Bioresource Technology, Volume 115(2012): 8–15**

Immobilization is a fundamental method to improve both enzyme activity and stability. In the present work, the process previously described for immobilizing laccase – an enzyme oxidizing phenolic compounds – onto fumed silica was optimized, in order to efficiently produce industrially relevant amounts of a nanobiocatalyst for biological micropollutant elimination, whilst saving 80% of surface modification agent (3-aminopropyl triethoxy silane) and 90% of cross-linker (glutaraldehyde). Minimized losses during preparation and favorable effects of immobilization yielded conjugates with drastically increased enzymatic activity (164% of invested activity). Long-term stability and activity regarding bisphenol A (2,2-bis(4-hydroxyphenyl)propane) removal of the synthesized biocatalyst were assessed under application-relevant conditions. With  $81.1 \pm 0.4\%$  residual activity after 7 days, stability of conjugates was drastically higher than of free laccase, which showed virtually no activity after 1.5 days.

These results illustrate the huge potential of fumed silica nanoparticles/laccase-composites for innovative biological wastewater treatment.

**Keywords:** Laccase; Fumed silica nanoparticles; Immobilization; Phenolic micropollutant; Wastewater treatment

**Ioannis V. Pavlidis<sup>a</sup>, Torge Vorhaben<sup>b</sup>, Theodoros Tsoufis<sup>c</sup>, Petra Rudolf<sup>c</sup>, Uwe T. Bornscheuer<sup>b</sup>, Dimitrios Gournis<sup>d</sup>, Haralambos Stamatis<sup>a</sup>** (<sup>a</sup> Laboratory of Biotechnology, Department of Biological Applications and Technologies, University of Ioannina, 45 110 Ioannina, Greece, <sup>b</sup> Department of Biotechnology and Enzyme Catalysis, Institute of Biochemistry, Greifswald University, Felix – Hausdorff-Str. 4, D-17487 Greifswald, Germany, <sup>c</sup> Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, NL-9747 AG Groningen, The Netherlands, <sup>d</sup> Department of Materials Science and Engineering, University of Ioannina, 45 110 Ioannina, Greece). **Development of effective nanobiocatalytic systems through the immobilization of hydrolases on functionalized carbon-based nanomaterials. *Bioresource Technology*, Volume 115 (2012): 164–171**

In this study we report the use of functionalized carbon-based nanomaterials, such as amine-functionalized graphene oxide (GO) and multi-walled carbon nanotubes (CNTs), as effective immobilization supports for various lipases and esterases of industrial interest. Structural and biochemical characterization have revealed that the curvature of the nanomaterial affect the immobilization yield, the catalytic behavior and the secondary structure of enzymes. Infrared spectroscopy study indicates that the catalytic behavior of the immobilized enzymes is correlated with their  $\alpha$ -helical content. Hydrolases exhibit higher esterification activity (up to 20-fold) when immobilized on CNTs compared to GO. The covalently immobilized enzymes exhibited comparable or even higher activity compared to the physically adsorbed ones, while they presented higher operational stability. The enhanced catalytic behavior observed for most of the hydrolases covalently immobilized on amine-functionalized CNTs indicate that these functionalized nanomaterials are suitable for the development of efficient nanobiocatalytic systems.

**Keywords:** Lipase; Esterase; Immobilization; Carbon nanotubes; Graphene oxide

Name of Journals

1. Acta Biotechnologica
2. Aerobiologia
3. Annual Review-Plant Pathology
4. Annual Review- Ecology and Systematics
5. Annual Review-Biochemistry
6. Annual Review-Biomedical Engineering
7. Annual Review-Biophysics and Biomolecular Structure
8. Annual Review-Microbiology
9. Annual Review-Pharmacology and Toxicology
10. Annual Review-Phytopathology
11. Annual Review-Physiology
12. Annual Review-Plant Physiology
13. Annual Review-Public Health
14. African Journal of Biotechnology
15. Applied and Environmental Microbiology
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17. Aquaculture
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35. Biotechnology Advances
36. Biotechnology and Applied Biochemistry
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39. Canadian Journal of Microbiology
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71. Indian Journal of Plant Physiology
72. International Biodeterioration & Biodegradation
73. International Journal of Biotechnology
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76. Journal of Agriculture and Environmental Ethics
77. Journal Biological Control

78. Journal of Bacteriology
79. Journal of Chemical Technology & Biotechnology
80. Journal of Environmental Management
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