



ENVIS CENTER on ENVIRONMENTAL BIOTECHNOLOGY

Abstract Vol. XVII

Sponsored by

**MINISTRY OF ENVIRONMENT AND FORESTS
GOVERNMENT OF INDIA
NEW DELHI**



**Department of Environmental Science
University of Kalyani
Nadia, West Bengal
December, 2010**



Published by:

Prof. S. C. Santra

Co-ordinator

ENVIS Centre of Environmental Biotechnology

Department of Environmental Science

University of Kalyani,

Kalyani –741235, Nadia,

West Bengal,

INDIA

Email: desku@envis.nic.in, scsantra@yahoo.com

envis@kuenvbiotech.org

Website: <http://www.kuenvbiotech.org>

ENVIS CENTRE

on

ENVIRONMENTAL BIOTECHNOLOGY

❖ Prof. S. C. Santra : *Coordinator, ENVIS Centre*

ENVIS's Staff

1. **Dr. Nandini Gupta** : *Programme Officer*
2. **Ms. Amrita Saha**
(Deb Chaudhury) : *Information Officer*
3. **Shri S. Bandyopadhyay** : *Data Entry Operator*

CONTENTS

Sl. No.	Title	Page No.
1.	Background	5
2.	Abstract format	6
3.	General information	7
4.	Abbreviation used	10
5.	Abstracts	
	Bioaccumulation	13
	Bioremediation	21
	Biotransformation	61
	Biomarker	69
	Biofertilizer	72
	Biocomposting	73
	Biopesticide	74
	Biodegradation	85
	Biosensor	121
	Bioengineering	132
	Pollen Biotechnology	133
	Biotechnology Policy Issue	133
	Agricultural Biotechnology	134
	Bioenergy	134
6.	Name of Journal	153
7.	Author Index	156

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. Now 78 centres are working under this network on various subject areas in the country. The focal point of this network is situated at the Ministry of Environment and Forest, Government of India, New Delhi.

This ENVIS Centre is established for studies on 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 fourteen subheads. 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 17th 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 December, 2010. 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 authors 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 14 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.

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	Ecotoxicol	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	Ethnopharmacol	Ethnopharmacology
Centl	Central	Expt	Experiment

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)		

Bioaccumulation

Ying Yin, Xiaorong Wang, Liuyan Yang, Yuanyuan Sun and Hongyan Guo. Bioaccumulation and ROS generation in Coontail *Ceratophyllum demersum* L. exposed to phenanthrene. *Ecotoxicology*, Volume 19(6) (2010): 1102-1110

Phenanthrene bioaccumulation, induction free radicals and their consequent biochemical responses in coontail (*Ceratophyllum demersum* L.) were examined. Plants were exposed to different levels (0.01, 0.02, 0.05, 0.07 and 0.1 mg/l) of phenanthrene for 10 days. Results showed that the phenanthrene concentration in the plants was exponentially correlated to exposure concentration ($R^2 = 0.958$) and phenanthrene exposure significantly increased the total free radicals and superoxide anion in the plants. The activities of antioxidant enzymes and the contents of glutathione were determined. The superoxide dismutase (SOD) activity and reduced glutathione (GSH) content were inhibited, while the catalase (CAT), peroxidase (POD), glutathione-*s*-transferase (GST) activities and oxidized glutathione (GSSG) content were significantly induced. Changes in the contents of chlorophyll and malondialdehyde (MDA) indicated that the MDA content was enhanced after phenanthrene exposure and the contents of chlorophyll were significantly increased in the 0.01 mg/l group. These experimental data demonstrated that the bioaccumulation of phenanthrene induced the production of free radicals and ROS, and changed the antioxidant defense system, ultimately resulting in oxidative damage in *C. demersum*.

Keywords: Bioaccumulation - *Ceratophyllum demersum* - Electron paramagnetic resonance (EPR) - Free radical - Phenanthrene

Shiming Su, Xibai Zeng, Lingyu Bai, Xiliang Jiang and Lianfang Li. Bioaccumulation and Biovolatilisation of Pentavalent Arsenic by *Penicillin janthinellum*, *Fusarium oxysporum* and *Trichoderma asperellum* Under Laboratory Conditions. *Current Microbiology*, Volume 61(4) (2010): 261-266

Some fungi are able to control and remediate arsenic (As)-contaminated soil, sediment, or water. Here, we investigate potential accumulation and volatilisation of As by three fungi strains. Results indicated that the highest level of As was accumulated by *Penicillin janthinellum* with 39.54 µg after 10 days in the culture system amended with 2,500 µg As(V), which represents 50 mg/l As. *Fusarium oxysporum* showed the highest amount of volatilised As with 304.06 µg after 15 days. The As content in the treated system (filter paper + As + fungi) was significantly higher than that in the control (filter paper + As; filter paper + fungi; filter paper). *Trichoderma asperellum* and *F. oxysporum* showed superior abilities for the absorption of extracellular As and accumulation of intracellular As, which accounted for 82.2 and 63.4% of the total accumulated As, respectively. However, *P. janthinellum* presented an equal distribution of intracellular and extracellular As. Scanning electron microscope (SEM) analysis suggested that little impact on mycelium growth of the three fungal strains was seen after exposure to 50 mg/l As(V) for 5 days, while the growth of fungi in the control was inhibited. The present results demonstrate that *P. janthinellum*, *F. oxysporum*, and *T. asperellum* would be expected to tackle As-contaminated environments.

Antonín Kouba, Miloš Buřič and Pavel Kozák. Bioaccumulation and Effects of Heavy Metals in Crayfish: A Review. *Water, Air, & Soil Pollution*, Volume 211(1-4) (2010): 5-16

Metal pollution is a global problem which represents a growing threat to the environment. Because of bioaccumulation and negative effects of heavy metals, their bioavailability needs to be monitored. Many studies showed accumulation of metals in crayfish tissues as dose- and time-dependent without significant differences in tissue concentration levels comparing males and females. Muscles and exoskeleton were considered as specific for accumulation of mercury and nickel, respectively. Cadmium, zinc, copper, lead, and chromium accumulated mainly in hepatopancreas. By analyzing these specific tissues, it is possible to deduce the bioavailability and, by presumption, the level of environmental pollution by specific metals. However, in the case of zinc and copper, their utility is limited to assessing bioavailability because rapid depuration of these metals renders them less useful for long-term environmental monitoring programs. The literature reporting heavy metal impacts on freshwater crayfish, with reference to accumulation levels, is reviewed and summarized with respect to their suitability as bioindicators. Summarized published data from unpolluted or control localities can be used as referential values in crayfish, and consequently help with evaluation of monitored sites.

Keywords: Aquatic environment - Bioindicator - Crustacea - Heavy metals contamination - Metal pollution

Ahmed M. Al-Ansari^a, Ammar Saleem^a, Linda E. Kimpe^a, Jim P. Sherry^b, Mark E. McMaster^b, Vance L. Trudeau^a and Jules M. Blais^a. (^a University of Ottawa, Department of Biology, Chemical and Environmental Toxicology, Ottawa, Ontario K1N 6N5, Canada, ^b Environment Canada, Water Science & Technology Directorate, Canada Centre for Inland Waters, Burlington, Ontario, Canada). Bioaccumulation of the pharmaceutical 17 α -ethynylestradiol in shorthead redhorse suckers (*Moxostoma macrolepidotum*) from the St. Clair River, Canada. *Environmental Pollution*, Volume 158(8) (2010): 2566-2571

17 α -ethynylestradiol (EE2), a synthetic estrogen prescribed as a contraceptive, was measured in Shorthead Redhorse Suckers (ShRHSs) (*Moxostoma macrolepidotum*) collected near a wastewater treatment plant (WWTP) in the St. Clair River (Ontario, Canada). We detected EE2 in 50% of the fish samples caught near the WWTP (Stag Island), which averaged 1.6 ± 0.6 ng/g (wet weight) in males and 1.43 ± 0.96 ng/g in females. No EE2 was detected in the samples from the reference site (Port Lambton) which was 26 km further downstream of the Stag Island site. Only males from Stag Island had VTG induction, suggesting the Corunna WWTP effluent as a likely source of environmental estrogen. EE2 concentrations were correlated with total body lipid content ($R^2 = 0.512$, $p < 0.01$, $n = 10$). Lipid normalized EE2 concentrations were correlated with $\delta^{15}\text{N}$ ($R^2 = 0.436$, $p < 0.05$, $n = 10$), suggesting higher EE2 exposures in carnivores. Our data support the hypothesis of EE2 bioaccumulation in wild fish.

Ethinylestradiol accumulation in wild fish.

Keywords: Bioaccumulation; *Moxostoma macrolepidotum*; St. Clair River; Ethinylestradiol; Steroids; Estrogens

Xianwei Liang^{a, b, c}, Shuzhen Zhu^{a, b, c}, Peng Chen^{a, b, c} and Lingyan Zhu^{a, b, c}. (^a The College of Environmental Science and Engineering, Nankai University, Weijin Road 94, Tianjin 300071, China, ^b Key Laboratory of Pollution Processes and Environmental Criteria

(Nankai University), Ministry of Education, Tianjin 300071, China, ^c Tianjin Key Laboratory of Urban Ecology Environmental Remediation and Pollution Control, Nankai University, Tianjin 300071, China). **Bioaccumulation and bioavailability of polybrominated diphenyl ethers (PBDEs) in soil. Environmental Pollution, Volume 158(7) (2010): 2387-2392**

Earthworms were exposed to artificially contaminated soils of DE-71 and DE-79 to investigate the bioaccumulation and bioavailability of PBDEs in soil. All major congeners were bioavailable to earthworms. The uptake and elimination rate coefficients of PBDEs decreased with their $\log K_{ow}$ s. The biota soil accumulation factors of PBDEs also declined with $\log K_{ow}$. These may be due to the large molecular size and the high affinity of PBDEs to soil particles. The concentrations extracted by Tenax for 6 h correlated very well with those found in earthworms, suggesting that the bioavailability of PBDEs in soil is related to the fraction of rapid desorption from soil. This also indicates that 6 h Tenax extraction is a good proxy for the bioavailability of PBDEs to earthworms in soil. The BSAFs of PBDEs in aged soil decreased 22–84% compared to freshly spiked soil, indicating that aging may diminish the bioavailability of PBDEs in soil significantly.

PBDEs are bioavailable to earthworms in soil and the uptake and elimination rate coefficients and BSAFs declined with their $\log K_{ow}$ s.

Keywords: Uptake; Elimination; PBDEs; Bioavailability; Desorption; Aging effect

Bioremediation

Surekha K. Satpute^a, Ibrahim M. Banat^c, Prashant K. Dhakephalkar^d, Arun G. Banpurkar^e and Balu A. Chopade^{a, b}. (^a Department of Microbiology, University of Pune, Pune 411007, Maharashtra, India, ^b Institute of Bioinformatics and Biotechnology, Pune 411007, Maharashtra, India, ^c School of Biomedical Sciences, University of Ulster, Coleraine, BT52 1SA, N. Ireland, UK, ^d Division of Microbial Sciences, Agharkar Research Institute, Pune 411004, India, ^e Center for Advanced Studies in Materials Science and Condensed Matter Physics, Department of Physics, University of Pune, Pune 411007, Maharashtra, India). **Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. Biotechnology Advances, Volume 28(4) (2010): 436-450**

Marine biosphere offers wealthy flora and fauna, which represents a vast natural resource of imperative functional commercial grade products. Among the various bioactive compounds, biosurfactant (BS)/bioemulsifiers (BE) are attracting major interest and attention due to their structural and functional diversity. The versatile properties of surface active molecules find numerous applications in various industries. Marine microorganisms such as *Acinetobacter*, *Arthrobacter*, *Pseudomonas*, *Halomonas*, *Myroides*, *Corynebacteria*, *Bacillus*, *Alteromonas* sp. have been studied for production of BS/BE and exopolysaccharides (EPS). Due to the enormity of marine biosphere, most of the marine microbial world remains unexplored. The discovery of potent BS/BE producing marine microorganism would enhance the use of

environmental biodegradable surface active molecule and hopefully reduce total dependence or number of new application oriented towards the chemical synthetic surfactant industry. Our present review gives comprehensive information on BS/BE which has been reported to be produced by marinemicroorganisms and their possible potential future applications.

Keywords: Marine; Biosurfactant; Bioemulsifier; Exopolysaccharides; Glycolipids; Lipopeptides; Applications

Satish V. Patil, Rahul B. Salunkhe, Chandrashekhar D. Patil, Deepak M. Patil and Bipinchandra K. Salunke. Bioflocculant Exopolysaccharide Production by *Azotobacter indicus* Using Flower Extract of *Madhuca latifolia* L. Applied Biochemistry and Biotechnology, Volume 162(4) (2010): 1095-1108

Efficacy of *Azotobacter indicus* ATCC 9540 strain for production exopolysaccharide (EPS) bioflocculant was investigated. Mahua flower extract (*Madhuca latifolia* L), a natural substrate at the concentration of 20 g L⁻¹, gave maximum recovery of EPS followed by sucrose and mannitol as compared to other carbon sources after 172 h. Yeast extract was found to be the most effective nitrogen source as compared to beef extract, sodium nitrate, ammonium sulfate, casein hydrolysate, and urea for the production of EPS. EPS production was increased in presence of nitrogen (5.51 g L⁻¹) as compared to nitrogen-free medium (3.51 g L⁻¹), and fermentation time was also reduced by 28 h. Maximum EPS production (6.10 g L⁻¹) was found in the presence of 20 g L⁻¹ flower extract and 0.5 g L⁻¹ yeast extract containing Ashby's media with 180 rpm at 30 °C at 144 h, under controlled conditions in 2.5 L fermenter using optimized medium. The isolated EPS showed cation-dependent flocculating activity. Concentration of EPS played an important role in bioflocculating activity which increased in a concentration-dependent manner up to a certain limit, with the maximum flocculation of 72% at 500 mg L⁻¹ concentration but remained almost static after this concentration. Extracted polymer was characterized by different chemical tests, FT-IR spectroscopy, and TLC which showed presence of uronic acids, *O*-acetyl groups, and Orcinol with suggestive indication of alginate like polymer. This study suggests that use of *M. latifolia* L. flowers can be a potential alternative bioresource for production of exopolysaccharide.

Keywords: Bioflocculant - *Azotobacter indicus* - Polysaccharide - EPS - Cations

Mariana Reategui, Holger Maldonado, Martha Ly and Eric Guibal. Mercury(II) Biosorption Using *Lessonia* sp. Kelp. Applied Biochemistry and Biotechnology, Volume 162(3) (2010): 805-822

Lessonia nigrescens and *Lessonia trabeculata* kelps have been tested for the sorption of mercury from aqueous solutions. A pretreatment (using CaCl₂) allowed stabilizing the biomass that was very efficient for removing Hg(II) at pH 6–7. Sorption isotherms were described by the Langmuir equation with sorption capacities close to 240–270 mg Hg g⁻¹ at pH 6. The temperature had a negligible effect on the distribution of the metal at equilibrium. The presence of chloride anions had a more marked limiting impact than sulfate and nitrate anions. The uptake kinetics were modeled using the pseudo-second-order equation that fitted better experimental data than the pseudo-first-order equation. The particle size hardly influenced sorption isotherms and uptake kinetics, indicating that sorption occurs in the whole mass of the biosorbent and that intraparticle mass transfer resistance was not the limiting rate. Varying the sorbent dosage and the initial metal concentration influenced the equilibrium, but the kinetic parameters were not

drastically modified. Metal can be eluted with hydrochloric acid, citric acid, or acidic KI solutions.

Keywords: Mercury(II) - *Lessonia* - Kelp - Isotherms - Kinetics - Pseudo-second-order equation - Desorption

Ghinwa Naja, Pierre Bouvrette, Julie Champagne, Roland Brousseau and John H.T. Luong. Activation of Nanoparticles by Biosorption for *E. coli* Detection in Milk and Apple Juice. Applied Biochemistry and Biotechnology, Volume 162(2) (2010): 460-475

Two types of silver nanoparticles were activated by specific sorption of biomolecules for the detection of *Escherichia coli*. The capture of this bacterium was performed using polyclonal antibodies (anti-*E. coli*) biosorbed onto nanospheres or nanorice through a protein-A layer. The bacterial detection was achieved using surface enhancement Raman scattering in order to compare the performance of these two nanoparticles. The activated silver nanospheres showed a better performance mainly due to the dimension of these nanoparticles. The detection limit has been established using the automated Raman mapping system. The technique was capable of detecting 10^3 cells/mL in milk and apple juice without any pre-enrichment. With an overall assay time less than 1 h, the process could be easily adapted to detect other pathogens by selecting the pertinent antibody. Furthermore, PCR was used for the DNA verification to assess whether the selected bacterial strain was identical before and after detection.

Keywords: Contaminated milk and apple juice - *Escherichia coli* - Nanoparticles - Detection limit - Bacterial capture - Biosorption

Wan Mohd Fazli Wan Nawawi^a, Parveen Jamal^a and Md. Zahangir Alam^a. (^a Bioenvironmental Engineering Research Unit (BERU), Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia (IIUM), P.O. Box 10, 50728 Kuala Lumpur, Malaysia). Utilization of sludge palm oil as a novel substrate for biosurfactant production. Bioresource Technology, Volume 101(23) (2010):9241-9247

This paper introduces sludge palm oil (SPO) as a novel substrate for biosurfactant production by liquid state fermentation. Potential strains of microorganism were isolated from various hydrocarbon-based sources at palm oil mill and screened for biosurfactant production with the help of drop collapse method and surface tension activity. Out of 22 isolates of microorganism, the strain S02 showed the highest bacterial growth with a surface tension of 36.2 mN/m and was therefore, selected as a potential biosurfactant producing microorganism. Plackett–Burman experimental design was employed to determine the important nutritional requirement for biosurfactant production by the selected strain under controlled conditions. Six out of 11 factors of the production medium were found to significantly affect the biosurfactant production. K_2HPO_4 had a direct proportional correlation with the biosurfactant production while sucrose, glucose, $FeSO_4$, $MgSO_4$, and $NaNO_3$ showed inversely proportional relationship with biosurfactant production in the selected experimental range.

Keywords: Sludge palm oil (SPO); Biosurfactant; Surface tension; Plackett–Burman

Yulan Ji^a, Yanhong Wang^{a,b}, Jinsheng Sun^a, Tingyan Yan^a, Jing Li^a, Tingting Zhao^a, Xiaohong Yin^b and Changjiang Sun^b. (^a School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, People's Republic of China, ^b School of Chemistry and Chemical Engineering, Tianjin University of Technology, Tianjin 300191, People's Republic of China). Enhancement of biological treatment of wastewater by magnetic field. *Bioresource Technology*, Volume 101(22) (2010): 8535-8540

In this study, effect of magnetic field on the activity of activated sludge in wastewater treatment was investigated. During the experiment, biodegradation duration, magnetic density, operating temperature and medium pH value were changed within the ranges from 0 to 60 h, 0 to 500.0 mT, 10.0 to 50.0 °C and 3.0 to 12.0, respectively. These results revealed that activated sludge acclimatization and organic pollutant biodegradation processes under magnetic field were stimulated, resulting in a higher efficiency of wastewater treatment. Biodegradation of organic compounds under magnetic field could reach stable states after 48 h. The organic compounds removal rate first roared up and then fell down with the increase of magnetic density of the field, turning at 20.0 mT. On account of application of magnetic field, the range of temperature and pH, which is suitable for the growth of microbe, were 20.0–40.0 °C and 6.0–10.0, respectively, which were expanded compared with those without the magnetic field.

Keywords: Wastewater treatment; Activated sludge; Magnetic field intensifying

Luminita Ghimici^a and Marieta Nichifor^a. (^a “Petru Poni” Institute of Macromolecular Chemistry, Aleea Grigore Ghica Voda, 41 A, 700487 Iasi, Romania). Novel biodegradable flocculating agents based on cationic amphiphilic polysaccharides. *Bioresource Technology*, Volume 101(22) (2010): 8549-855

Flocculation properties of a series of cationic polysaccharides, with N-alkyl-N,N-dimethyl-N-(2-hydroxypropyl)ammonium chloride pendent groups attached to a dextran backbone, were evaluated in clay dispersions with respect to length of the alkyl substituent, molar mass, the charge density, and polycation dose. According to turbidimetric results, the alkyl chain length greatly influenced the optimum polymer dose as well as the width of the flocculation window since both increased from an ethyl to a butyl group and decreased for when octyl or dodecyl group was present. The residual turbidity values also varied with the charge density but no dramatic effect was observed with the molar masses investigated. These findings together with the negative value of the zeta potential at the optimum polymer dose and floc size measurements, point to contributions from both patch and bridging mechanisms for the flocculation process.

Keywords: Amphiphilic polysaccharide; Flocculation; Turbidity; Zeta potential; Floc size

Mingxue Liu^{a,c}, Faqin Dong^b, Xiuying Yan^c, Wenming Zeng^c, Liangyu Hou^c and Xiaofeng Pang^a. (^a School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, People's Republic of China, ^b National Defense Key Discipline Laboratory of the Nuclear Waste and Environmental Safety of the Commission of Science, Technology and Industry for National Defense, Southwest University of Science and Technology, Mianyang 621010, People's Republic of China, ^c Life Science and Engineering College, Southwest University of Science and Technology, Mianyang 621010, People's Republic of China). Biosorption of uranium by *Saccharomyces*

***cerevisiae* and surface interactions under culture conditions. *Bioresource Technology*, Volume 101(22) (2010): 8573-8580**

Few studies have focused on biosorption by microorganisms under culture conditions. To explore the biosorption of uranium by *Saccharomyces cerevisiae* under culture conditions, the *S. cerevisiae* growth curve, biosorption capacity and surface interaction under batch culture conditions were investigated in this study. The growth curve showed that uranium ($<300 \text{ mg L}^{-1}$) did not markedly inhibit the growth of *S. cerevisiae* under short culture time. The maximum scavenging efficiency reached 92.4% under 6–10 h culture conditions, and the adsorption quantity of *S. cerevisiae* increased with initial uranium concentration. Centrifuging and drying after biosorption caused the volume reduction ratio to reach 99%. Scanning electron microscope results demonstrated that uranium interacted with yeast cell surfaces, as well as culture medium, and produced uranium precipitate on cell surfaces. Fourier transformed infrared spectra revealed that cell walls were the major sorption sites, and $-\text{O}-\text{H}$, $-\text{C}=\text{O}$ and $-\text{PO}_2^-$ contributed to the major binding groups.

Keywords: *Saccharomyces cerevisiae*; Biosorption; Uranium; Surface interaction

Hanjun Guo^{a, b}, Shenglian Luo^{a, b, c, d}, Liang Chen^{a, b}, Xiao Xiao^{a, b}, Qiang Xi^c, Wanzhi Wei^c, Guangming Zeng^{a, b}, Chengbin Liu^c, Yong Wan^{a, b}, Juiliang Chen^{a, b} and Yejuan He^c. (^a College of Environmental Science and Engineering, Hunan University, Changsha 410082, People's Republic of China, ^b Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, People's Republic of China, ^c State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, People's Republic of China, ^d School of Environment and Chemical Engineering, Nanchang Hangkong University, Nanchang 330063, People's Republic of China). **Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium *Bacillus* sp. L14. *Bioresource Technology*, Volume 101(22) (2010): 8599-8605**

Heavy metal bioremediation by a multi-metal resistant endophytic bacteria L14 (EB L14) isolated from the cadmium hyperaccumulator *Solanum nigrum* L. was characterized for its potential application in metal treatment. 16S rDNA analysis revealed that this endophyte belonged to *Bacillus* sp. The hormesis of EB L14 were observed in presence of divalent heavy metals (Cu (II), Cd (II) and Pb (II)) at a relatively lower concentration (10 mg/L). Such hormesis was the side effect of abnormal activities increases of ATPase which was planned to provide energy to help EB L14 reduce the toxicity of heavy metals by exporting the cations. Within 24 h incubation, EB L14 could specifically uptake 75.78%, 80.48%, 21.25% of Cd (II), Pb (II) and Cu (II) under the initial concentration of 10 mg/L. However, nearly no chromium uptake was observed. The mechanism study indicated that its remediation efficiencies may be greatly promoted through inhibiting the activities of ATPase. The excellent adaptation abilities and promising remediation efficiencies strongly indicated the superiority of this endophyte in heavy metal bioremediation at low concentrations, which could be useful for developing efficient metal removal system.

Keywords: Endophytic bacteria; Heavy metals; Bioremediation; Hormesis

Jongmin Park^a, Hai-Feng Jin^a, Byung-Ran Lim^b, Ki-Young Park^c and Kisay Lee^a. (^a Dept. of Environmental Engineering and Biotechnology, Myongji University, Yongin 449-728, Republic of Korea, ^b Environmental Materials Education Center, Seoul National University of Technology, Seoul, Republic of Korea, ^c Dept. of Civil and Environmental System Engineering, Konkuk University, Seoul, Republic of Korea). **Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus* sp. Bioresource Technology, Volume 101(22) (2010): 8649-8657**

The green alga *Scenedesmus* was investigated for its ability to remove nitrogen from anaerobic digestion effluent possessing high ammonium content and alkalinity in addition to its growth characteristics. Nitrate and ammonium were indistinguishable as a nitrogen source when the ammonium concentration was at normal cultivation levels. Ammonium up to 100 ppm NH₄-N did not inhibit cell growth, but did decrease final cell density by up to 70% at a concentration of 200–500 ppm NH₄-N. Inorganic carbon of alkalinity in the form of bicarbonate was consumed rapidly, in turn causing the attenuation of cell growth. Therefore, maintaining a certain level of inorganic carbon is necessary in order to prolong ammonia removal. A moderate degree of aeration was beneficial to ammonia removal, not only due to the stripping of ammonium to ammonia gas but also due to the stripping of oxygen, which is an inhibitor of regular photosynthesis. Magnesium is easily consumed compared to other metallic components and therefore requires periodic supplementation. Maintaining appropriate levels of alkalinity, Mg, aeration along with optimal an initial NH₄⁺/cell ratio were all necessary for long-term semi-continuous ammonium removal and cell growth.

Keywords: Ammonia removal; Microalgae; Anaerobic digestion effluent; Alkalinity; Semi-continuous operation

Marimuthu Jeya^{a, c}, Ngoc-Phuong-Thao Nguyen^a, Hee-Jung Moon^b, Sang-Hwan Kim^a and Jung-Kul Lee^{a, c}. (^a Department of Chemical Engineering, Konkuk University, Seoul 143-701, Republic of Korea, ^b Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea, ^c Institute of SK-KU Biomaterials, Konkuk University, Seoul 143-701, Republic of Korea). **Conversion of woody biomass into fermentable sugars by cellulase from *Agaricus arvensis*. Bioresource Technology, Volume 101(22) (2010): 8742-8749**

Agaricus arvensis, a newly isolated basidiomycetous fungus, was found to secrete efficient cellulases. The strain produced the highest endoglucanase (EG), cellobiohydrolase (CBH) and β-glucosidase (BGL) activities of 0.3, 3.2 and 8 U/mg-protein, respectively, with rice straw as the carbon source. Saccharification of the woody biomass with *A. arvensis* cellulase as the enzyme source released a high level of fermentable sugars. Enzymatic hydrolysis of the poplar biomass was optimized using the response surface methodology in order to study the influence of the variables (pH, temperature, cellulases concentration and substrate concentration). The enzyme and substrate concentrations were identified as the limiting factors for the saccharification of poplar wood biomass. A total reducing sugar level of 29 g/L (293 mg/g-substrate) was obtained at an enzyme concentration of 65 FPU/g-substrate after optimization of the hydrolysis parameters. The model validation showed a good agreement between the experimental results and the predicted responses. *A. arvensis* could be a good candidate for the production of reducing sugars from a cellulosic biomass.

Keywords: *Agaricus arvensis*; Cellulase; Poplar wood; RSM optimization; Saccharification

Jian M. Chen^a, Run Y. Zhu^a, Wei B. Yang^a and Li L. Zhang^a. (^a School of Biological and Environmental Engineering, Zhejiang University of Technology, No. 6 District, Zhaohui, Hangzhou 310032, People's Republic of China). **Treatment of a BTo-X-contaminated gas stream with a biotrickling filter inoculated with microbes bound to a wheatbran/red wood powder/diatomaceous earth carrier. Bioresource Technology, Volume 101(21) (2010): 8067-8073**

Microbes bound to a wheat bran/red wood powder/diatomaceous earth carrier were used as inoculants for a biotrickling filter (BTF) for treating gases contaminated with a mixture of benzene, toluene, and *o*-xylene (BTo-X). An overall removal efficiency of more than 87.9% was achieved after a start-up period of as low as 4 days. At BTo-X loading rates (LRs) below 60.0 g/m³ h, the BTF's performance was similar for EBRTs of 90, 60, 45 and 30 s with an elimination capacity (EC) almost approaching the LR; stable REs above 91.3% for benzene and toluene and above 82.8% for *o*-xylene were achieved. A maximum EC of 97.7 g/m³ h was obtained at inlet load of 146.4 g/m³ h. The mass ratio of carbon dioxide produced to the BTo-X removed was approximately 2.62, which confirmed complete degradation of BTo-X. The results demonstrate that microbes bound to a solid carrier can be an alternative to traditional liquid inoculums applied in BTFs and highlight their potential applicability to BTF technologies.

Keywords: Solid carrier; Microbial consortia; Inocula; BTo-X; Biotrickling filter

T.T. More^a, S. Yan^a, R.D. Tyagi^a and R.Y. Surampalli^b. (^a Université du Québec, Institut National de la Recherche Scientifique, Centre Eau, Terre and Environnement, 490 de la Couronne, Québec (QC), Canada G1K 9A9, ^b US Environmental Protection Agency, P.O. Box 17-2141, Kansas City, KS 66117, USA). **Potential use of filamentous fungi for wastewater sludge treatment. Bioresource Technology, Volume 101(20) (2010): 7691-7700**

Specific filamentous fungi (FF) have been recognized for sludge treatment and possibly these strains can be utilized for simultaneous bioflocculation, solids and pathogens reduction and, removal and degradation of toxic compounds. Based on current research work and findings, this review provides the state-of-art knowledge on the role of FF (or moulds) in sludge treatment. The proposed theories are presented, critically analyzed and future scope for specific research on utilization of FF for treatment of sludge is recommended.

Keywords: Filamentous fungi; Sludge settling and dewatering; Degradation; Pathogen removal; Detoxification

Huosheng Li^a, Shaoqi Zhou^{a, b, c}, Yanbo Sun^a and Jiang Lv^d. (^a College of Environmental Science and Engineering, South China University of Technology, Guangzhou Higher Education Mega Center, 510006, PR China, ^b State Key Laboratory of Subtropical Building Sciences, South China University of Technology, Guangzhou 510641, PR China, ^c Key Laboratory of Environmental Protection and Eco-Remediation of Guangdong Regular Higher Education Institutions, South China University of Technology, Guangzhou Higher Education Mega Center, 510006, PR China, ^d College of Civil Engineering and Transportation, South China University of Technology, Guangzhou 510640, PR China). **Nitrogen and carbon removal from Fenton-treated leachate by denitrification and biofiltration. Bioresource Technology, Volume 101(20) (2010): 7736-7743**

Treatment of a Fenton-treated leachate was carried out by denitrification of nitrate using an upflow anaerobic sludge blanket (UASB) biofilm reactor and subsequent biofiltration of the residual COD using a biological aerated filter (BAF). Methanol was selected as the optimal external carbon source because of its good performance, low cost and simple operability. Central composite design (CCD) and response surface methodology (RSM) were used to evaluate the effect of three factors namely hydraulic retention time (HRT), nitrogen concentration and COD/N (C/N) ratio on denitrification performance. Process optimization matched well with the model prediction and allowed 94% reduction of COD and up to 98% removal of total nitrogen (TN). The 21-day biofiltration experiments in a BAF showed that an effluent with COD concentration below 100 mg/L and TN concentration less than 40 mg/L was achieved. Our results indicated that the combination process of UASB-biofilter/BAF is effective for nitrate and residual carbon removal.

Keywords: Nitrogen removal; Landfill leachate; Denitrification; Response surface methodology (RSM); Biofiltration

H.Y. Kang^a, P.Y. Yang^a, W.G. Dominy^b and C.S. Lee^b. (^a Department of Molecular Bioscience and Bioengineering, University of Hawaii at Manoa, Honolulu, HI 96822, USA, ^b Oceanic Institute, Waimanalo, HI 96795, USA). **Bioprocessing papaya processing waste for potential aquaculture feed supplement – Economic and nutrient analysis with shrimp feeding trial. Bioresource Technology, Volume 101(20) (2010): 7973-7979**

Papaya processing waste (PPW), a major fruit processing waste in the Hawaii islands, served as substrate for yeast (*Saccharomyces cerevisiae*) growth. The fermented PPW products containing nutrients of 45% crude protein and various fat, fiber, lignin, cellulose, and minerals were advantages to nutrients of yeast alone. Three experimental diets controlled at 35% protein formulation containing different levels of inclusion of PPW products and a commercial control diet were fed to shrimps for 8 weeks. The 50% inclusion of PPW diets were comparable to commercial feed in weight, growth, feed conversion ratio (FCR) and survival rate. Such bioprocess treatment system would be economically feasible with the control of annual cost and increase of the amount of PPW treated. The selling price of PPW products and annual operation and maintenance cost were the most influential factors to additional profits. This study presented a promising alternative for environmental-friendly treatment of organic wastes as well as the sustainability of local agriculture and aquaculture industries.

Keywords: Fruit processing wastes; Value-added product; Yeast production; Shrimp feeding trial; Economic analysis

Tamer Akar^a and Melike Divriklioglu^a. (^a Department of Chemistry, Faculty of Arts and Science, Eskişehir Osmangazi University, 26480 Eskişehir, Turkey). **Biosorption applications of modified fungal biomass for decolorization of Reactive Red 2 contaminated solutions: Batch and dynamic flow mode studies. Bioresource Technology, Volume 101(19) (2010): 7271-7277**

Biosorption characteristics of a surfactant modified macro fungus were investigated for decolorization of Reactive Red 2 contaminated solutions. Better biosorption efficiency was obtained with a small amount of fungal biomass after modification process. Operating variables like pH, biomass amount, contact time, temperature, dye concentration, flow rate and column size were explored. The biosorption process followed the pseudo-second-order kinetic and

Langmuir isotherm models. Thermodynamic data confirm that the biosorption process is spontaneous and endothermic in nature. Under optimized batch conditions, up to 141.53 mg dye g⁻¹ could be removed from solution in a relatively short time. Modification process was confirmed by FTIR spectroscopy and zeta potential studies. Possible dye-biosorbent interactions were discussed. Good dynamic flow biosorption potential was observed for the suggested biosorbent in simulated wastewater. Overall, batch and continuous mode data suggest that this environmentally friendly and efficient biosorbent may be useful for the removal of reactive dyes from aqueous media.

Keywords: Biosorption; Equilibrium; Isotherms; Kinetics; Modification

Meral Yüce^a, Hasan Nazır^b and Gönül Dönmez^c. (^a Ankara University, Institute of Biotechnology, 06100 Beşevler-Ankara, Turkey, ^b Ankara University, Faculty of Science, Department of Chemistry, 06100 Beşevler-Ankara, Turkey, ^c Ankara University, Faculty of Science, Department of Biology, 06100 Beşevler-Ankara, Turkey). **Using of *Rhizopus arrhizus* as a sensor modifying component for determination of Pb(II) in aqueous media by voltammetry. Bioresource Technology, Volume 101(19) (2010): 7551-7555**

For the sensitive determination of Pb(II) from aqueous solutions, a new voltammetric biosensor based on carbon paste electrode modified with *Rhizopus arrhizus* was developed. The preconcentrated ions at open circuit were reduced by using differential pulse stripping voltammetry technique. The obtained current values were related to the concentration of Pb(II) in the solutions. The best results were achieved at pH 7 with 0.01 M Tris-HCl buffer solution applying a preconcentration time of 12 min. The linear range for the biosensor was found to be within 1.0×10^{-7} – 1.25×10^{-5} M, with a detection limit of 0.5×10^{-8} M. The selectivity of the microbial biosensor was explored by adding interfering heavy metals to accumulation medium one by one, and their matrix effects were also investigated in the model metal solutions. Energy dispersive X-ray spectra analysis were applied to show the specific effect of the fungal biomass on the Pb(II) determination.

Keywords: Microbial biosensor; Environmental control; Lead; Voltammetry; *Rhizopus arrhizus*

Lin Hui^a, Cheng Wan^b, Ding Hai-tao^a, Chen Xue-jiao^a, Zhou Qi-fa^c and Zhao Yu-hua^a. (^a Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou 310058, China, ^b College of Science, Jiangxi Agriculture University, Nanchang 330045, China, ^c Institute of Plant Science, College of Life Sciences, Zhejiang University, Hangzhou 310058, China). **Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of *Aspergillus oryzae* A-4 in solid-state fermentation. Bioresource Technology, Volume 101(19) (2010): 7556-7562**

Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of *Aspergillus oryzae* A-4 in solid-state fermentation (SSF) was investigated. In submerged fermentation, *A. oryzae* A-4 accumulated lipid to 15–18.15% of biomass when pure cellulose was utilized as the sole substrate. In SSF of the wheat straw and bran mixture, *A. oryzae* A-4 yielded lipid of 36.6 mg/g dry substrate (gds), and a cellulase activity of 1.82 FPU/gds with 25.25% of holocellulose utilization in the substrates were detected on the 6th day. The lipid yield reached

62.87 mg/gds in SSF on the 6th day under the optimized conditions from Plackett–Burman design (PBD). Cellulase secretion of *A. oryzae* A-4 was found to influence the lipid yield. Dilute acid pretreatment of the straw and addition of some agro-industrial wastes to the straw could enhance lipid production of *A. oryzae* A-4.

Keywords: Microbial lipid; Direct microbial conversion; *Aspergillus oryzae* A-4; Cellulase; Solid-state fermentation

Yanna Liang^a, Yi Cui^a, Jesse Trushenski^b and James W. Blackburn^c (Error! Bookmark not defined.. (^a Department of Civil & Environmental Engineering, 1230 Lincoln Dr., Southern Illinois University Carbondale, Carbondale, IL 62901, USA, ^b Fisheries and Illinois Aquaculture Center, Department of Zoology, 1125 Lincoln Dr., Southern Illinois University Carbondale, Carbondale, IL 62901, USA, ^c Department of Mechanical Engineering and Energy Processes, 1230 Lincoln Dr., Southern Illinois University Carbondale, Carbondale, IL 62901, USA). **Converting crude glycerol derived from yellow grease to lipids through yeast fermentation. Bioresource Technology, Volume 101(19) (2010): 7581-7586**

Cryptococcus curvatus, an oleaginous yeast was observed to grow on crude glycerol derived from yellow grease. When cultured in a one-stage fed-batch process wherein crude glycerol and nitrogen source were fed intermittently for 12 days, the final biomass density and lipid content were 31.2 g/l and 44.2%, respectively. When cultured in a two-stage fed-batch operation wherein crude glycerol was supplemented at different time points while nitrogen source addition was discontinued at the middle of the experiment, the biomass density was 32.9 g/l and the lipid content was 52% at the end of 12 days. Compared with other oil feedstocks for biodiesel production, lipid accumulated by *C. curvatus* grown on glycerol has high concentration of monounsaturated fatty acid, which makes it an excellent source for biodiesel use.

Keywords: Crude glycerol; Lipids; *Cryptococcus curvatus*; Fed-batch; Biodiesel

Jian-guang Yang^{a,b}. (^a Department of Metallurgical Science and Engineering, Central South University, Changsha 410083, China, ^b Institute of Powder Metallurgy Research, Central South University, Changsha 410083, China). **Heavy metal removal and crude bio-oil upgrading from *Sedum plumbizincicola* harvest using hydrothermal upgrading process[†]. Bioresource Technology, Volume 101(19) (2010): 7653-7657**

The main aim of this study was to separate heavy metals and yield crude bio-oil from a heavy metals hyperaccumulator harvest, *Sedum plumbizincicola*, through hydrothermal upgrading process. Parameters such as granularity, temperature, pressure, and duration were examined for their effect on the removal efficiency of heavy metals and upgrading efficacy of crude bio-oil. Maximum heavy metal removal efficiency of >99% and crude bio-oil upgrading efficiency of >63% were attained with an 18 mesh (1 mm) granularity, and 22.1 MPa at 370 °C in the presence of 10 mg/L additives for 60 s. Under these optimized conditions, an oil phase (mostly composed of phenolic hydrocarbons and derivatives), a water phase raffinate containing Zn²⁺ (0.39 g/L), Pb²⁺ (0.10 g/L), Cu²⁺ (0.16 g/L), and a solid phase (the hydrothermal upgrading residue, which completely satisfies the limit set by China legislation related to biosolids disposal, were obtained).

Keywords: *Sedum plumbizincicola*; Biomass; Hydrothermal upgrading process; Heavy metals; Hyperaccumulator

D. Moldes^{a,b}, E.M. Cadena^a and T. Vidal^a. (^a Department of Textile and Paper Engineering, Universitat Politècnica de Catalunya, Colom 11, E-08222 Terrassa, Spain, ^b Department of Chemical Engineering, Universidade de Vigo, Lagoas Marcosende, E-36310 Vigo, Spain). **Biobleaching of eucalypt kraft pulp with a two laccase-mediator stages sequence. Bioresource Technology, Volume 101(18) (2010): 6924-6929**

A new biobleaching sequence, with two enzymatic stages based on the application of laccase-mediator systems, was tested (L₁EL₂QPo) in order to increase the effectiveness of enzyme delignification on eucalypt kraft pulp. Different synthetic – 1-hydroxybenzotriazole (HBT) and violuric acid (VA) – and natural –syringaldehyde (SyAl) – mediators were used in the laccase stages and the biobleached pulp were compared in terms of chemical, optical and physico-mechanical properties. The pulp bleached with HBT or VA showed similar delignification (64.1% and 65.9% respectively) and optical properties (86.4% and 86.1% ISO brightness respectively) than an industrial TCF pulp (68.3% delignification and 84.8% ISO brightness). SyAl improved these properties in a lower extent (56.71% delignification and 80.52% ISO brightness). Regarding physico-mechanical properties of pulp, the biobleaching sequence had no a negative effect, even some slight improvements were observed in very specific cases.

Keywords: Laccase-mediator system; Kraft pulp; Biobleaching; Pulp properties

Zhongjun Xu^a, Na Qin^a, Jinggang Wang^a and Hua Tong^a. (^a Department of Environmental Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, China). **Formaldehyde biofiltration as affected by spider plant. Bioresource Technology, Volume 101(18) (2010): 6930-6934**

The kinetic process of formaldehyde biodegradation in a biofilter packed with a mixture of compost, vermiculite powder and ceramic particles was investigated in this study. The results showed that more than 60% of formaldehyde was removed by the first 5 cm high biofilter bed at 406 L h⁻¹ flowrate within the range of 5–207 mg m⁻³ inlet concentrations. A macrokinetic model was applied to describe the kinetic process of formaldehyde biodegradation and the experimentally determined elimination capacity for the biofilter agreed well with the model predicted values. The data on the effect of spider plant (*Chlorophytum comosum* L.) on formaldehyde removal indicated that formaldehyde biofiltration might be stimulated by spider plant since formaldehyde was assimilated by spider plant roots and microbial formaldehydedegradation was enhanced by the root exudates.

Keywords: Formaldehyde; Biofiltration; Macrokinetics; Spider plant

Lin Ke^{a,b}, Lijuan Luo^{b,c}, Ping Wang^b, Tiangang Luan^c and Nora Fung-Yee Tam^b. (^a College of Environmental Science and Engineering, South China University of Technology, Guangzhou, Guangdong 510006, China, ^b Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong SAR, China, ^c State Key Laboratory of Biocontrol/MOE Key Laboratory of Aquatic Product

Safety, School of Life Science, Sun Yat-Sen University, Guangzhou 510275, China). Effects of metals on biosorption and biodegradation of mixed polycyclic aromatic hydrocarbons by a freshwater green alga *Selenastrum capricornutum*. Bioresource Technology, Volume 101(18) (2010): 6950-6961

The effects of metals (cadmium, zinc, copper and nickel in a mixture) on biosorption and biodegradation of five mixed polycyclic aromatic hydrocarbons (PAHs), namely fluorene (FLU), phenanthrene (PHE), fluoranthrene (FLA), pyrene (PYR) and benzo[a]pyrene (BAP), by *Selenastrum capricornutum* were investigated. Exposure to metals significantly influenced the interactions between cells and PAHs and, such impact was PAH species dependent. For low molecular weight PAHs (FLU and PHE), both metal dosage and exposure time posed a significant, positive effect on their removal, with up to 99% of FLU and 89% of PHE were removed from the medium in seven days, which was mainly due to the cellular degradation induced by metal stress. For high molecular weight PAHs (FLA, PYR and BAP), the presence of metals did not affect the removal efficiency, but the uptake in the ethyl acetate-extractable fraction of the biomass was increased.

Keywords: Microalgae; Co-contamination; Bioaccumulation; PAH intermediates

Mokded Rabhi^a, Siwar Ferchichi^a, Jihène Jouini^a, Mohamed Hédi Hamrouni^b, Hans-Werner Koyro^c, Annamaria Ranieri^d, Chedly Abdelly^a and Abderrazak Smaoui^a. (^a Laboratory of Plant Adaptation to Abiotic Stresses (LAPSA), Biotechnology Centre of Borj Cedria, P.O. Box 901, 2050 Hammam-Lif, Tunisia, ^b Ministry of Agriculture – Direction of Soils, 17 Hédi Karray Street, 2080 Ariana, Tunisia, ^c Institute for Plant Ecology, Justus-Liebig-University Giessen, D-35392 Giessen, Germany, ^d Università di Pisa, Dipartimento di Chimica e Biotechnologie Agrarie, Via del Borghetto 80, 56124 Pisa, Italy). Phytodesalination of a salt-affected soil with the halophyte *Sesuvium portulacastrum* L. to arrange in advance the requirements for the successful growth of a glycophytic crop. Bioresource Technology, Volume 101(17) (2010): 6822-6828

In the present work, we studied the potential of the obligate halophyte, *Sesuvium portulacastrum* L., to desalinize an experimentally-salinized soil after the following criteria: (i) decrease in soil salinity and sodicity, (ii) plant biomass capacity to accumulate sodium ions, and (iii) phytodesalinated soil quality (equivalent to growth of a glycophytic test culture of *Hordeum vulgare* L.). The cultivation of the halophyte on the salinized soil (phytodesalination culture) led to a marked absorption of Na⁺ ions by *S. portulacastrum* roots and their accumulation in the above-ground biomass up to 872 mg plant⁻¹ and 4.36 g pot⁻¹ (about 1 t ha⁻¹). The decrease in salinity and sodicity of the phytodesalinated soil significantly reduced the negative effects on growth of the test culture of *H. vulgare*. Furthermore, the phytodesalination enabled *H. vulgare* plants to keep a high water content and to develop a higher biomass with relatively high K and low Na contents.

Keywords: Arid and semi-arid regions; Halophyte; Non-leaching conditions; Phytodesalination; Sodium accumulation

Jingyu Ran^a, Shaoyi Jia^a, Yong Liu^a, Wei Zhang^a, Songhai Wu^a and Xiaolei Pan^b. (^a School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China). A

facile method for improving the covalent crosslinking adsorption process of catalase immobilization. *Bioresource Technology*, Volume 101(16): (2010): 6285-6290

In this paper, we introduced a polydiol (mixture of 1,2-propanediol, 1,3-propanediol, and 2,3-butanediol) to improve the covalent crosslinking adsorption process of immobilized catalase onto chitosan beads. The adsorption behavior was investigated by means of adsorption kinetics and adsorption isotherm. The protein content in crosslinking agent required for approximately 45 min to reach the relative equilibrium, and the protein content in solution of the control group and the pretreated group were 6.63 $\mu\text{g/mL}$ and 6.20 $\mu\text{g/mL}$, respectively. The maximum catalase adsorption capacity of the control group and the pretreated group were observed as 23.118 $\mu\text{g/g}$ and 25.688 $\mu\text{g/g}$ at pH 7.0, respectively. Temperature profiles showed that 40 °C was the ideal temperature for active domain of catalase, and the relative activity of pretreated group was 1.12 times higher than that of the control group. The K_m value of the control group (67 mM) was higher than that of the pretreated group (54 mM). Thermal stability, operational stability, and the effect of surfactant on catalase adsorption were also explored in this study.

Keywords: Catalase; Immobilization; Chitosan beads; Polydiol; Adsorption

Lei Yan^a, Huanhuan Yin^a, Shuang Zhang^b, Jiangong Duan^a, Yongquan Li^a, Peng Chen^a and Hongyu Li^a. (^a MOE Key Laboratory of Arid and Grassland Ecology, School of Life Sciences, Lanzhou University, Lanzhou 730000, PR China, ^b College of Life Science and Technology, Heilongjiang August First Land Reclamation University, Daqing 163319, PR China). **Organoarsenic resistance and bioremoval of *Acidithiobacillus ferrooxidans*. *Bioresource Technology*, Volume 101(16) (2010): 6572-6575**

The tolerance and bioremoval of dimethylarsinic acid (DMA^{V}) by *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) were investigated here. The inhibitory concentration (IC) of DMA^{V} was determined for *A. ferrooxidans*. The effects of various parameters such as pH, contact time, initial DMA^{V} concentration, biosorbent dose and temperature were systematically examined to study the biosorption processes. Results indicated that Langmuir model fitted better than Freundlich model to the equilibrium data. Analysis of kinetic data showed that the biosorption processes of DMA^{V} involved pseudo-second-order kinetics. Thermodynamic analysis showed that the biosorption of DMA^{V} onto *A. ferrooxidans* was feasible, spontaneous, endothermic and chemisorptive under examined conditions. Fourier transform infrared spectroscopy (FTIR) showed the involvement of $-\text{OH}$, $-\text{NH}$ and $-\text{SO}_3$ groups in the biosorption process.

Keywords: Tolerance; Biosorption; Organoarsenic; *Acidithiobacillus ferrooxidans*; Kinetics

D.M. Al-Mailem^a, N.A. Sorkhoh^a, M. Marafie^a, H. Al-Awadhi^a, M. Eliyas^a and S.S. Radwan^a. (^a Department of Biological Sciences, Faculty of Science, Kuwait University, Kuwait). **Oil phytoremediation potential of hypersaline coasts of the Arabian Gulf using rhizosphere technology. *Bioresource Technology*, Volume 101(15) (2010): 5786-5792**

The rhizosphere and phyllosphere of the halophyte *Halonemum strobilaceum* naturally inhabiting hypersaline coastal areas of the Arabian Gulf harbor up to $8.1 \times 10^4 \text{ g}^{-1}$ and

$3 \times 10^2 \text{ g}^{-1}$, respectively, of extremely halophilic oil-utilizing microorganisms. Such organisms were 14- to 38-fold more frequent in the rhizosphere than in the plant-free soil. Frequent genera in the rhizosphere were affiliated to the archaea *Halobacterium* sp. and *Halococcus* sp., the firmicute *Brevibacillus borstenlensis*, and the proteobacteria *Pseudoalteromonas ruthenica* and *Halomonas sinaensis*. The phyllospheric microflora consisted of the dimorphic yeast *Candida utilis* and the two proteobacteria *Ochrobactrum* sp. and *Desulfovibrio* sp. Individual strains grew on a range of pure aliphatic and aromatic hydrocarbons, as sole sources of carbon and energy. All the strains, except *C. utilis* which could not tolerate salinities $>2 \text{ M NaCl}$, grew also in media with salinities ranging between 1 and 4 M NaCl, with optimum growth between 1 and 2 M NaCl. With the exception of the two archaeal genera, all isolates could grow in a nitrogen-free medium. The total rhizospheric and phyllospheric microbial consortia could attenuate crude oil in complete (nitrogen-containing) medium, but also equally well in a nitrogen-free medium. It was concluded that *H. strobilaceum* could be a valuable halophyte for phytoremediation of oil-polluted hypersaline environments via rhizosphere technology.

Keywords: Halophyte; Hypersaline; Oil-attenuation; Phyllosphere; Rhizosphere

Nosheen Mirza^a, Qaisar Mahmood^a, Arshid Pervez^a, Raza Ahmad^a, Robina Farooq^a, Mohammad Maroof Shah^a and Muhammad Rashid Azim^b. (^a Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan, ^b Department of Botany, Federal Government Post Graduate College H-8, Islamabad, Pakistan). **Phytoremediation potential of *Arundo donax* in arsenic-contaminated synthetic wastewater. *Bioresource Technology*, Volume 101(15) (2010): 5815-5819**

The present study reports the potential of *Arundo donax* for phytoextraction of arsenic from synthetic wastewater. *A. donax* plants were grown under greenhouse conditions in pots containing a nutrient solution amended with increasing doses of As (0, 50, 100, 300, 600 and 1000 $\mu\text{g L}^{-1}$) for 21 days in a completely randomized design. Shoot and roots dry matter production, growth parameters, arsenic and nutrient tissue concentrations were measured at the end of the experiment. Increasing As concentration in nutrient solution caused an increase in shoot and root biomass without toxicity symptoms in *A. donax* growing under a range of As concentration from 50 to 600 $\mu\text{g L}^{-1}$. Elevated oxidative stress was observed at As supplied level of 1000 $\mu\text{g L}^{-1}$. The As doses up to 600 $\mu\text{g L}^{-1}$ did not affect the growth of *A. donax*. It is suggested that *A. donax* plants may be employed to treat contaminated waters containing arsenic concentrations up to 600 $\mu\text{g L}^{-1}$.

Keywords: *Arundo donax*; Arsenic pollution; Bioaccumulation; Oxidative stress; Phytoextraction

Ge Lei^a, Hongqiang Ren^{a,b}, Lili Ding^{a,b}, Feifei Wang^a and Xingsong Zhang^b. (^a State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210093, PR China, ^b Institution for Environmental Science and Technology, Nanjing University-Yixing, Wuxi 214200, PR China). **A full-scale biological treatment system application in the treated wastewater of pharmaceutical industrial park. *Bioresource Technology*, Volume 101(1) (2010): 5852-5861**

A full-scale combined biological system is used for the treatment of treated wastewater discharged from a pharmaceutical industrial park. This treated water is rich in NH_4^+-N

(average in 86.4 mg/L), low in $\text{COD}/\text{NH}_4^+-\text{N}$ (average in 3.4) and low in BOD_5/COD ratio (average in 0.24) with pH varying from 7.16 to 7.78. The final effluent of the combined treatment process was stably below 100 mg/L COD and 20 mg/L NH_4^+-N , separately, with organic loading rate of 4954 kg COD/d and 92.5 kg $\text{NH}_4^+-\text{N}/\text{d}$. It is found that the BOD_5/COD ratio could be raised from 0.24 to 0.35, and the production of total VFAs account for 9.57% of the total COD via the treatment of hydrolysis/acidification. MBBR and oxidation ditch represent 35.4% and 60.7% of NH_4^+-N removal, 30.2% and 61.5% of COD removal, separately, of the total treatment process. PCR-DGGE is used for microbial community analysis of MBBR and oxidation ditch.

Keywords: Full-scale; MBBR; Hydrolysis/acidification; Oxidation ditch; Pharmaceutical industrial park (PIP)

Umar Farooq^{a,b}, Janusz A. Kozinski^a, Misbahul Ain Khan^{b,c} and Makshoof Athar^c. (^a College of Engineering, University of Saskatchewan, SK, Canada S7N 5A9, ^b Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, ^c Institute of Chemistry, University of the Punjab, Lahore, Pakistan). **Biosorption of heavy metal ions using wheat based biosorbents – A review of the recent literature. Bioresource Technology, Volume 101(14) (2010): 5043-5053**

Conventional technologies for the removal/remediation of toxic metal ions from wastewaters are proving expensive due to non-regenerable materials used and high costs. Biosorption is emerging as a technique offering the use of economical alternate biological materials for the purpose. Functional groups like carboxyl, hydroxyl, sulphhydryl and amido present in these biomaterials, make it possible for them to attach metal ions from waters.

Every year, large amounts of straw and bran from *Triticum aestivum* (wheat), a major food crop of the world, are produced as by-products/waste materials. The purpose of this article is to review rather scattered information on the utilization of straw and bran for the removal/minimization of metal ions from waters. High efficiency, high biosorption capacity, cost-effectiveness and renewability are the important parameters making these materials as economical alternatives for metal removal and waste remediation. Applications of available adsorption and kinetic models as well as influences of change in temperature and pH of medium on metal biosorption by wheat straw and wheat bran are reviewed. The biosorption mechanism has been found to be quite complex. It comprises a number of phenomena including adsorption, surface precipitation, ion-exchange and complexation.

Keywords: *Triticum aestivum*; Straw; Bran; Biosorption; Metal ions

Mohamed Ali Wahab^a, Salah Jellali^a and Naceur Jedidi^a. (^a Water Research and Technologies Centre (CERTE), Wastewater Treatment and Recycling Laboratory, Tunisia). **Ammonium biosorption onto sawdust: FTIR analysis, kinetics and adsorption isotherms modeling. Bioresource Technology, Volume 101(14) (2010): 5070-5075**

Sawdust, an available and renewable biomass, was investigated as a novel ammonium biosorbent. Biosorption occurred over a pH range of 6–10, reached an

equilibrium state within only 20 min, and can be described by a pseudo-second-order model predicting a chemisorption process. Equilibrium data were very well represented by Langmuir isotherm and confirm monolayer coverage. FTIR analysis before and after biosorption of ammonium onto sawdust suggested that the main mechanisms involved in the removal of ammonium might be the ionic exchange and complexation. The use of sawdust presents an interesting option for both tertiary wastewater treatment (as a possible non-conventional biosorbent for the removal of ammonium), and waste recycling (as a fertilizer and compost).

Keywords: Biosorption; Ammonium; Sawdust; FTIR; Modeling

Aderval S. Luna^a, André L.H. Costa^a, Antonio Carlos A. da Costa^a and Cristiane A. Henriques^a. (^a Rio de Janeiro State University (UERJ), Programa de Pós-graduação em Engenharia Química, Rua São Francisco Xavier, 524, Maracanã, Rio de Janeiro, RJ, CEP 20550-900, Brazil). **Competitive biosorption of cadmium(II) and zinc(II) ions from binary systems by *Sargassum filipendula*. *Bioresource Technology*, Volume 101(14) (2010): 5104-5111**

The present work describes our study on the competitive biosorption of Cd(II) and Zn(II) ions onto *Sargassum filipendula* from single component and binary systems. Results showed that *S. filipendula* was an efficient biosorbent for the removal of Cd(II) and Zn(II) ions from an aqueous solution. The equilibrium experimental data for the single component system for the two metallic species fitted well to Langmuir and Langmuir–Freundlich isotherm models. Seven isotherm models were tested with the equilibrium data for modeling of the binary system. Based on the sum of squares error, the Langmuir–Freundlich isotherm model showed the best fit to the binary adsorption data. The influence of the additional cation on the behavior of the biosorption of Cd²⁺ and Zn²⁺ was analyzed comparing single component and binary isotherms. It was observed that Zn²⁺ had a relevant effect on the Cd²⁺ biosorption, but the interference of Cd²⁺ on the sorption of Zn²⁺ was considerably less intense.

Keywords: Competitive biosorption; *Sargassum filipendula*; Cadmium(II); Zinc(II); Equilibrium isotherms

R.A. Pandey^a, P.R. Joshi^a, S.N. Mudliar^a and S.C. Deshmukh^a. (^a National Environmental Engineering Research Institute, Nehru Marg, Nagpur 440020, India). **Biological treatment of waste gas containing mixture of monochlorobenzene (MCB) and benzene in a bench scale biofilter. *Bioresource Technology*, Volume 101(14) (2010): 5168-5174**

The paper outlines treatment of waste gas containing monochlorobenzene (MCB) and benzene in a mixture using biofilter packed with compost and woodchips seeded with *Acinetobacter calcoaceticus*. The biofilter could treat waste gas containing MCB and benzene effectively with an efficiency of (99 ± 5%) and (97 ± 6%) at optimal empty bed contact time (EBCT) of 3 min with a loading of 57 g/m³/h of MCB and 2 g/m³/h of benzene. At optimum loading of MCB and benzene, the biofilter showed total bacterial count of 13 × 10⁵ CFU/g of compost, while the MCB and benzene degrading bacterial count was 71 × 10⁴ CFU/g and 5 × 10⁴ CFU/g compost respectively. The experimental removal efficiency of MCB and benzene were in good agreement with the model predicted value.

Keywords: Monochlorobenzene; Benzene; Biofilter; Treatment; Waste gas

Xuwei Yang^a, Yelin Zeng^a, Fuying Ma^a, Xiaoyu Zhang^a and Hongbo Yu^a. (^a Key Laboratory of Molecular Biophysics of MOE, Huazhong University of Science and Technology, Luoyu Road 1037, Wuhan, PR China). Effect of biopretreatment on thermogravimetric and chemical characteristics of corn stover by different white-rot fungi. *Bioresource Technology*, Volume 101(14) (2010): 5475-5479

The thermogravimetric and chemical characterization of corn stover biopretreated by three different species of white-rot fungi have been studied in this research. Results indicated that biopretreatment can optimize the thermal decomposition, decrease the reaction temperature and reduce the gas contamination (SO_x), making the biomass pyrolysis more efficient and environmentally friendly. Biopretreatment can decrease the activation energy and reacting temperature of the hemicellulose and cellulose pyrolysis (up to 36 °C), shorten the temperature range of the active pyrolysis (up to 14 °C), and increase the thermal decomposition rate, greatly promoting the reaction and making the biomass pyrolysis easier to start and carry on. On the other hand, by biopretreatment, the sulphur content can decrease up to 46.15%, which can considerably reduce the inventory of SO_x emission. Moreover, the mechanism of the biopretreatment was also explored that the deconstruction and depolymerization of the recalcitrant linkages of lignin and cellulose by biopretreatment can make the structure of biomass incompact and easier to be pyrolyzed.

Keywords: Biopretreatment; White-rot fungi; Pyrolysis characteristics; Corn stover

Prabha K. Padmavathiamma^a and Loretta Y. Li^b. (^a Faculty of Land and Food Systems, University of British Columbia, 2357 Main Mall, Vancouver, B.C, Canada V6T 1Z4, ^b Department of Civil Engineering, University of British Columbia, 6250 Applied Science Lane, Vancouver, B.C, Canada V6T 1Z4). Phytoavailability and fractionation of lead and manganese in a contaminated soil after application of three amendments. *Bioresource Technology*, Volume 101(14) (2010): 5667-5676

Studies were conducted to determine the best management practice for immobilisation of toxic Pb and Mn in soil and the interaction of these metal contaminants with the associated plants. The research protocol comprises addition of soil amendments to accelerate physico-chemically driven sorption processes and growth of appropriate plant species to reduce physiologically driven uptake of Pb and Mn. *Lolium perenne* L (perennial rye grass), *Festuca rubra* L (creeping red fescue) and *Poa pratensis* L (Kentucky blue grass) were tested in the presence of soil amendments (lime, phosphate and compost, both individually and in combination). The effectiveness of treatments in stabilizing metals was assessed on the basis of metal speciation in soil, partitioning of metals in plants, and metal uptake. Significant partitioning of Pb in immobile forms was noticed by the growth of *P. pratensis* and Mn by the growth of *L. perenne*. Lime application lowered plant Pb and Mn, while phosphate decreased plant Pb and increased plant Mn. Combined amendment addition resulted in a significant decrease in the exchangeable (mobile) metal fraction in soils growing *Poa* for Pb and in soils growing *Lolium* for Mn. EC_{root} (ratio of root concentration to soil concentration) and EC_{shoot} (ratio of shoot concentration to soil concentration) for Pb in *Poa* decreased by 72% and 60% with combined application of amendments, while the corresponding decreases for Mn in *Lolium* were 48% and 43%.

Keywords: Phytostabilisation; Soil-amendment-plant interaction; Metal fractionation; Translocation properties; Biometric characters

Márcia Maria Rosa, Sâmia Maria Tauk-Tornisielo, Pedro Eduardo Rampazzo and Sandra Regina Ceccato-Antonini. Evaluation of the biological control by the yeast *Torulaspora globosa* against *Colletotrichum sublineolum* in sorghum. World Journal of Microbiology and Biotechnology, Volume 26(8) (2010): 1491-1502

The yeasts are microorganisms with great potential for biotechnological applications in diverse areas. The biological control of phytopathogens by yeasts has showed satisfactory results under laboratory conditions, and it has already produced commercial formulations. With this as focus, this work aims to perform in vitro and in vivo evaluations of the action of a *Torulaspora globosa* yeast strain (1S112), isolated from sugarcane rhizosphere, against the phytopathogenic mold *Colletotrichum sublineolum*, the causative agent of anthracnose in sorghum. In vitro experiments included the antagonism test in Petri dishes with morphological hyphal evaluation; yeast killer activity; siderophore, volatile compound and hydrolytic enzyme production. In vivo experiments were conducted in greenhouse conditions with a sorghum variety susceptible to *C. sublineolum* by evaluating the anthracnose disease for 6 weeks. The results indicated that the yeast strain significantly controlled the fungal growth, either in vitro or in vivo. The strain of *T. globosa* exhibited killer activity against two sensitive strains, which is a novel capacity for this species. The yeast did not produce siderophores, volatile compounds or hydrolytic enzymes, although it has reduced the mycelial growth, resulting in hyphal deformities but not cell death. The yeast controlled the anthracnose disease in sorghum, either inoculated before or after the fungal spores, suggesting that the competition for space and nutrients to dominate the mold and killer toxin production, altering the hyphal morphology, are mechanisms utilized by the yeast in the biocontrol.

Keywords: Biological control - Yeasts - *Torulaspora globosa* - *Colletotrichum sublineolum* - Killer yeast

Agnieszka Mroziak^a and Zofia Piotrowska-Seget^b. (^a Department of Biochemistry, University of Silesia, Jagiellońska 28, 40-032 Katowice, Poland, ^b Department of Microbiology, University of Silesia, Jagiellońska 28, 40-032 Katowice, Poland). Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. Microbiological Research, Volume 165(5) (2010): 363-375

The contamination of soil with aromatic compounds is of particular environmental concern as they exhibit carcinogenic and mutagenic properties. One of the methods of their removal from soil is bioaugmentation, defined as a technique for improvement of the degradative capacity of contaminated areas by introduction of specific competent strains or consortia of microorganisms. The efficiency of bioaugmentation is determined by many abiotic and biotic factors discussed in this paper. The first include chemical structure, concentration and availability of pollutants as well as physico-chemical properties of soil. In turn, among biotic factors the most important is the selection of proper microorganisms that can not only degrade contaminants but can also successfully compete with indigenous microflora.

Several strategies are being developed to make augmentation a successful technology particularly in soils without degrading indigenous microorganisms. These approaches involve the use of genetically engineered microorganisms and gene bioaugmentation. The enhancement

of bioaugmentation may be also achieved by delivering suitable microorganisms immobilized on various carriers or use of activated soil.

Keywords: Bioaugmentation; Microorganisms; Soil; Aromatic compounds; Gene transfer

Valérie Bert, Piet Seuntjens, Winnie Dejonghe, Sophie Lacherez, Hoang Thi Thanh Thuy and Bart Vandecasteele. Phytoremediation as a management option for contaminated sediments in tidal marshes, flood control areas and dredged sediment landfill sites. Environmental Science and Pollution Research, Volume 16(7) (2010): 745-764

Background, aim and scope

Polluted sediments in rivers may be transported by the river to the sea, spread over river banks and tidal marshes or managed, i.e. actively dredged and disposed of on land. Once sedimented on tidal marshes, alluvial areas or control flood areas, the polluted sediments enter semi-terrestrial ecosystems or agro-ecosystems and may pose a risk. Disposal of polluted dredged sediments on land may also lead to certain risks. Up to a few years ago, contaminated dredged sediments were placed in confined disposal facilities. The European policy encourages sediment valorisation and this will be a technological challenge for the near future. Currently, contaminated dredged sediments are often not valorisable due to their high content of contaminants and their consequent hazardous properties. In addition, it is generally admitted that treatment and re-use of heavily contaminated dredged sediments is not a cost-effective alternative to confined disposal. For contaminated sediments and associated disposal facilities used in the past, a realistic, low cost, safe, ecologically sound and sustainable management option is required. In this context, phytoremediation is proposed in the literature as a management option. The aim of this paper is to review the current knowledge on management, (phyto)remediation and associated risks in the particular case of sediments contaminated with organic and inorganic pollutants.

Main features

This paper deals with the following features: (1) management and remediation of contaminated sediments and associated risk assessment; (2) management options for ecosystems on polluted sediments, based on phytoremediation of contaminated sediments with focus on phytoextraction, phytostabilisation and phytoremediation of organic pollutants and (3) microbial and mycorrhizal processes occurring in contaminated sediments during phytoremediation.

Results

In this review, an overview is given of phytoremediation as a management option for semi-terrestrial and terrestrial ecosystems affected by polluted sediments, and the processes affecting pollutant bioavailability in the sediments. Studies that combine contaminated sediment and phytoremediation are relatively recent and are increasing in number since few years. Several papers suggest including phytoremediation in a management scheme for contaminated dredged sediments and state that phytoremediation can contribute to the revaluation of land-disposed contaminated sediments. The status of sediments, i.e. reduced or oxidised, highly influences contaminant mobility, its (eco)toxicity and the success of phytoremediation. Studies are

performed either on near-fresh sediment or on sediment-derived soil. Field studies show temporal negative effects on plant growth due to oxidation and subsequent ageing of contaminated sediments disposed on land. The review shows that a large variety of plants and trees are able to colonise or develop on contaminated dredged sediment in particular conditions or events (e.g. high level of organic matter, clay and moisture content, flooding, seasonal hydrological variations). Depending on the studies, trees, high-biomass crop species and graminaceous species could be used to degrade organic pollutants, to extract or to stabilise inorganic pollutants. Water content of sediment is a limiting factor for mycorrhizal development. In sediment, specific bacteria may enhance the mobilisation of inorganic contaminants whereas others may participate in their immobilisation. Bacteria are also able to degrade organic pollutants. Their actions may be increased in the presence of plants.

Discussion

Choice of plants is particularly crucial for phytoremediation success on contaminated sediments. Extremely few studies are long-term field-based studies. Short-term effects and resilience of ecosystems is observed in long-term studies, i.e. due to degradation and stabilisation of pollutants. Terrestrial ecosystems affected by polluted sediments range from riverine tidal marshes with several interacting processes and vegetation development mainly determined by hydrology, over alluvial soils affected by overbank sedimentation (including flood control areas), to dredged sediment disposal facilities where hydrology and vegetation might be affected or managed by human intervention. This gradient is also a gradient of systems with highly variable soil and hydrological conditions in a temporal scale (tidal marshes) versus systems with a distinct soil development over time (dredged sediment landfill sites).

Conclusions

In some circumstances (e.g. to avoid flooding or to ensure navigation) dredging operations are necessary. Management and remediation of contaminated sediments are necessary to reduce the ecological risks and risks associated with food chain contamination and leaching. Besides disposal, classical remediation technologies for contaminated sediment also extract or destroy contaminants. These techniques imply the sediment structure deterioration and prohibitive costs. On the contrary, phytoremediation could be a low-cost option, particularly suited to in situ remediation of large sites and environmentally friendly. However, phytoremediation is rarely included in the management scheme of contaminated sediment and accepted as a viable option.

Perspectives

Phytoremediation is still an emerging technology that has to prove its sustainability at field scale. Research needs to focus on optimisations to enhance applicability and to address the economic feasibility of phytoremediation.

Keywords: Bioaccumulation - Bioavailability - Biomass recovery - Contaminated sediment - Ecosystem - Flooding - Management option - Microorganisms - Phytostabilisation - Phytoextraction - Rhizodegradation - Risk assessment - Seasonal hydrological variations

Jaco Vangronsveld, Rolf Herzig, Nele Weyens, Jana Boulet, Kristin Adriaensen, Ann Ruttens, Theo Thewys, Andon Vassilev, Erik Meers and Erika Nehnevajova, et al.

Phytoremediation of contaminated soils and groundwater: lessons from the field. Environmental Science and Pollution Research, Volume 16(7) (2010): 765-794**Background, aim, and scope**

The use of plants and associated microorganisms to remove, contain, inactivate, or degrade harmful environmental contaminants (generally termed phytoremediation) and to revitalize contaminated sites is gaining more and more attention. In this review, prerequisites for a successful remediation will be discussed. The performance of phytoremediation as an environmental remediation technology indeed depends on several factors including the extent of soil contamination, the availability and accessibility of contaminants for rhizosphere microorganisms and uptake into roots (bioavailability), and the ability of the plant and its associated microorganisms to intercept, absorb, accumulate, and/or degrade the contaminants. The main aim is to provide an overview of existing field experience in Europe concerning the use of plants and their associated microorganisms whether or not combined with amendments for the revitalization or remediation of contaminated soils and undepend groundwater. Contaminations with trace elements (except radionuclides) and organics will be considered. Because remediation with transgenic organisms is largely untested in the field, this topic is not covered in this review. Brief attention will be paid to the economical aspects, use, and processing of the biomass.

Conclusions and perspectives

It is clear that in spite of a growing public and commercial interest and the success of several pilot studies and field scale applications more fundamental research still is needed to better exploit the metabolic diversity of the plants themselves, but also to better understand the complex interactions between contaminants, soil, plant roots, and microorganisms (bacteria and mycorrhiza) in the rhizosphere. Further, more data are still needed to quantify the underlying economics, as a support for public acceptance and last but not least to convince policy makers and stakeholders (who are not very familiar with such techniques).

Keywords: Field experiments - Metals - Organic contaminants - Phytodegradation - Phytoextraction - Phytoremediation - Phytostabilization - Rhizodegradation - Trace elements

Peter Schröder, Lyudmila Lyubenova and Christian Huber. Do heavy metals and metalloids influence the detoxification of organic xenobiotics in plants? Environmental Science and Pollution Research, Volume 16(7) (2010): 795-804**Background, aim and scope**

Mixed pollution with trace elements and organic industrial compounds is characteristic for many spill areas and dumping sites. The danger for the environment and human health from such sites is large, and sustainable remediation strategies are urgently needed. Phytoremediation seems to be a cheap and environmentally sound option for the removal of unwanted compounds, and the hyperaccumulation of trace elements and toxic metals is seemingly independent from the metabolism of organic xenobiotics. However, stress reactions, ROS formation and depletion of antioxidants will also cause alterations in xenobiotic detoxification. Here, we investigate the

capability of plants to detoxify chlorophenols via glutathione conjugation in a mixed pollution situation.

Materials and methods

Typha latifolia and *Phragmites australis* plants for the present study were grown under greenhouse conditions in experimental ponds. A *Picea abies* L. suspension culture was grown in a growth chamber. Cadmium sulphate, sodium arsenate and lead chloride in concentrations from 10 to 500 μM were administered to plants. Enzymes of interest for the present study were: glutathione transferase (GST), glutathione reductase, ascorbate peroxidase and peroxidase. Measurements were performed according to published methods. GST spectrophotometric assays included the model substrates CDNB, DCNB, NBC, NBoC and the herbicide Fluorodifen.

Results

Heavy metals lead to visible stress symptoms in higher plants. Besides one long-term experiment of 72 days duration, the present study shows time and concentration-dependent plant alterations already after 24 and 72 h Cd incubation. *P. abies* spruce cell cultures react to CdSO_4 and Na_2HAsO_4 with an oxidative burst, similar to that observed after pathogen attack or elicitor treatment. Cd application resulted in a reduction in GSH and GSSG contents. When a heavy metal mixture containing Na_2HAsO_4 , CdSO_4 and PbCl_2 was applied to cultures, both GSH and GSSG levels declined. Incubation with 80 μM arsenic alone doubled GSSG values. Based on these results, further experiments were performed in whole plants of cattail and reed, using cadmium in *Phragmites* and cadmium and arsenic in *Typha* as inducers of stress. In *Phragmites australis*, GST activities for CDNB and DCNB were significantly reduced after short-term Cd exposure (24 h). In the same samples, all antioxidant enzymes increased with rising heavy metal concentrations. *Typha latifolia* rhizome incubation with Cd and As leads to an increase in glutathione reductase and total peroxidase activity and to a decrease in ascorbate peroxidase activity. Measurements of the same enzymes in leaves of the same plants show increased GR activities, but no change in peroxidases. GST conjugation for CDNB was depressed in both cattail rhizomes and leaves treated with Cd. After As application increased, DCNB enzyme activities were detected.

Discussion

T. latifolia and *P. australis* are powerful species for phytoremediation because they penetrate a large volume of soil with their extensive root and rhizome systems. However, an effective remediation process will depend on active detoxifying enzymes, and also on the availability of conjugation partners, e.g. glutathione and its analogues. Species-specific differences seem to exist between the regulations of primary defence enzymes like SOD, catalase, peroxidases, whereas others prefer to induce the glutathione-dependent enzymes. As long as the pollutant mix encountered is simple and dominated by heavy metals, plant defence might be sufficient. When pollution plumes contain heavy metals and organic xenobiotics at the same time, this means that part of the detoxification capacity, at least of glutathione-conjugating reactions, is withdrawn from the heavy metal front to serve other purposes. In fact, glutathione *S*-transferases show strong reactions in stressed plants or in the presence of heavy metals. The spruce cell culture was a perfect model system to study short-term responses on heavy metal impact. Overall, and on the canopy level, this inhibitory effect might result in a lower detoxification capacity for organic pollutants and thus interfere with phytoremediation.

Conclusions

We present evidence that pollution with heavy metals will interfere with both the oxidative stress defence in plants, and with their ability to conjugate organic xenobiotics. Despite plant-species-dependent differences, the general reactions seem to include oxidative stress and an induction of antioxidative enzymes. Several processes seem to depend on direct binding of heavy metals to enzyme proteins, but effects on transcription are also observed. Induction of xenobiotic metabolism will be obtained at high heavy metal concentrations, when plant stress is elevated.

Recommendations and perspectives

Plants for phytoremediation of complex pollution mixtures have to be selected according to three major issues: uptake/accumulation capacity, antioxidative stress management, and detoxification/binding properties for both the trace elements and the organic xenobiotics. By way of this, it might be possible to speed up the desired remediation process and/or to obtain the desired end products. And, amongst the end products, emphasis should be laid on industrial building materials, biomass for insulation or biogas production, but not for feed and fodder. Each of these attempts would increase the chances for publicly accepted use of phytoremediation and help to cure the environment.

Keywords: Detoxification - Heavy metals - Inducible metabolism - Multiple pollution - Organic xenobiotics - Phytoremediation

Valérie Page and Jean-Paul Schwitzguébel. The role of cytochromes P450 and peroxidases in the detoxification of sulphonated anthraquinones by rhubarb and common sorrel plants cultivated under hydroponic conditions. Environmental Science and Pollution Research, Volume 16(7) (2010): 805-816

Background, aim and scope

Sulphonated anthraquinones are precursors of many synthetic dyes and pigments, recalcitrant to biodegradation and thus not eliminated by classical wastewater treatments. In the development of a phytotreatment to remove sulphonated aromatic compounds from dye and textile industrial effluents, it has been shown that rhubarb (*Rheum rabarbarum*) and common sorrel (*Rumex acetosa*) are the most efficient plants. Both species, producing natural anthraquinones, not only accumulate, but also transform these xenobiotic chemicals. Even if the precise biochemical mechanisms involved in the detoxification of sulphonated anthraquinones are not yet understood, they probably have cross talks with secondary metabolism, redox processes and plant energy metabolism. The aim of the present study was to investigate the possible roles of cytochrome P450 monooxygenases and peroxidases in the detoxification of several sulphonated anthraquinones.

Materials and methods

Both plant species were cultivated in a greenhouse under hydroponic conditions, with or without sulphonated anthraquinones. Plants were harvested at different times and either microsomal or cytosolic fractions were prepared. The monooxygenase activity of cytochromes P450 toward

several sulphonated anthraquinones was tested using a new method based on the fluorimetric detection of oxygen consumed during cytochromes P450-catalysed reactions. The activity of cytosolic peroxidases was measured by spectrophotometry, using guaiacol as a substrate.

Results

A significant activity of cytochromes P450 was detected in rhubarb leaves, while no (rhizome) or low (petioles and roots) activity was found in other parts of the plants. An induction of this enzyme was observed at the beginning of the exposition to sulphonated anthraquinones. The results also indicated that cytochromes P450 were able to accept as substrate the five sulphonated anthraquinones, with a higher activity toward AQ-2,6-SS (0.706 nkat/mg protein) and AQ-2-S (0.720 nkat/mg protein). An activity of the cytochromes P450 was also found in the leaves of common sorrel (1.212 nkat/mg protein (AQ-2,6-SS)), but no induction of the activity occurred after the exposition to the pollutant. The activity of peroxidases increased when rhubarb was cultivated in the presence of the five sulphonated anthraquinones (0.857 nkat/mg protein). Peroxidase activity was also detected in the leaves of the common sorrel (0.055 nkat/mg protein), but in this plant, no significant difference was found between plants cultivated with and without sulphonated anthraquinones.

Discussion

Results indicated that the activity of cytochromes P450 and peroxidases increased in rhubarb in the presence of sulphonated anthraquinones and were involved in their detoxification mechanisms.

Conclusions

These results suggest the existence in rhubarb and common sorrel of specific mechanisms involved in the metabolism of sulphonated anthraquinones. Further investigation should be performed to find the next steps of this detoxification pathway.

Recommendations and perspectives

Besides these promising results for the phytotreatment of sulphonated anthraquinones, it will be of high interest to develop and test, at small scale, an experimental wastewater treatment system to determine its efficiency. On the other hand, these results reinforce the idea that natural biodiversity should be better studied to use the most appropriate species for the phytotreatment of a specific pollutant.

Keywords: Common sorrel - Cytochromes P450 monooxygenases - Detoxification pathway - Dye industry - Hydroponic systems - Peroxidases - Phytoremediation - *Rheum rabarbarum* - Rhubarb - *Rumex acetosa* - Sulphonated anthraquinones - Textile industry - Xenobiotic chemicals

Martina Mackova, Petra Prouzova, Petr Stursa, Edita Ryslava, Ondrej Uhlik, Katarina Beranova, Jan Rezek, Veronika Kurzawova, Katerina Demnerova and Tomas Macek. Phyto/rhizoremediation studies using long-term PCB-contaminated soil. Environmental Science and Pollution Research, Volume 16(7) (2010): 817-829

Purpose

Polychlorinated biphenyls (PCBs) represent a large group of recalcitrant environmental pollutants, differing in the number of chlorine atoms bound to biphenyl ring. Due to their excellent technological properties, PCBs were used as heat-transfer media, for filling transformers and condensers, as paint additives, etc. With increasing knowledge of their toxicity, transfer to food chains and accumulation in living organisms, their production ended in most countries in the 1970s and in 1984 in the former Czechoslovakia. But even a quarter of century after the PCB production ceased, from contaminated areas, the volatile PCBs evaporate and contaminate much larger areas even at very distant parts of the world. For this reason, PCBs still represent a global problem. The main method of PCB removal from contaminated environment is at present the expensive incineration at high temperatures. With the aim of finding effective alternative approaches, we are studying biological methods for PCB removal from the environment. In this paper, we summarise 10 years of studies using long-term PCB-contaminated soil from a dumpsite in South Bohemia, targeted for the use of plants (phytoremediation) and their cooperation with microorganisms in the root zone (rhizoremediation).

Materials and methods

Long-term contaminated soil from Lhenice dumpsite, more than hundred kilograms of homogenised material, was used in microcosms (pots and buckets), and field plots were established at the site. Tested plants include among others tobacco, black nightshade, horseradish, alfalfa and willow. Aseptic plant cell and tissue cultures were from the collection of the IOCB. Microorganisms were our own isolates. The paper summarises experiments done between 1998 and 2008 with real contaminated soil, both vegetated and non-vegetated. PCB analysis was performed by GC-ECD, metabolic products identified mostly using 2D-GC/MS-MS and synthetic standards, whereas molecular methods included quantitative PCR and sequencing.

Results

The soil was used both for preparation of field plots at the site and for greenhouse and laboratory tests in microcosms. The results include analyses of changes in PCB content in untreated and vegetated soil, PCB uptake and distribution in different parts of various plant species, analysis of products formed, identification and characterisation of cultivable and non-cultivable bacteria both in rhizosphere and in bulk soil. Different treatments and amendments were also tested. Experiments in real contaminated soil were accompanied by *in vitro* experiments using aseptic cultures of plant biomass, genetically modified (GM) plants and bacteria, to allow identification of players responsible for PCB metabolisation in soil. The time-span of the experiments allows extrapolating some of the results and drawing conclusions concerning the effectivity of exploitation of various plant species and treatments to remove PCBs from soils.

Discussion

The approach using plants proved to represent a viable alternative to costly incineration of PCB-contaminated soils. The recent studies using molecular methods show that plants are responsible

for the composition of consortia of microorganisms present in their root zone, including those with ability to degrade the chlorinated aromatic compounds.

Conclusions

In addition to uptake, accumulation and partial metabolisation of PCBs by plants, compounds produced by plants allow survival of microorganisms even in poor soils, serve as carbon and energy source, and can even induce the degradation pathways of different xenobiotics. Thus, the choice of proper plant species is crucial for effective cleaning of different polluted sites. Our study shows how the efficiency of PCB removal is dependent on the plant used.

Recommendations and perspectives

The use of plants in biological remediation of different organic xenobiotics proved to be a useful approach. Further improvement can be expected by application of specifically tailored GM plants and use of selective conditions ensuring high remediation potential based on optimal composition of the soil microbial consortia designed for the needs of given site.

Keywords: COST - PCB-contaminated soil - PCB uptake - Phytoremediation - Rhizoremediation - Toxicity

Eleni Manousaki and Nicolas Kalogerakis. Phytoextraction of Pb and Cd by the Mediterranean saltbush (*Atriplex halimus* L.): metal uptake in relation to salinity. *Environmental Science and Pollution Research*, Volume 16(7) (2010): 844-854

Background, aim, and scope

The success of phytoextraction depends upon the identification of suitable plant species that hyperaccumulate heavy metals and produce large amounts of biomass using established agricultural techniques. In this study, the Mediterranean saltbush *Atriplex halimus* L., which is a C4 perennial native shrub of Mediterranean basin with an excellent tolerance to drought and salinity, is investigated with the main aim to assess its phytoremediation potential for Pb and Cd removal from contaminated soils. In particular, the influence of soil salinity in metal accumulation has been studied as there is notable evidence that salinity changes the bioavailability of metals in soil and is a key factor in the translocation of metals from roots to the aerial parts of the plant.

Materials and methods

Three pot experiments were conducted under greenhouse conditions for a 10-week period with *A. halimus* grown in soil artificially polluted with 20 ppm of Cd and/or 800 ppm of Pb and irrigated with three different salt solutions (0.0%, 0.5%, and 3.0% NaCl). Soil measurements for soil characterization were performed with the expiration of the first week of plant exposure to metals and NaCl, and at the end of the experimental period, chlorophyll content, leaf protein content, leaf specific activity of guaiacol peroxidase (EC 1.11.1.7), shoot water content, biomass, and Cd and Pb content in the plant tissues were determined. Additionally, any symptoms of metal or salt toxicity exhibited by the plants were visually noted during the whole experimental period.

Results

The experimental data suggest that increasing salinity increases cadmium uptake by *A. halimus* L. while in the case of lead there was not a clear effect of the presence of salt on lead accumulation in plant tissues. *A. halimus* developed no visible signs of metal toxicity; only salt toxicity symptoms were observed in plants irrigated with 3% NaCl solutions. Chlorophyll content, leaf protein content, shoot water content, and biomass were not negatively affected by the metals; instead, there was even an increase in the amount of photosynthetic pigments in plants treated with both metals and salinity. The specific activity of guaiacol peroxidase seems to have a general tendency for increase in plants treated with the metals in comparison with the respective controls but a statistically significant difference exists only in plants treated with the metal mixture and saline conditions.

Discussion

The data revealed that lead and cadmium accumulation in plant tissues was kept generally at low levels. Salinity was found to have a positive effect on cadmium uptake by the plant and this may be related to a higher bioavailability of the metal in soil due to decreased Cd sorption on soil particles. On the other hand, salinity did not influence in a clear way the uptake of Pb by the plant probably because of lead's limited mobility in soils and plant tissues. Cd and Pd usually decrease the chlorophyll content and biomass and change water relations in plants; however, *A. halimus* was found not to be affected indicating that it is a Cd- and Pb-tolerant plant. Guaiacol peroxidase activity as one of the parameters expressing oxidative damage and extent of stress in plants was not generally found to be significantly affected under the presence of metals in most plants suggesting that the extent of stress in plants was minimal, while only for plants treated with the metal mixture and low salinity the enzyme activity was elevated confirming that this enzyme serves as an antioxidative tool against the reactive oxygen species produced by the metals.

Conclusions

Atriplex halimus L. is a Pb- and Cd-tolerant plant but metal concentrations achieved in plant tissues were kept generally at low levels; however, metal accumulation in shoots, especially for Cd, considered together with its high biomass production, rapid growth, and deep root system able to cope with poor structure and xeric characteristics of several polluted soils suggest that this plant deserves further investigation.

Recommendations and perspectives

Phytoextraction by halophytes is a promising alternative for the remediation of heavy metal contaminated sites affected by salinity since saline depressions often indicate sites of industrial effluents accumulation, contaminated by heavy metals, including Pb and Cd. Halophytes are also promising candidates for the removal of heavy metals from non-saline soils. Furthermore, the use of such plants can be potentially viewed as an alternative method for soil desalination where salt is removed from the soil instead of being washed downwards by water or other solutions.

Keywords: *Atriplex halimus* L. - Cadmium - COST—phytoremediation of contaminated soils - Halophytes - Heavy metal tolerance - Lead - Phytoextraction - Pot experiment - Salinity - Stress

Michel Mench, Jean-Paul Schwitzguébel, Peter Schroeder, Valérie Bert, Stanislaw Gawronski and Satish Gupta. Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification and sequestration, and consequences for food safety. Environmental Science and Pollution Research, Volume 16(7) (2010): 876-900

Purpose

The term “phytotechnologies” refers to the application of science and engineering to provide solutions involving plants, including phytoremediation options using plants and associated microbes to remediate environmental compartments contaminated by trace elements (TE) and organic xenobiotics (OX). An extended knowledge of the uptake, translocation, storage, and detoxification mechanisms in plants, of the interactions with microorganisms, and of the use of “omic” technologies (functional genomics, proteomics, and metabolomics), combined with genetic analysis and plant improvement, is essential to understand the fate of contaminants in plants and food, nonfood and technical crops. The integration of physicochemical and biological understanding allows the optimization of these properties of plants, making phytotechnologies more economically and socially attractive, decreasing the level and transfer of contaminants along the food chain and augmenting the content of essential minerals in food crops. This review will disseminate experience gained between 2004 and 2009 by three working groups of COST Action 859 on the uptake, detoxification, and sequestration of pollutants by plants and consequences for food safety. Gaps between scientific approaches and lack of understanding are examined to suggest further research and to clarify the current state-of-the-art for potential end-users of such green options.

Conclusion and perspectives

Phytotechnologies potentially offer efficient and environmentally friendly solutions for cleanup of contaminated soil and water, improvement of food safety, carbon sequestration, and development of renewable energy sources, all of which contribute to sustainable land use management. Information has been gained at more realistic exposure levels mainly on Cd, Zn, Ni, As, polycyclic aromatic hydrocarbons, and herbicides with less on other contaminants. A main goal is a better understanding, at the physiological, biochemical, and molecular levels, of mechanisms and their regulation related to uptake–exclusion, apoplastic barriers, xylem loading, efflux–influx of contaminants, root-to-shoot transfer, concentration and chemical speciation in xylem/phloem, storage, detoxification, and stress tolerance for plants and associated microbes exposed to contaminants (TE and OX). All remain insufficiently understood especially in the case of multiple-element and mixed-mode pollution. Research must extend from model species to plants of economic importance and include interactions between plants and microorganisms. It remains a major challenge to create, develop, and scale up phytotechnologies to market level and to successfully deploy these to ameliorate the environment and human health.

Keywords: Contaminated soil - Contaminated water - Food safety - Genes - Microorganisms - Organic xenobiotics - Phytoremediation - Plant species - Root uptake - Tolerance - Trace elements

Xin Wang and Youngfeng Jia. Study on adsorption and remediation of heavy metals by poplar and larch in contaminated soil. Environmental Science and Pollution Research, Volume 17(7) (2010): 1331-1338

Introduction

Field experiments at the Shenyang Experimental Station of Ecology were conducted to study the adsorption, accumulation, and remediation of heavy metals by poplar and larch grown in artificially contaminated soil.

Materials and methods

The soil was spiked with a combination of Cd, Cu, and Zn at concentrations of 1.5, 100, and 200 mg·kg⁻¹, respectively.

Results

The results showed that the biomass of poplar (*Populus canadensis* Moench) was lower by 26.0% in the soil spiked with a mixture of Cd, Cu, and Zn, compared with the control. Concentrations of Cd in poplar leaf and Cu in poplar roots in the treated soil were 4.11 and 14.55 mg kg⁻¹, respectively, which are much greater than in corresponding controls. The migration of heavy metals in woody plant body was in the order Cd > Zn > Cu. Poplar had higher metal concentrations in aboveground tissues and a higher biomass compared with larch of the same age and therefore is potentially more suitable for remediation. In the heavy metal-polluted soil of this study, phytoremediation by poplar may take 56 and 245 years for Cd and Cu, respectively, for meeting the soil standards of heavy metals, and the corresponding phytoremediation times by larch would take 211 and 438 years.

Conclusion

The research findings could be used as a basis to develop ecological engineering technologies for environmental control and remediation of pollution caused by heavy metals in soils.

Keywords: Heavy metals - Adsorption - Accumulation - Woody plant - Remediation
Responsible editor: Elena Maestri

M. Nazaré P. F. S. Couto, Emanuela Monteiro and M. Teresa S. D. Vasconcelos. Mesocosm trials of bioremediation of contaminated soil of a petroleum refinery: comparison of natural attenuation, biostimulation and bioaugmentation. Environmental Science and Pollution Research, Volume 17(7) (2010): 1339-1346

Purpose

Contamination with petroleum hydrocarbons (PHC) is a global problem with environmental implications. Physico-chemical treatments can be used for soil cleanup, but they are expensive, and can have implications for soil structure and environment. Otherwise, biological remediation treatments are cost-effective and restore soil structure. Several remediation experiments have been carried out in the lab and in the field; however, there is the challenge to achieve as good or

better results in the field as in the laboratory. In the ambit of a project aiming at investigating suitable biological remediation approaches for recovering a refinery contaminated soil, we present here results obtained in bioremediation trials. The approaches biostimulation and bioaugmentation were tested, in parallel, and compared with natural attenuation. For this purpose, mesocosm experiments were carried out inside the refinery area, which constitutes a real asset of this work.

Methods

Soil contaminated with crude oil was excavated, re-contaminated with turbine oil, homogenised and used to fill several 0.5 m³ high-density polyethylene containers. The efficiency of procedures as follows: (1) natural attenuation; (2) manual aeration; (3) biostimulation by adding (3.1) only nutrients; and (3.2) nutrients and a non-ionic surfactant; and (4) bioaugmentation in the presence of added (4.1) nutrients or (4.2) nutrients and a non-ionic surfactant were evaluated after a 9-month period of experiment. For bioaugmentation, a commercial bacterial product was used. In addition to physico-chemical characterization, initial and final soil contents in total petroleum hydrocarbons (TPH) (by Fourier transform infrared spectrophotometry) and the total number of bacteria (by total cell counts) were carried out. For TPH degradation evaluation the soil was divided in four fractions corresponding to different depths: 0–5; 5–10; 10–15; and 15–20 cm. Mean values of percentages of PHC degradation varied between 20 and 50% at surface and between 10 and 35% below 5-cm depth. Natural attenuation was as efficient as most of the tested treatments (about 30% TPH degradation) being exceeded only by bioaugmentation combined with nutrient and surfactant amendments (about 50% TPH degradation). Higher TPH degradation at surface suggests that a combination of sufficient dioxygen, propitious for aerobically degradation, with sunlight required for production of strong photochemical oxidants like ozone, contributed for enhancing degradation. Indeed, the atmosphere of the refineries is relatively rich in volatile organic compounds and nitrogen dioxide (a side-product of the combustion of residual volatile PHC released by the chimneys), which are precursors of O₃ and other photochemical oxidants produced in sunny days, which are very common in Portugal. The fact that natural attenuation was as efficient as most of the soil treatments tested was very probably a result of the presence, in the initial soil, of physiologically adapted native microorganisms, which could be efficient in degrading PHC.

Conclusions

A cost-effective way to reduce half-life for the degradation of PHC of contaminated soil of the refinery will be a periodic revolving of the soil, like tillage, in order to expose to the oxidative atmosphere the different layers of contaminated soil. A combination of soil revolving with bioaugmentation together with nutrients and surfactant amendments may result in an additional improvement of PHC degradation rate. However, this last procedure will raise markedly the price of the remediation treatment.

Keywords: Petroleum hydrocarbons - Refinery soil - Biostimulation - Bioaugmentation
Responsible editor: Elena Maestri

Yun-Shan Liang, Xing-Zhong Yuan, Guang-Ming Zeng, Chen-Lu Hu, Hua Zhong, Dan-Lian Huang, Lin Tang and Jia-Jia Zhao. Bidelignification of rice straw by *Phanerochaete chrysosporium* in the presence of dirhamnolipid. Biodegradation, Volume 21(4) (2010): 615-624

Lignin degradation by white-rot fungi has received considerable attention as a means for reducing accumulation of lignocellulosic wastes in the environment. The stimulatory effect of surfactants on fungal lignocellulose bioconversion also has attracted wide interest. In this study the influence of dirhamnolipid biosurfactant on biodegradation of rice straw by *Phanerochaete chrysosporium* was investigated. It was shown that the biodelignification process of rice straw can be significantly enhanced by the presence of dirhamnolipid biosurfactant. In particular, the dirhamnolipid at the concentration of 0.007% increased the peak activity of lignin peroxidase (LiP) by 86% without affecting the manganese peroxidase (MnP) activity. The water-soluble organic carbon (WSOC) contents in the straw substrates as well as the microbial growth and activity were effectively improved by dirhamnolipid, while the degradation rate of lignin increased by 54% with dirhamnolipid of 0.007%. Observed chemical structural and morphological changes showed that the straw substrates were delignified in the presence of dirhamnolipid with the formation of terrace-like fragments separated from the inner cellular fibers and the release of simple compounds. Variation partitioning analysis revealed that the dirhamnolipid addition induced a significant straw biodelignification which explained 22.1% ($P = 0.013$) of the variance.

Keywords: Lignin - Biodelignification - Dirhamnolipid - Biosurfactant - *Phanerochaete chrysosporium*

Zeinab Salehi, Farzaneh Vahabzadeh, Morteza Sohrabi, Shohreh Fatemi and Hussein Tawfiq Znad. Statistical medium optimization and biodegradative capacity of *Ralstonia eutropha* toward *p*-nitrophenol. Biodegradation, Volume 21(4) (2010): 645-657

The effect of *p*-nitrophenol (PNP) concentration with or without glucose and yeast extract on the growth and biodegradative capacity of *Ralstonia eutropha* was examined. The chemical constituents of the culture medium were modeled using a response surface methodology. The experiments were performed according to the central composite design arrangement considering PNP, glucose and yeast extract as the selected variables whose influences on the degradation was evaluated (shaking in reciprocal mode, temperature of 30°C, pH 7 and test time of about 9 h). Quadratic polynomial regression equations were used to quantitatively explain variations between and within the models (responses: the biodegradation capacity and the biomass formation). The coefficient of determination was high ($R_{\text{adjusted}}^2 = 0.9783$), indicating the constructed polynomial model for PNP biodegradative capacity explains the variation between the regressors fairly well. A PNP removal efficiency of 74.5% occurred within 9 h (15 mg/L as the initial concentration of PNP with use of yeast extract at 0.5 g/L).

Keywords: *Ralstonia eutropha* - Biodegradation - *p*-nitrophenol - Central composite design - Yeast extract - Glucose

Shaik Basha, Santlal Jaiswar and Bhavanath Jha. On the biosorption, by brown seaweed, *Lobophora variegata*, of Ni(II) from aqueous solutions: equilibrium and thermodynamic studies. Biodegradation, Volume 21(5) (2010): 661-680

The biosorption equilibrium isotherms of Ni(II) onto marine brown algae *Lobophora variegata*, which was chemically-modified by CaCl₂ were studied and modeled. To predict the biosorption isotherms and to determine the characteristic parameters for process design, twenty-three one-

two-, three-, four- and five-parameter isotherm models were applied to experimental data. The interaction among biosorbed molecules is attractive and biosorption is carried out on energetically different sites and is an endothermic process. The five-parameter Fritz–Schluender model gives the most accurate fit with high regression coefficient, R^2 (0.9911–0.9975) and F -ratio (118.03–179.96), and low standard error, SE (0.0902–0.0.1556) and the residual or sum of square error, SSE (0.0012–0.1789) values to all experimental data in comparison to other models. The biosorption isotherm models fitted the experimental data in the order: Fritz–Schluender (five-parameter) > Freundlich (two-parameter) > Langmuir (two-parameter) > Khan (three-parameter) > Fritz–Schluender (four-parameter). The thermodynamic parameters such as ΔG^0 , ΔH^0 and ΔS^0 have been determined, which indicates the sorption of Ni(II) onto *L. variegata* was spontaneous and endothermic in nature.

Keywords: *Lobophora variegata* - Biosorption - Isotherm models - Nickel - Thermodynamics

R. Rajeshkannan, M. Rajasimman and N. Rajamohan. Optimization, equilibrium and kinetics studies on sorption of Acid Blue 9 using brown marine algae *Turbinaria conoides*. Biodegradation, Volume 21(5) (2010): 713-727

In the present study, the parameters, temperature, adsorbent dose, contact time, adsorbent size and agitation speed were optimized for Acid Blue 9 removal from aqueous medium by using response surface methodology (RSM). The optimum conditions for maximum removal of Acid Blue 9 from an aqueous solution of 100 mg/l were found as follows: temperature (33°C), adsorbent dose (3 g/l), contact time (225 min), adsorbent size (85 mesh (0.177 mm)) and agitation speed (226 rpm). At these optimized conditions, batch adsorption experiments were conducted to study the effect of pH and initial dye concentration for the removal Acid Blue 9 dye. Kinetic and equilibrium studies were carried out for the experimental results. From the kinetic studies it was found that pseudo second order model suits the system well. From the equilibrium studies, the Freundlich and Redlich-Peterson isotherm fit the data well.

Keywords: Optimization - Isotherm - Kinetics - *Turbinaria conoides* - Acid Blue 9

Kan Wang, Yongding Liu and Dunhai Li. Biosorption of Copper by Cyanobacterial Bloom-Derived Biomass Harvested from the Eutrophic Lake Dianchi in China. Current Microbiology, Volume 61(4) (2010): 340-345

Biomass of cyanobacterial bloom from Lake Dianchi was used as a biosorbent for copper removal from aqueous solution. The maximum capacity was found at conditions of pH 4, initial concentration of copper was 10 mg/l and initial dose of biomass was 1.0 g/l. HNO_3 demonstrated the highest desorption efficiency compared with HCl, EDTA, and citric acid. Physical adsorption was assumed not to be the dominant mechanism of biosorption as revealed by scanning electron microscopy and surface area measurement of the biomass. Infrared ray spectra analysis of the biomass suggested that ion-exchange is the principal mechanism for biosorption. Considering the advantages—low cost, easy to collect, and huge in quantity—the *Microcystis* bloom biomass could be used as a sorbent for copper and other heavy metals removal.

Victoria Gesheva, Erko Stackebrandt and Evgenia Vasileva-Tonkova. Biosurfactant Production by Halotolerant *Rhodococcus fascians* from Casey Station, Wilkes Land, Antarctica. Current Microbiology, Volume 61(2) (2010): 112-117

Isolate A-3 from Antarctic soil in Casey Station, Wilkes Land, was characterized for growth on hydrocarbons. Use of glucose or kerosene as a sole carbon source in the culture medium favoured biosynthesis of surfactant which, by thin-layer chromatography, indicated the formation of a rhamnose-containing glycolipid. This compound lowered the surface tension at the air/water interface to 27 mN/m as well as inhibited the growth of *B. subtilis* ATCC 6633 and exhibited hemolytic activity. A highly hydrophobic surface of the cells suggests that uptake occurs via a direct cell–hydrocarbon substrate contact. Strain A-3 is Gram-positive, halotolerant, catalase positive, urease negative and has rod–coccus shape. Its cell walls contained meso-diaminopimelic acid. Phylogenetic analysis based on comparative analysis of 16S rRNA gene sequences revealed that strain A-3 is closely related to *Rhodococcus fascians* with which it shares 100% sequence similarity. This is the first report on rhamnose-containing biosurfactant production by *Rhodococcus fascians* isolated from Antarctic soil.

C.L. Brierley^a. (^a Brierley Consultancy LLC, P.O. Box 630012, Highlands Ranch, Colorado 80163-0012, USA). Biohydrometallurgical prospects. Hydrometallurgy, Volume 104(3-4) (2010): 324-328

Bioleaching, also referred to as minerals biooxidation, and bioremediation have been widely employed commercially for heap and dump bioleaching of secondary copper sulfide ores, sulfidic-refractory gold concentrates and treatment of acid rock drainage. Technical and commercial challenges, identified in this paper, remain for bioleaching of primary sulfides and complex ores. New frontiers for the technology exist in processing massive sulfides, silicate-locked minerals and in the more distant future in-situ leaching. Decommissioning of cyanide heap leach operations and stabilizing mine wastes using biotechnology are opportunities requiring intensive and focused research, development and engineering efforts.

Keywords: Biohydrometallurgy; Bioremediation; Bioleaching; Minerals biooxidation; Heap leaching; Models; Complex ores; Secondary copper sulfide; Chalcopyrite

A. Schippers^a, A. Breuker^a, A. Blazejak^a, K. Bosecker^a, D. Kock^a and T.L. Wright^a. (^aBundesanstalt für Geowissenschaften und Rohstoffe (BGR), Stilleweg 2, 30655 Hannover, Germany). The biogeochemistry and microbiology of sulfidic mine waste and bioleaching dumps and heaps, and novel Fe(II)-oxidizing bacteria. Hydrometallurgy, Volume 104(3-4) (2010): 342-350

More than 70 microbiological studies of sulfidic mine dumps and heaps have been published. The microbiology and the relevant biogeochemical processes in the dumps and heaps are reviewed and have to be understood for improving copper heap leaching operations and to develop and control countermeasures for the formation of acid mine drainage (AMD). Pyrite or pyrrhotite oxidation is the most relevant biogeochemical process in sulfidic mine waste dumps and heaps and different techniques have been applied to measure reaction rates: column experiments, humidity cells, heat flow measurements, or oxygen consumption measurements. Acidophilic Fe(II)- and sulfur-oxidizing microorganisms are most relevant for metal sulfide oxidation. Anaerobic biogeochemical processes in sulfidic mine dumps and heaps are Fe(III)- and sulfate reduction, but little is known about the reaction rates. Fe(III)-reducing microorganisms dissolve Fe(III)(hydr)oxides and may thereby release adsorbed or precipitated metals. Sulfate-reducing microorganisms precipitate and immobilize many metals. Mainly

culturing approaches were used to study the microbial communities in sulfidic mine dumps and heaps. More recently, molecular biological techniques have been applied to investigate the microbial diversity and to quantify and monitor particular microorganisms. Since acidophilic Fe(II)-oxidizing bacteria and archaea are most relevant for metal sulfide oxidation, several novel cultures were enriched from sulfidic mine dumps from 10 different countries and maintained in the geomicrobiology laboratory of the Bundesanstalt für Geowissenschaften und Rohstoffe (BGR). Of 68 analyzed acidophilic Fe(II)-oxidizing cultures, 40 almost full (> 1300 bp) 16S rRNA gene sequences were obtained and subjected to phylogenetic analysis. Most of the cultivated Fe(II)-oxidizers belonged to four genera. The Gram-positive strains related to *Acidimicrobium* or *Ferrimicrobium* were phylogenetically more diverse than the strains of the Gram-negative genera *Acidithiobacillus* and *Leptospirillum* and partly represent so far undescribed bacteria. Several cultures also contained novel representatives of the heterotrophic genus *Acidiphilium*, often previously found in co-culture with *Acidithiobacillus ferrooxidans*.

Keywords: Mine dumps; Waste rock; Tailings; Pyrite oxidation; Acid mine drainage; Bacteria; Bioleaching; Biomining

S. Groudev^a, I. Spasova^a, M. Nicolova^a and P. Georgiev^a. (^a **University of Mining and Geology “Saint Ivan Rilski”, Sofia 1700, Bulgaria**). ***In situ* bioremediation of contaminated soils in uranium deposits. Hydrometallurgy, Volume 104(3-4) (2010): 518-523**

Experimental plots consisting of acidic and alkaline soils heavily contaminated with radionuclides (mainly U and Ra) and non-ferrous metals (mainly Cu, Zn, Cd and Pb) were treated *in situ* under real field conditions using the activity of the indigenous soil microflora. This activity was enhanced by suitable changes of some essential environmental factors such as pH and water, oxygen and nutrient contents of the soil. The treatment was connected with solubilization and removal of contaminants from the top soil layers (horizon A) due to the joint action of the soil microorganisms and leach solutions used to irrigate the soils (mainly acidophilic chemolithotrophic bacteria and diluted sulphuric acid in the acidic soil, and various heterotrophs and bicarbonate and soluble organics in the alkaline soil). The dissolved contaminants were removed from the soil profile through the drainage soil effluents or were transferred to the deeply located soil subhorizon B₂ where they were precipitated as the relevant insoluble forms (uranium as uraninite, and the non-ferrous metals as the relevant sulphides) as a result of the activity of the sulphate-reducing bacteria inhabiting this soil subhorizon.

Keywords: Uranium; Non-ferrous metals; Soil bioremediation; Uranium complexes; Sulphate-reducing bacteria; Iron-reducing bacteria

H. Ciftci^a and A. Akcil^a. (^a **Mineral Processing Division, Department of Mining Engineering, Suleyman Demirel University, Isparta TR 32260, Turkey**). **Effect of biooxidation conditions on cyanide consumption and gold recovery from a refractory gold concentrate. Hydrometallurgy, Volume 104(2) (2010): 142-149**

This study investigates the biooxidation of a refractory gold concentrate using a mixed culture of acidophilic mesophiles, moderate thermophiles and extreme thermophiles and their effect on the subsequent cyanidation and gold recovery. The experiments with high % solids using mixed mesophiles showed better oxidation potential compared to moderate thermophiles and extreme thermophiles. However, the extreme thermophiles performed better than mesophiles and moderate thermophiles during the biooxidation with < 5% solids (w/v). The biooxidized residues

obtained from the experiments with extremophiles, gave a maximum gold recovery of 92% after cyanidation. The NaCN consumption during cyanidation of the biooxidized residues obtained from experiments with extremophiles was lower than bioresidues obtained from experiments with mesophiles and moderate thermophiles. The highest consumption of NaCN of 17.5 kg/ton was from the experiment with 10% solids (w/v) using mesophiles. X-ray diffraction studies and chemical analysis of the biooxidized residues confirms the precipitation of jarosites at high redox potential which produces some ferricyanide, thereby explaining the reason for high cyanide consumption during cyanidation.

Keywords: Biooxidation; Mesophiles; Thermophiles; Gold recovery; Cyanidation; Jarosite

Chris O. Nwoko. (Department of Environmental Technology, Federal University of Technology, Owerri. PMB 1526. Nigeria. E-mail: Obix04@yahoo.co.uk. Tel: +2348037097613). Trends in phytoremediation of toxic elemental and organic pollutants. African Journal of Biotechnology Vol. 9 (37) (2010): 6010-6016

Phytoremediation is the use of plants to extract, sequester or mineralize pollutants. This process is seen as an ecologically sound strategy for management of contaminated ecosystems. In this review, current status of several subsets of phytoremediation are discussed which includes: (a) Phytoextraction – which is a process in which high biomass pollutant accumulating plants are used to accumulate and transport pollutants from the soil to harvestable parts of plants. (b) Phytofiltration – which is a process in which plant roots are used to precipitate and concentrate pollutants from effluents. (c) Phytostabilization - here plants stabilize pollutants, thus rendering them harmless. (d) Phytovolatilization –plants absorb pollutants and convert them into gaseous components via transpiration. The advantages inherent in these technologies are also discussed. There is need for further understanding on the processes that affect pollutant availability, rhizosphere processes, pollutant uptake and sequestration.

Key words: Phytoremediation, xenobiotics, metal, pollution, decontamination.

Abbreviations: PCBs, Polychlorinated biphenyls; PAHs, polycyclic aromatics hydrocarbons; TNT, trinitrotoluene; TCE, trichloroethene; SC, selenium; VOC, volatile organic compounds; MTs, metallothioneins; PCs, phytochelatins; R-SH, organic sulphur; CEC, cations-cation exchange capacity; EDTA, ethylene diamine tetra acetic acid; TSCF, transpiration stream concentration factor; SeCys, selenoaminoacids selen-cysteine; SeMet, selenomethionine; DMS₂, dimethylselenide; MerA, mercury reductase.

Yaohui Bai, Qinghua Sun, Cui Zhao, Donghui Wen and Xiaoyan Tang. Bioaugmentation treatment for coking wastewater containing pyridine and quinoline in a sequencing batch reactor. Applied Microbiology and Biotechnology, Volume 87(5) (2010): 1943-1951

Two pyridine-degrading bacteria and two quinoline-degrading bacteria were introduced for bioaugmentation to treat the coking wastewater. Sequencing batch reactors (SBRs) were used for a comparative study on the treatment efficiency of pyridine, quinoline, and chemical oxygen demand. Results showed that the treatment efficiency with coking-activated sludge plus a mixture of the four degrading bacteria was much better than that ones with coking-activated sludge only or mixed degrading bacteria only. Moreover, a 52-day continuous operation of the

bioaugmented and general SBRs was investigated. The bioaugmented SBR showed better treatment efficiency and stronger capacity to treat high pyridine and quinoline shock loading. The general SBR failed to cope with the shock loading, and the biomass of the activated sludge decreased significantly. In order to monitor the microbial ecological variation during the long-term treatment, the bacterial community in both reactors was monitored by the amplicon length heterogeneity polymerase chain reaction technique. The diversity of the bacterial community decreased in both reactors, but the introduced highly efficient bacteria were dominant in the bioaugmented SBR. Our experiment showed clearly that the use of highly efficient bacteria in SBR process could be a feasible method to treat wastewater containing pyridine or/and quinoline.

Keywords: Bioaugmentation - Pyridine - Quinoline - SBR - Bacterial community

Josângela do Carmo Trezena de Araújo and Clístenes Williams Araújo do Nascimento. Phytoextraction of Lead from Soil from a Battery Recycling Site: The Use of Citric Acid and NTA. *Water, Air, & Soil Pollution*, Volume 211(1-4) (2010): 113-120

Phytoextraction is a soil remediation technique involving plants that concentrate heavy metals in their shoots, which may be removed from the area by harvest. The application of synthetic chelants to soil increases metal solubility, and therefore enhances phytoextraction. However, synthetic chelants degrade poorly in soil, and metal leaching poses a threat to human and animal health. The aim of this study is to assess the use of two biodegradable chelants (citric acid and nitrilotriacetic acid (NTA)) for Pb phytoextraction by maize from a soil contaminated by battery-casing disposal. In order to assess the behavior of a non-degradable chelant, ethylenediaminetetraacetic acid (EDTA) was also included in the experiment. The chelants NTA and EDTA were applied to soil pots at rates of 0, 3, 5, 7, and 10 mmol kg⁻¹ of soil. The rates used to citric acid were 0, 5, 10, 15, and 30 mmol kg⁻¹. Maize plants were grown for 72 days and chelants were applied 9 days before harvest. Soil samples were extracted with CaCl₂ to assess the Pb solubility after chelants application. The results showed that NTA was more efficient than citric acid to solubilize Pb from soil; however, citric acid promoted higher net removal of Pb (120 mg pot⁻¹) than NTA (57 mg pot⁻¹). Thus, the use of citric acid, a biodegradable organic acid, could be feasible for enhancing the phytoextraction of Pb from the site studied with no environmental constraints.

Keywords: Phytoremediation - Synthetic chelants - Soil contamination

Vishal Mishra, Chandrajit Balomajumder and Vijay Kumar Agarwal. Biosorption of Zn (II) onto the Surface of Non-living Biomasses: A Comparative Study of Adsorbent Particle Size and Removal Capacity of Three Different Biomasses. *Water, Air, & Soil Pollution*, Volume 211(1-4) (2010): 489-500

Present research has delineated the biosorption potential of three different nonliving biomasses namely eucalyptus bark saw dust, mango bark saw dust, and pineapple fruit peel with respect to Zn (II) ion removal from liquid phase through batch experiments. The efficacy of Zn (II) ion biosorption onto surface of biosorbents was judged and correlated with biosorbent particle size, surface chemistry, and surface texture. Maximum metal ion uptake capacity, percentage removal, and minimum equilibrium concentration as 1.688 mg/g, 84.4%, and 1.56 mg/l, respectively, was obtained using eucalyptus bark saw dust mediated biosorption followed by mango bark saw dust as 1.028 mg/g, 51.4%, and 4.867 mg/l and pineapple fruit peel as

0.45 mg/g, 22.9%, and 7.71 mg/l, respectively, at a particle size of 0.5 mm. Additionally, present investigation also proved that biosorption efficiency and metal ion interaction with adsorbent surface also depends upon presence of functional groups involved in metal ion adsorption and surface porosity.

Keywords: Heavy metal - Biosorption - Biomass - Particle size - Eucalyptus bark - Zn (II)

Bon-Jun Koo^a, Weiping Chen^b, Andrew C. Chang^c, Albert L. Page^c, Thomas C. Granato^d and Robert H. Dowdy^e. (^a Department of Natural and Mathematical Sciences, California Baptist University, Riverside, CA 92504, USA, ^b State Key Lab of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China, ^c Department of Environmental Sciences, University of California, Riverside, CA 92521, USA, ^d Metropolitan Water Reclamation District of Greater Chicago, Chicago, IL 60611, USA, ^e Department of Soil, Water and Climate, University of Minnesota, St Paul, MN 55108, USA). **A root exudates based approach to assess the long-term phytoavailability of metals in biosolids-amended soils. Environmental Pollution, Volume 158(8) (2010): 2582-2588**

Organic acids present in the rhizosphere of growing plants are widely recognized to be responsible for dissolving the solid phase metals in the soil and making them available for plant absorption. We proposed a root exudates-based model to assess the long-term phytoavailability of metals in biosolids-amended soils. The phytoavailability of biosolids-borne metals was defined in terms of a capacity factor and an intensity factor. The plant available metal pool, C_0 (capacity factor, mg kg^{-1}), can be estimated by fitting the successive organic acids extraction data to an exponential decay kinetic equation. The field metal removal rate, k (intensity factor, yr^{-1}), can be estimated from the successive extraction-based metal release rate through an effective annual organic acid production in the rhizosphere which was found to be characteristic of plant species. The protocol was successfully used to assess the long-term phytoavailability of metals in biosolids-amended soil from two biosolids land application sites.

A new approach based on root exudates extraction was developed to assess the phytoavailability of metals in biosolids-amended soils.

Keywords: Phytoavailability; Model; Heavy metal; Biosolids-amended soil; Rhizosphere; Root exudates

Julien Laurent^a, Magali Casellas^a, Marie-Noëlle Pons^b and Christophe Dagot^a. (^aGroupement de Recherche Eau Sol Environnement, Université de Limoges, ENSIL, 16 rue Atlantis, Parc ESTER Technopôle, 87068 Limoges Cedex, France, ^b Laboratoire des Sciences du Génie Chimique, CNRS, Nancy Université, INPL, 1 rue Granville, BP 451, F-54001 Nancy, France). **Cadmium biosorption by ozonized activated sludge: The role of bacterial flocs surface properties and mixed liquor composition. Journal of Hazardous Materials, Volume 183(1-3) (2010): 256-263**

Cadmium uptake by activated sludge was studied following modifications of sludge composition and surface properties induced by ozone treatment. Ozone leads to the solubilization of sludge compounds as well as their mineralization, especially humic like substances. Small particles

were formed following floc disintegration, leading to a decrease of average floc size. The study of surface properties underlined the mineralization as the number of surface binding sites decreased with the increase of ozone dose. Depending on ozone dose, cadmium uptake by activated sludge flocs was either increased or decreased. Different mechanisms were involved: below 10 mg O₃/g TS, the increase of floc specific surface area following floc size decrease as well as the release of phosphate ions yielded an increase by 75% of cadmium uptake, due to the better availability of biosorption sites and the increase of precipitation. Inversely, at higher ozone doses, the number of biosorption sites decreased due to oxidation by ozone. Moreover, dissolved organic matter concentration increased and provided ligands for metal complexation. Cadmium uptake was therefore limited for ozone doses ranging from 10 to 16.8 mg O₃/g TS.

Keywords: Ozone; Activated sludge minimization; Surface properties; Biosorption; Heavy metals

Yaohui Xu^a and Mang Lu^b. (^a Department of Geochemistry, Yangtze University, Nanhuan Road, Jingzhou 434023, China, ^b School of Materials Science and Engineering, Jingdezhen Ceramic Institute, Jingdezhen 333001, Jiangxi Province, China). Bioremediation of crude oil-contaminated soil: Comparison of different biostimulation and bioaugmentation treatments. *Journal of Hazardous Materials*, Volume 183(1-3) (2010): 395-401

Biostimulation with inorganic fertilizer and bioaugmentation with hydrocarbon utilizing indigenous bacteria were employed as remedial options for 12 weeks in a crude oil-contaminated soil. To promote oil removal, biocarrier for immobilization of indigenous hydrocarbon-degrading bacteria was developed using peanut hull powder. Biodegradation was enhanced with free-living bacterial culture and biocarrier with a total petroleum hydrocarbon removal ranging from 26% to 61% after a 12-week treatment. Oil removal was also enhanced when peanut hull powder was only used as a bulking agent, which accelerated the mass transfer rate of water, oxygen, nutrients and hydrocarbons, and provided nutrition for the microflora. Dehydrogenase activity in soil was remarkably enhanced by the application of carrier material. Metabolites of polycyclic aromatic hydrocarbons were identified by Fourier transform ion cyclotron resonance mass spectrometry.

Keywords: Peanut hull; Dehydrogenase activity; Bulking; Toxicity; Fourier transform ion cyclotron resonance mass spectrometry

Zhenze Li^a, Takeshi Katsumi^a, Shigeyoshi Imaizumi^b, Xiaowu Tang^c and Toru Inui^a. (^aGSGES, Kyoto University, Sakyo, Kyoto 606-8501, Japan, ^b Dep. Adv. Interdiscip. Sci., Utsunomiya University, Yoto, Utsunomiya 321-8585, Japan, ^c MOE Key Laboratory of Soft Soils and Geoenvironmental Engineering, Zhejiang University, Hangzhou 310058, China). Cd(II) adsorption on various adsorbents obtained from charred biomaterials. *Journal of Hazardous Materials*, Volume 183(1-3) (2010): 410-420

Cadmium could cause severe toxicant impact to living beings and is especially mobile in the environment. Biomass is abundant and effective to adsorb heavy metals, but is easy to be decomposed biologically which affects the reliability of long-run application. Several biomasses were charred with and without additives at temperatures less than 200 °C in this study. The prepared adsorbents were further testified to remove Cd(II) from aqueous solution. Equilibrium and kinetic studies were performed in batch conditions. The effect of several experimental parameters on the cadmium adsorption kinetics namely: contact time, initial cadmium

concentration, sorbent dose, initial pH of solution and ionic strength was evaluated. Kinetic study confirmed (1) the rapid adsorption of Cd(II) on GC within 10 min and (2) the following gradual intraparticle diffusion inwards the sorbent at neutral pH and outwards at strong acidic solution. The grass char (GC) was selected for further test according to its high adsorption capacity (115.8 mg g^{-1}) and affinity (Langmuir type isotherm). The Cd(II) removal efficiency was increased with increasing solution pH while the highest achieved at sorbent dosage 10.0 g L^{-1} . The ionic strength affects the sorption of Cd(II) on GC to a limited extent whereas calcium resulted in larger competition to the sorption sites than potassium. Spectroscopic investigation revealed the adsorption mechanisms between Cd(II) and surface functional groups involving amine, carboxyl and iron oxide. The long-term stability of the pyrolyzed grass char and the potential application in engineering practices were discussed.

Keywords: Cadmium; Adsorption; Charred biomaterials; Kinetics; Equilibrium; Modeling

Dhanus Suryaman^{a, b} and Kiyoshi Hasegawa^b. (^a Agency for the Assessment and Application of Technology, M.H. Thamrin No. 8, Jakarta 10340, Indonesia, ^b Department of Chemical and Biochemical Engineering, Faculty of Engineering, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan). **Biological and photocatalytic treatment integrated with separation and reuse of titanium dioxide on the removal of chlorophenols in tap water. Journal of Hazardous Materials, Volume 183(1-3) (2010): 490-496**

We investigated biological, photocatalytic, and combination of biological and photocatalytic treatments in order to remove a mixture of 2-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, and pentachlorophenol in tap water (total: 100 mg L^{-1} , each: 25 mg L^{-1}). The removal of chlorinated phenols was conducted with a flow biological treatment and a circulative flow photocatalytic treatment under black light and sunlight irradiations integrated with titanium dioxide separation and reuse. The combined biological–photocatalytic treatment significantly shortened the degradation and mineralization time of both the biological treatment and the photocatalytic treatment. The removed chlorophenols per hour by the combined biological–photocatalytic treatment was 25.8 mg h^{-1} , whereas by the combined photocatalytic–biological treatment was 10.5 mg h^{-1} . After a large portion of biodegradable 2-chlorophenol and 2,4-dichlorophenol, and around half amount of slightly biodegradable 2,4,5-trichlorophenol were removed by the biological treatment, the remained three chlorophenols, biorecalcitrant pentachlorophenol, and biodegradation products were completely removed by the subsequent photocatalytic treatment. Since titanium dioxide particles in tap water spontaneously sedimented on standing after the photocatalytic treatment, the combined treatment can be operated by integrating with the titanium dioxide separation and reuse. The TiO_2 particles were recovered and reused at least three times without significantly decreasing the removal efficiency.

Keywords: Photocatalysis; TiO_2 reuse; Biodegradation; Chlorophenol; Wastewater

Hanen Zaier^{a, 1}, Tahar Ghnaya^{a, 1}, Abelbasset Lakhdar^a, Rawdha Baioui^a, Rim Ghabriche^a, Majda Mnasri^a, Souhir Sghair^a, Stanley Lutts^b and Chedly Abdely^a. (^aLaboratoire d'Adaptation des Plantes aux Stress Abiotiques, Centre de Biotechnologie de Borj-Cédria, BP 901, 2050 Hammam-lif, Tunisia, ^b Groupe de Recherche en Physiologie Végétale (GRPv), Earth and Life Institute, Université Catholique de Louvain, Louvain-la-Neuve, Belgium). **Comparative study of Pb-phytoextraction potential in *Sesuvium portulacastrum***

and *Brassica juncea*: Tolerance and accumulation. *Journal of Hazardous Materials*, Volume 183(1-3) (2010): 609-615

Lead phytoextraction from salty soils is a difficult task because this process needs the use of plants which are able to tolerate salt and accumulate Pb^{2+} within in their shoots. It has recently been suggested that salt-tolerant plants are more suitable for heavy metals extraction than salt-sensitive ones commonly used in this approach.

The aim of this study was to investigate Pb-phytoextraction potential of the halophyte *Sesuvium portulacastrum* in comparison with *Brassica juncea* commonly used in Pb-phytoextraction. Seedlings of both species were exposed in nutrient solution to 0, 200, 400, 800 and 1000 μM Pb^{2+} for 21 days. Lead strongly inhibited growth in *B. juncea* but had no impact on *S. portulacastrum*. Exogenous Pb^{2+} reduced nutrients uptake mainly in *B. juncea* as compared to *S. portulacastrum*. Lead was preferentially accumulated in roots in both species. *S. portulacastrum* accumulated more Pb^{2+} in the shoot than *B. juncea*. Hence, the amounts of Pb^{2+} translocated at 1000 μM Pb^{2+} were 3400 $\mu g\ g^{-1}$ DW and 2200 $\mu g\ g^{-1}$ DW in *S. portulacastrum* and *B. juncea*, respectively. These results suggest that *S. portulacastrum* is more efficient to extract Pb^{2+} than *B. juncea*.

Keywords: Pb^{2+} ; Halophyte; Glycophyte; Phytoextraction; Translocation

Francesca Beolchini^a, Laura Rocchetti^a, Francesco Regoli^b and Antonio Dell'Anno^a. (^aDepartment of Marine Sciences, Polytechnic University of Marche, Via Brece Bianche, 60131 Ancona, Italy, ^b Department of Biochemistry, Biology and Genetic, Polytechnic University of Marche, Via Ranieri, 60131 Ancona, Italy). Bioremediation of marine sediments contaminated by hydrocarbons: Experimental analysis and kinetic modeling. *Journal of Hazardous Materials*, Volume 182(1-3) (2010): 403-407

This work deals with bioremediation experiments on harbor sediments contaminated by aliphatic and polycyclic aromatic hydrocarbons (PAHs), investigating the effects of a continuous supply of inorganic nutrients and sand amendments on the kinetics of microbial growth and hydrocarbon degradation. Inorganic nutrients stimulated microbial growth and enhanced the biodegradation of low and high molecular weight hydrocarbons, whereas sand amendment increased only the removal of high molecular weight compounds. The simultaneous addition of inorganic nutrients and sand provided the highest biodegradation (>70% for aliphatic hydrocarbons and 40% for PAHs). A semi-empirical kinetic model was successfully fitted to experimental temporal changes of hydrocarbon residual concentrations and microbial abundances. The estimated values for parameters allowed to calculate a doubling time of 2.9 d and a yield coefficient biomass/hydrocarbons 0.39 g C biomass g⁻¹C hydrocarbons, for the treatment with the highest hydrocarbon biodegradation yield. A comparison between the organic carbon demand and temporal profiles of hydrocarbons residual concentration allowed also to calculate the relative contribution of contaminants to carbon supply, in the range 5–32%. This suggests that C availability in the sediments, influencing prokaryotic metabolism, may have cascade effects on biodegradation rates of hydrocarbons. Even if these findings do not represent a general rule and site-specific studies are needed, the approach used here can be a relevant support tool when designing bioremediation strategies on site.

Keywords: Kinetic models; Bioremediation; Hydrocarbon; Marine sediment

Abbreviations: *C*, Total aliphatic hydrocarbon concentration (μg aliphatic hydrocarbon g^{-1} sediment); HMW, high molecular weight aliphatic hydrocarbons ($C > 24\text{--}40$); *k*, first-order rate constant (d^{-1}); K_0 , semi-empirical model parameter (Eq. (1), d^{-2}); LMW, low molecular weight aliphatic hydrocarbons ($C > 12\text{--}24$); PAHs, polycyclic aromatic hydrocarbons; *t*, time (d); *T_d*, doubling time (d); *X*, prokaryotic abundance (10^8 cells g^{-1} sediment); *Y*, semi-empirical model parameter (Eq. (1)) related to yield coefficient (10^8 cells μg^{-1} aliphatic hydrocarbons), (g C biomass g^{-1} C aliphatic hydrocarbons); β , semi-empirical model parameter (Eq. (1)) related to inhibiting factor (g sediment 10^{-8} cells)

M. Cea^a, M. Jorquera^a, O. Rubilar^a, H. Langer^b, G. Tortella^a and M.C. Diez^a. (^aScientific and Technological Bioresources Nucleus, Universidad de La Frontera, Av. Francisco Salazar 01145, Casilla 54-D, Temuco, Chile, ^b Agrícola Nacional SACEI (ANASAC), Panamericana 5 Sur km 645, Lote 1 Parque Industrial, Lautaro, Chile). **Bioremediation of soil contaminated with pentachlorophenol by *Anthracophyllum discolor* and its effect on soil microbial community.** *Journal of Hazardous Materials*, Volume 181(1-3) (2010): 315-323

Bioaugmentation is a promising technology to clean up sites contaminated with recalcitrant chemicals. White-rot fungi have proven to be effective in the degradation of pentachlorophenol. Here, we report the bioremediation of soil contaminated with pentachlorophenol (PCP) by *Anthracophyllum discolor* and its impact on the soil microbial community. In this study three types of microcosms were established: fresh soil (C_0), fresh soil plus wheat straw (WS_0) and, fresh soil plus wheat straw inoculated with *A. discolor* ($WSAD_0$). Additionally, similar treatments and a control of sterile soil spiked with PCP (C_{250} , WS_{250} and $WSAD_{250}$) were used to evaluate the remediation and adsorption of PCP. The PCP removal, total microbial activity, and enzymatic activities were evaluated. This study also investigated the structure of soil microbial community by denaturing gradient gel electrophoresis (DGGE), identifying some of the dominant bacterial and fungal species. The results showed that PCP was effectively degraded in soils by *A. discolor* and by indigenous soil microorganisms. The addition of wheat straw increased the PCP degradation and enzymatic activities. Only laccase activity was negatively affected by PCP contamination. The PCP degradation was associated with changes in microbial communities, mainly stimulation of members of bacterial phylum *Proteobacteria* (*Xanthomonadaceae*, *Burkholderiaceae* and *Enterobacteriaceae*), and fungal phylum *Ascomycota* and *Basidiomycota*. This study shows the ability of *A. discolor* to degrade PCP from contaminated soil, and demonstrates that agricultural residues, such as wheat straw, can be used as growth substrate by microorganisms in PCP-contaminated soil, demonstrating a great potential of autochthonous microorganisms for soil remediation.

Keywords: *Anthracophyllum discolor*; DGGE; Pentachlorophenol; Microbial community

Luminita Ghimici^a, Marieta Constantin^a and Gheorghe Fundueanu^a. (^a Petru Poni Institute of Macromolecular Chemistry, 41-A Grigore Ghica Voda Alley, 700487 Iasi, Romania). **Novel biodegradable flocculating agents based on pullulan.** *Journal of Hazardous Materials*, Volume 181(1-3) (2010): 351-358

New copolymers with different grafted cationic chain content and length were synthesized by graft-polymerization of (3-acrylamidopropyl)-trimethylammonium chloride onto pullulan in

aqueous solution, using potassium persulfate as an initiator. Their flocculation efficiency was studied in a clay suspension as a function of the grafted chains content and length as well as of some flocculation parameters: the polycation dose, the settling time, the parent solution concentration of polycation. All the polysaccharide samples showed flocculation ability: the higher the grafted cationic chain content and length the lower the amount of pullulan derivative was required for reaching the maximum clarity degree (of about 100%). This finding together with the negative value of the zeta potential and floc size measurements, at the optimum polymer dose, point to contributions from both neutralization and bridging mechanisms for flocculation process. The residual turbidity values also varied with the settling time and the parent solution concentration of polymer.

Keywords: Graft copolymer; Pullulan; Flocculation; Turbidimetry; Zeta potential

Shuhe Wei^a, Shanshan Wang^{a, b}, Qixing Zhou^a, Jie Zhan^c, Lihui Ma^d, Zhijie Wu^a, Tieheng Sun^a and M.N.V. Prasad^e. (^a Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, PR China, ^b Graduate School of Chinese Academy of Sciences, Beijing 100039, PR China, ^c Department of Biotechnology, Liaoning University of Traditional Chinese Medicine, Shenyang 110101, PR China, ^d Huayou Industrial Company, Liaohe Petroleum Exploration Bureau, Panjin 124010, PR China, ^e Department of Plant Sciences, University of Hyderabad, Hyderabad 500046, India). **Potential of *Taraxacum mongolicum* Hand-Mazz for accelerating phytoextraction of cadmium in combination with eco-friendly amendments. Journal of Hazardous Materials, Volume 181(1-3) (2010): 480-484**

Phytoextraction and phytostabilization are well-established sub-processes of phytoremediation that are being followed for *in situ* remediation of soils contaminated with toxic metals. *Taraxacum mongolicum* Hand-Mazz, a newly reported Cd accumulator has shown considerable potential for phytoextracting Cd. This paper investigated the effects of urea and chicken manure on *T. mongolicum* phytoextracting Cd from soil using pot culture experiments. The results showed that urea application did not affect the Cd concentrations in root, leaf, inflorescence and shoot of *T. mongolicum*, but chicken manure significantly decreased them ($p < 0.05$) by 23.5%, 31.5%, 24.8% and 30.4% owing to decreased extractable Cd. Urea and chicken manure significantly increased ($p < 0.05$) the phytoextraction capacities ($\mu\text{g pot}^{-1}$) of *T. mongolicum* to Cd by 3–5-fold due to the increase in shoot biomass (increased 4–7 folds). Further, addition of urea and chicken manure increased organic matter, nitrogen, phosphorus and potassium, the microorganism count, urease and phosphatase activities of soil indicating their eco-friendly function. Urea is ideal for optimizing phytoextraction of *T. mongolicum* to Cd, while chicken manure is appropriate for phytostabilization.

Keywords: Urea; Chicken manure; Phytoremediation; *Taraxacum mongolicum* Hand-Mazz; Cd (Cadmium)

Ping Li^a, Ya-Juan Su^a, Yan Wang^a, Bo Liu^a and Li-Ming Sun^a. (^a School of Life Science and Technology, Tongji University, Siping Road 1239#, Shanghai 200092, China). **Bioadsorption of methyl violet from aqueous solution onto Pu-erh tea powder. Journal of Hazardous Materials, Volume 179(1-3) (2010): 43-48**

Chinese unique Pu-erh tea powder (PTP), with leached active ingredients, was used here to adsorb methyl violet (MV), a cationic dye. The effects of several variables on the removal of

methyl violet were studied at 25 °C, including pH value, contact time, quantity of the adsorbent, initial concentration, and particle size of the adsorbent. The results showed that the particle size of the adsorbent significantly affected the adsorption process, and the nano-sized PTP particles had the best adsorption efficiency. The equilibrium data was analyzed using Langmuir, Freundlich, and Tempkin isotherms models. The pseudo-second-order kinetics model best explained the MV adsorption by PTP of any particle size. The intra-particle diffusion model was also used to analyze the adsorption process, and it was found that smaller adsorbent particles had a bigger boundary layer effect.

Keywords: Pu-erh tea powder; Methyl violet; Bioadsorption

Huifen Li^a, Yanbing Lin^a, Wumeng Guan^a, Jiali Chang^a, Lin Xu^a, Junkang Guo^a and Gehong Wei^a. (^a College of Life Sciences, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwest A & F University, Yangling, Shaanxi 712100, China). **Biosorption of Zn(II) by live and dead cells of *Streptomyces ciscaucasicus* strain CCNWHX 72-14. Journal of Hazardous Materials, Volume 179(1-3) (2010): 151-159**

The biosorption characteristics of Zn(II) using live and dead cells of *Streptomyces ciscaucasicus* strain CCNWHX 72-14 as biosorbents have been investigated in the present research. Optimum conditions for biosorption were determined to be: pH adjusted to 5.0, agitated at 90 rpm and at a dose of 2 g/L. For initial zinc concentrations of 1–150 mg/L, batch biosorption data of live biomass preferred to be simulated with Freundlich model while those of dead strain fit Langmuir isotherm well. Experimental maximum biosorption capacity turned out to be 42.75 mg/g (0.654 mmol/g) for living material and 54 mg/g (0.826 mmol/g) for dead sorbents, respectively. The pseudo-second-order equation, instead of the pseudo-first-order one, was chosen to describe the time course biosorption process. In contrast to live biosorbents, dead biomass seemed to have lower binding strength with higher desorption efficiency at pH 1.0. Competitive biosorption revealed the order of competing metal ion to be: $\text{Cu}^{2+} > \text{Cd}^{2+} > \text{Ni}^{+}$. FT-IR analysis indicated that more functional groups were involved in the biosorption process of dead adsorbents, compared with those linked to live biomass. Taken together, it can be concluded that dead cells of CCNWHX 72-14 were better and cheaper biosorbents than live ones.

Keywords: *Streptomyces*; Biosorption; Zinc; Live; Dead

Ying-Shuiian Shen^a, Shan-Li Wang^a, Shih-Tsuen Huang^{a, b}, Yu-Min Tzou^a and Jang-Hung Huang^a. (^a Department of Soil and Environmental Sciences, National Chung Hsing University, 250 Kuo Kuang Road, Taichung 40227, Taiwan, ^b Department of Science Application and Dissemination, National Taichung University, Taichung, Taiwan). **Biosorption of Cr(VI) by coconut coir: Spectroscopic investigation on the reaction mechanism of Cr(VI) with lignocellulosic material. Journal of Hazardous Materials, Volume 179(1-3) (2010): 160-165**

In this study, the removal mechanism of Cr(VI) from water by coconut coir (CC) was investigated using X-ray photoelectron spectroscopy (XPS), Cr K-edge X-ray absorption near edge structure (XANES) and FTIR spectroscopy. The results showed that, upon reaction with CC at pH 3, Cr(VI) was reduced to Cr(III), which was either bound to CC or released back into solution. As revealed by the FTIR spectra of CC before and after reacting with Cr(VI), the

phenolic methoxyl and hydroxyl groups of lignin in CC are the dominant drivers of Cr(VI) reduction, giving rise to carbonyl and carboxyl groups on CC. These functional groups can subsequently provide binding sites for Cr(III) resulting from Cr(VI) reduction. In conjunction with forming complexes with carbonyl and carboxyl groups, the formation of Cr(III) hydroxide precipitate could also readily occur as revealed by the linear combination fitting of the Cr *K*-edge XANES spectrum using a set of reference compounds. The phenolic groups in lignin are responsible for initiating Cr(VI) reduction, so lignocellulosic materials containing a higher amount of phenolic groups are expected to be more effective scavengers for removal of Cr(VI) from the environment.

Keywords: Lignocellulose; Hexavalent chromium; Reduction; Sorption; Trivalent chromium; Surface precipitation

Changzhou Yan^a, Guoxin Li^a, Peiying Xue^a, Qunshan Wei^a and Qingzhao Li^a. (^a Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China). **Competitive effect of Cu(II) and Zn(II) on the biosorption of lead(II) by *Myriophyllum spicatum*. Journal of Hazardous Materials, Volume 179(1-3) (2010): 721-728**

Batch experiments were conducted to assess the effects of Cu(II) and Zn(II) on the biosorption of Pb(II) ions by fresh tissues of *Myriophyllum spicatum*. The biosorption of Pb(II) was examined for single, binary and ternary solutions at different initial concentrations and different pH values. The experimental results showed that the biosorption capacity increased with increasing pH from 2.0 to 6.0. Both Cu(II) and Zn(II) ions were found to have an adverse effect on the biosorption of Pb(II). The biosorption equilibrium data for single-metal solution were fitted to three isotherm models: Langmuir, Freundlich and Sips, and the Sips isotherm model gave the best fit for the experimental data. The maximum biosorption of Pb(II) in Pb–Cu binary system decreased with increasing concentration of copper ions, and the biosorption equilibrium data for the binary metal solution fitted the Langmuir competitive model well. Comparison between biosorption of Pb(II) and Cu(II) by *M. spicatum* in the binary solution could lead to the conclusion that the biosorbent (*M. spicatum*) has no preference of Pb(II) over Cu(II). Fourier transform infrared (FT-IR) spectroscopy was used to characterize the interaction between *M. spicatum* and Pb(II) ions. The results revealed that the carboxyl, hydroxyl and carbonyl groups are the main binding sites for Pb(II).

Keywords: Biosorption; Competitive biosorption; Lead; Aquatic plant; Langmuir competitive model

Biotransformation

Johann F. Osma^a, José L. Toca-Herrera^b and Susana Rodríguez-Couto^{c, d}. (^a Departament d'Enginyeria Química, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Spain, ^b Biosurfaces Unit, CICBiomaGUNE. Paseo Miramón 182, 20009 San Sebastian, Spain, ^c CEIT, Unit of Environmental Engineering, Paseo Manuel de Lardizábal 15, 20018 San Sebastian, Spain, ^d IKERBASQUE, Basque Foundation for Science, Alameda de Urquijo 36, 48011 Bilbao, Spain). **Transformation pathway of Remazol**

Brilliant Blue R by immobilised laccase. Bioresource Technology, Volume 101(22) (2010): 8509-8514

This study deals with the biotransformation products obtained from the transformation of the anthraquinonic dye Remazol Brilliant Blue R (RBBR) by immobilised laccase from the white-rot fungus *Trametes pubescens*. A decolouration percentage of 44% was obtained in 42 h. RBBR transformation products were investigated using ultraviolet–visible (UV–vis) spectrum scan and High Performance Liquid Chromatography/Mass Spectrometry (LC–MS) analysis. Two compounds were identified as the transformation intermediates (m/z 304.29 and m/z 342.24) and other two as the final transformation products (m/z 343.29 and m/z 207.16). As a result a metabolic pathway for RBBR transformation by laccase was proposed. No backward polymerisation of the transformation products resulting in recurrent colouration was observed after laccase treatment of RBBR. It was also found that the biotransformation products of RBBR showed less phytotoxicity than the dye itself.

Keywords: Remazol Brilliant Blue R; Biotransformation; Laccase; Immobilisation; *Trametes pubescens*

Li Ye^{a,1}, Chao-Qun Zhou^{a,1}, Wei Zhou^b, Pei Zhou^a, Dao-Feng Chen^a, Xiao-Hui Liu^a, Xun-Long Shi^a and Mei-Qing Feng^a. (^a School of Pharmacy, Fudan University, 826 Zhangheng Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China, ^b Zhejiang Hisun Pharmaceutical Co., Ltd., Taizhou 318000, China). **Biotransformation of ginsenoside Rb1 to ginsenoside Rd by highly substrate-tolerant *Paecilomyces bainier* 229-7. Bioresource Technology, Volume 101(20) (2010): 7872-787**

Paecilomyces bainier 229-7 was obtained after UV irradiation for 8 min in the presence of 0.4% LiCl and selection on potato dextrose media containing 30 mg/mL saponin from *Panax notoginseng* leaves (SPNL). The mutant produces ginsenoside Rd from ginsenoside Rb1 with a bioconversion rate as high as 94.9% under optimized culture conditions in shake flasks when supplied with 20 mg/mL of SPNL. Scale-up in 10-L fermenter resulted in an 89% bioconversion rate. Ginsenoside Rd was purified from the culture medium by a macroporous resin with a chromatographic purity of 92.6%. These results suggest that *P. bainier* 229-7 could be useful for the preparation of ginsenoside Rd in the pharmaceutical industry.

Keywords: Biotransformation; Ginsenoside Rd; *Paecilomyces bainier*; Scale-up fermentation; Purification

Lin Yang, Chunyan Yan, Jianhua Zhu, Liyan Song and Rongmin Yu. Biotransformation of eugenol by suspension cultures of transgenic crown galls of *Panax quinquefolium* and suspension cultures of *Nicotiana tabacum*. World Journal of Microbiology and Biotechnology, Volume 26(7) (2010): 1201-1205

The biocatalytic ability of transgenic crown galls of *Panax quinquefolium* was evaluated by using eugenol (**1**) as a substrate and suspension cultures of *Nicotiana tabacum* as control system. Three biotransformed products, namely: 2-methoxy-4-(2-propenyl)phenyl-*O*- β -D-glucopyranoside (**2**, 67.11%), 2-methoxy-4-(2-propenyl)phenyl-*O*- β -D-glucopyranosyl (6' \rightarrow 1'')- β -D-xylopyranoside (**3**, 2.85%) and methyl eugenol (**4**, 14.30%) were obtained after

5 days of administration of eugenol to the suspension cultures of transgenic crown galls of *P. quinquefolium*. In contrast, only one product, compound **2** (15.41%), was obtained in suspension cultures of *N. tabacum* after 5 days of incubation. The results indicated that the glycosylation ability of transgenic crown galls of *P. quinquefolium* was much higher than that of the cultured cells of *N. tabacum*.

Keywords: Biotransformation - Transgenic crown galls of *Panax quinquefolium* - *Nicotiana tabacum* - Eugenol - Suspension cultures

Takayuki Hata, Shingo Kawai, Hideo Okamura and Tomoaki Nishida. Removal of diclofenac and mefenamic acid by the white rot fungus *Phanerochaete sordida* YK-624 and identification of their metabolites after fungal transformation. Biodegradation, Volume 21(5) (2010): 681-689

The non-steroidal anti-inflammatory drugs diclofenac (DCF) and mefenamic acid (MFA) were treated with the white rot fungus *Phanerochaete sordida* YK-624. DCF completely disappeared and MFA decreased by about 90% after 6 days of treatment. It was also confirmed that the fungus almost completely removed the acute lethal toxicity of DCF and MFA towards the freshwater crustacean *Thamnocephalus platyurus* after 6 days of treatment. Mass spectrometric and ¹H nuclear magnetic resonance analyses demonstrated that two mono-hydroxylated DCFs (4'-hydroxydiclofenac and 5-hydroxydiclofenac) and one di-hydroxylated DCF (4',5-dihydroxydiclofenac) were formed via fungal transformation. The four metabolites of MFA were identified as 3'-hydroxymethylmefenamic acid (mono-hydroxylated MFA), 3'-hydroxymethyl-5-hydroxymefenamic acid (di-hydroxylated MFA), 3'-hydroxymethyl-6'-hydroxymefenamic acid (di-hydroxylated MFA) and 3'-carboxymefenamic acid. These results suggest that hydroxylation catalyzed by cytochrome P450 (CYP) in *P. sordida* YK-624 may be involved in the elimination and detoxification of DCF and MFA. This notion was further supported by the fact that smaller decreases in DCF and MFA were observed in cultures of *P. sordida* YK-624 incubated with 1-aminobenzotriazole, a known inhibitor of CYP.

Keywords: Diclofenac - Mefenamic acid - Hydroxylation - Cytochrome P450 - White rot fungus

Suman Patel, Rashmi Gaur, Priyanka Verma, Rajendra S. Bhakuni and Archana Mathur. Biotransformation of artemisinin using cell suspension cultures of *Catharanthus roseus* (L.) G.Don and *Lavandula officinalis* L. Biotechnology Letters, Volume 32(8) (2010): 1167-1171

Artemisinin, an antimalarial compound, at 5 mg/40 ml, was transformed by cell suspension cultures of *Catharanthus roseus* (L.) G.Don and *Lavandula officinalis* L. into deoxyartemisinin with yields >78% (3.93 mg deoxyartemisinin from 5 mg artemisinin). Maximum conversion (78.6 and 78%) occurred after 6 and 7 days of adding artemisinin to 20 and 9 days old cultures of *C. roseus* and *L. officinalis*, respectively. The procedure was scaled up by and 500 mg artemisinin was transformed into 390 mg deoxyartemisinin. Addition of artemisinin at the beginning of the culture cycle resulted in >50% reduction in dry biomass production with no bioconversion. Conversion of artemisinin occurred intracellularly followed by leaching of the product into the medium.

Keywords: Artemisinin - Bioconversion - *Catharanthus roseus* - Cell suspension cultures - Deoxyartemisinin - *Lavandula officinalis*

K. Hanumantha Rao^a, A. Vilinska^a and I.V. Chernyshova^b. (^a Division of Mineral Processing, Department of Chemical Engineering and Geosciences, Luleå University of Technology, SE-971 87 LULEÅ, Sweden, ^b Department of Earth and Environmental Engineering, Henry Krumb School of Mines, Columbia University, NY 10027, USA). *Hydrometallurgy*, Volume 104(3-4) (2010): 465-470

Microorganisms have a tremendous influence on their environment through the transfer of energy, charge, and materials across a complex biotic mineral–solution interface. The biomodification of mineral surfaces involves the complex action of microorganism on the mineral surface. The manner, in which bacteria affect the surface reactivity and the mechanism of bacteria adsorption, is still unknown and accumulation of the primary data in this area is only starting. The bioflotation and bioflocculation processes concern the mineral response to the bacterium presence, which is essentially an interplay between microorganism and the physicochemical properties of the mineral surface, such as the atomic and electronic structures, the net charge/potential, the acid–base properties, and the wettability of the surface. There is an urgent need for developing basic knowledge that would underpin biotechnological innovations in the natural resource (re)processing technologies that deliver competitive solutions.

Research highlights

► Bioflotation and bioflocculation of metal sulfides and oxides. ► Interface science at mineral-bacteria interfaces contacted with aqueous solutions. ► Redox reactions of mineral surfaces in the presence of bacteria. ► In-situ spectroscopic measurements at mineral-bacteria-solution interfaces.

Keywords: Microorganisms; Oxide and sulfide minerals; Flotation; Flocculation

Eun-Ju Kim, Jong-Rok Jeon, Young-Mo Kim, Kumarasamy Murugesan and Yoon-Seok Chang. *Mineralization and transformation of monofluorophenols by *Pseudonocardia benzenivorans**. *Applied Microbiology and Biotechnology*, Volume 87(4) (2010): 1569-1577

The aerobic metabolism of monofluorophenols (mono-FPs) by the actinomycete, *Pseudonocardia benzenivorans*, was studied. This strain was able to grow on 4-fluorophenol (4-FP) and readily transform 2- and 3-fluorophenol to the corresponding metabolites. The detailed mechanism of mono-FPs degradation by *P. benzenivorans* was elucidated from enzymatic assays and the identification of reaction intermediates by high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry. Two types of fluorocatechols (i.e., 3- and 4-fluorocatechol) were identified as the key transformation products. During 4-FP degradation, only 4-fluorocatechol was detected, and a stoichiometric level of fluoride was released. Both fluorocatechols were observed together in cultures containing 3-fluorophenol (3-FP), while only 3-fluorocatechol was found to accumulate in 2-fluorophenol (2-FP)-containing cultures. Whole-cell extracts of *P. benzenivorans* expressed catechol 1,2-dioxygenase activity, indicating that the transformation of the three tested mono-FPs proceeded via *ortho*-cleavage pathway. The results presented in this paper provide comprehensive information regarding the metabolism of mono-FPs by a single bacterium.

Keywords: Biodegradation - Monofluorophenols - 4-Fluorophenol -
benzenivorans

Pseudonocardia

C.R. Rekha, G. Vijayalakshmi. Bioconversion of isoflavone glycosides to aglycones, mineral bioavailability and vitamin B complex in fermented soymilk by probiotic bacteria and yeast. Journal of Applied Microbiology. Volume 109(4) (2010): 1198–1208

Aim: To study the role of β -glucosidase producing probiotic bacteria and yeast in the biotransformation of isoflavone glycosides to aglycones, mineral bioavailability and vitamin B complex in fermented soymilk.

Methods and Results: Five isolates of probiotic lactic acid bacteria (LAB), *Lactobacillus acidophilus* B4496, *Lactobacillus bulgaricus* CFR2028, *Lactobacillus casei* B1922, *Lactobacillus plantarum* B4495 and *Lactobacillus fermentum* B4655 with yeast *Saccharomyces boulardii* were used to ferment soymilk to obtain the bioactive isoflavones, genistein and daidzein. High-performance liquid chromatography was used to analyse the concentration of isoflavones. Bioactive aglycones genistein and daidzein after 24 and 48 h of fermentation ranged from 97.49 to 98.49% and 62.71 to 92.31% respectively with different combinations of LAB with yeast. Increase in bioavailability of minerals and vitamin B complex were also observed in fermented soymilk.

Conclusions: LAB in combination with yeast *S. boulardii* has great potential for the enrichment of bioactive isoflavones, enhancing the viability of LAB strains, decreasing the antinutrient phytic acid and increasing the mineral bioavailability in soymilk fermentation.

Significance and Impact of the Study: Fermentation of soymilk with probiotic organisms improves the bioavailability of isoflavones, assists in digestion of protein, provides more soluble calcium, enhances intestinal health and supports immune system. Increased isoflavone aglycone content in fermented soymilk improves the biological functionality of soymilk.

Keywords: β -glucosidase; isoflavones; *Lactobacillus*; *Saccharomyces boulardii*; soymilk fermentation; starter cultures

Maria C. Hansson^{a, b, 1}, Maria E. Persson^{b, 1}, Per Larsson^c and Torbjörn von Schantz^d. (a)Department of Environmental Science, Ecology Building, Lund University, SE-223 62 Lund, Sweden, (b)Department of Ecology, Section for Chemical Ecology and Ecotoxicology, Ecology Building, Lund University, SE-223 62 Lund, Sweden, (c)Department of Aquatic Ecology, University of Kalmar, SE-39182 Kalmar, Sweden, (d)Department of Ecology, Section for Animal Ecology, Ecology Building, Lund University, SE-223 62 Lund, Sweden). Polychlorinated biphenyl (PCB) load, lipid reserves and biotransformation activity in migrating Atlantic salmon from River Mörrum, Sweden. Environmental Pollution, Volume 157(12) (2009): 3396-3403

Atlantic salmon accumulate high levels of contaminants such as polychlorinated biphenyls (PCBs) in their lipids during the adult growth phase spent at sea. The lipids are later utilized during migration for swimming and biological adaptations. We hypothesize that migrating salmon's biotransformation processes are affected by the high levels of built-up PCBs compared to salmon that in a pre-migrational stage. For these analyses we sampled adult Atlantic salmon during migration in the Swedish River Mörrum and measured the 21 most common PCB

congeners (Σ PCB) and lipid levels in muscle tissue, aryl hydrocarbon receptor (AHR2) and cytochrome P4501A1 (CYP1A1) transcript levels as well as ethoxyresorufin-O-deethylase activity (EROD) in liver. We also determined which AHR2 genotypes the salmon carried. We show that EROD activity is correlated to CYP1A1 level but not to Σ PCB concentration. Σ PCB concentration does not predict levels of neither the AHR2 nor CYP1A1 genes. We find no associations between specific AHR2 transcription levels and AHR2 genotypes or a correlation between AHR2 and CYP1A1 transcription levels, which is in direct contrast to pre-migrational adult salmon from the Baltic Sea. When we compare River Mörrum to salmon we have previously sampled in the Baltic Sea we show that migrating salmon have significantly lower lipid levels in their muscles; higher muscle concentrations of Σ PCB on a lipid basis; and significantly lower CYP1A1 and EROD levels compared to salmon from the Baltic Sea. Also, transcript levels of three out of four AHR2 genes are significantly different. In conclusion, migrating Swedish Atlantic salmon carry higher concentrations of PCBs in their lipids compared to salmon in the Baltic Sea, but have lower activation of biotransformation genes and enzymes. Our results indicate that accumulated pollutants from the Baltic Sea are deactivated inside the migrating salmon's lipid tissues and increase in concentration when migration is initiated thereby limiting their impact on biotransformation processes.

Migrating Atlantic salmon in the Swedish River Mörrum carry high PCB concentrations in their lipids but have low activation of biotransformation enzymes in the liver compared with salmon from the Baltic Sea

Keywords: Atlantic salmon; Migration; PCB; CYP1A1; AHR

Juliane Ventura-Lima^{a, b}, Daniele Fattorini^c, Francesco Regoli^c and José M. Monserrat^{a, b}. (^aInstituto de Ciências Biológicas, Universidade Federal do Rio Grande – FURG, Rio Grande, RS, Brazil, ^bPrograma de Pós-Graduação em Ciências Fisiológicas – Fisiologia Animal Comparada (FURG), Rio Grande, RS, Brazil, ^cIstituto di Biologia e Genetica, Università Politecnica delle Marche, 60100, Ancona, Italy). **Effects of different inorganic arsenic species in *Cyprinus carpio* (Cyprinidae) tissues after short-time exposure: Bioaccumulation, biotransformation and biological responses. Environmental Pollution, Volume 157(12) (2009): 3479-3484**

Differences in the toxicological and metabolic pathway of inorganic arsenic compounds are largely unknown for aquatic species. In the present study the effects of short-time and acute exposure to As^{III} and As^V were investigated in gills and liver of the common carp, *Cyprinus carpio* (Cyprinidae), measuring accumulation and chemical speciation of arsenic, and the activity of glutathione-S-transferase omega (GST Ω), the rate limiting enzyme in biotransformation of inorganic arsenic. Oxidative biomarkers included antioxidant defenses (total glutathione-S-transferases, glutathione reductase, glutathione, and glucose-6-phosphate dehydrogenase), total scavenging capacity toward peroxyl radicals, reactive oxygen species (ROS) measurement and lipid peroxidation products. A marked accumulation of arsenic was observed only in gills of carps exposed to 1000 ppb As^V. Also in gills, antioxidant responses were mostly modulated through a significant induction of glucose-6-phosphate dehydrogenase activity which probably contributed to reduce ROS formation; however this increase was not sufficient to prevent lipid peroxidation. No changes in metal content were measured in liver of exposed carps, characterized by lower activity of GST Ω compared to gills. On the other hand,

glutathione metabolism was more sensitive in liver tissue, where a significant inhibition of glutathione reductase was concomitant with increased levels of glutathione and higher total antioxidant capacity toward peroxy radicals, thus preventing lipid peroxidation and ROS production. The overall results of this study indicated that exposure of *C. carpio* to As^{III} and As^V can induce different responses in gills and liver of this aquatic organism.

Common carp (*Cyprinus carpio*) presented marked differences between gills and liver after arsenic exposure in terms of antioxidant responses and also in biotransformation.

Keywords: Arsenate; Arsenite; Carp; Glutathione-S-transferase omega; Antioxidant system

Ana Novo and Célia M. Manaia. Factors influencing antibiotic resistance burden in municipal wastewater treatment plants. *Applied Microbiology and Biotechnology*, Volume 87(3) (2010): 1157-1166

Municipal wastewater treatment plants are recognized reservoirs of antibiotic-resistant bacteria. Three municipal wastewater treatment plants differing on the dimensions and bio-treatment processes were compared for the loads of amoxicillin-, tetracycline-, and ciprofloxacin-resistant heterotrophic bacteria, enterobacteria, and enterococci in the raw inflow and in the treated effluents. The sewage received by each plant, in average, corresponded to 85,000 inhabitant equivalents (IE), including pretreated industrial effluents ($\leq 30\%$) in plant activated sludge, 105,000 IE, including pretreated hospital effluents ($\leq 15\%$) in plant trickling filter, and 2,000 IE, exclusively of domestic sewage, in plant submerged aerated filter. The presence of pretreated industrial effluents or of pretreated hospital sewage in the raw inflow did not imply significantly higher densities (per milliliter or per IE) of antibiotic-resistant bacteria in the raw wastewater. Longer hydraulic residence periods (24 h) corresponded to higher bacterial removal rates than shorter periods (12 and 9 h), although such efficiency did not imply significant average decreases in the antibiotic resistance prevalence of the treated effluent. The bacterial loads in the treated effluent could be ranked according to the treatment efficiency, suggesting that the characteristics of the raw inflow may have less relevance on the quality of the treated wastewater than other aspects, such as the inflow volume, the type of biological treatment, or the hydraulic residence time.

Keywords: Wastewater treatment - Antibiotic resistance - Amoxicillin - Tetracycline - Ciprofloxacin

Biomarker

J. W. Summers, K. F. Gaines, N. Garvin, W. L. Stephens, J. C. Cumbee and G. L. Mills. Feathers as bioindicators of PCB exposure in clapper rails. *Ecotoxicology*, Volume 19(6) (2010): 1003-1011

In this study we used feathers to biomonitor exposure to the polychlorinated biphenyl (PCB) Aroclor 1268 congener mixture in clapper rails (*Rallus longirostris*). This species has been used as an indicator species of environmental damage for the LCP superfund site located in Brunswick, GA, USA which is contaminated with Aroclor 1268, a congener mixture that has been used in limited amounts elsewhere and therefore can be used as a contaminant marker. The Aroclor 1268 congener mixture, including congener profiles, were quantified in feathers using

gas chromatography (GC). Concurrently, each sample was quantified for the total Aroclor 1268 congener mixture using an enzyme-linked immunosorbent assay (ELISA) and compared to the GC results to determine if ELISA was an efficient method for quantifying or qualifying PCBs in feathers. ELISA consistently quantified PCB loads over an order of magnitude lower than the GC. Based on sample replication, extraction recovery, and sample spike, it appears that GC is the more reliable method of detection and that ELISA methods may be more suitable for qualitative exposure assessment for this particular Aroclor. Moreover, since all clapper rails from the LCP site had the Aroclor 1268 congener mixture in their feathers, this experiment showed that birds were returning to the site to breed despite the adverse effects experienced by this population from the contamination revealed in previous studies. This study also supports the utility of feathers as a non-lethal mechanism by which to biomonitor PCBs in the environment.

Keywords: Aroclor 1268 - ELISA - Clapper rail - Organochlorine - LCP – PCBs

Ruey-an Doong^a, Pin-Shang Lee^a and Kowthalam Anitha^a. (^a 101, Sec. 2, Kuang Fu Road, Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu 30013, Taiwan). Simultaneous determination of biomarkers for Alzheimer's disease using sol-gel-derived optical array biosensor. *Biosensors and Bioelectronics*, Volume 25(11) (2010): 2464-2469

The sol-gel-derived optical array-based biosensor for the simultaneous analysis of multiple analytes as potential markers for Alzheimer's disease was fabricated. β -Amyloid, acetylcholine and glutamate were selected as the biosensing probes. The fluorescent dye, carboxy SNARF-1-dextran, was co-immobilized with glutamate dehydrogenase and acetylcholinesterase for sensing glutamate and acetylcholine, respectively, while Amplex red and FITC-dextran were immobilized with horseradish peroxidase for determination of β -amyloid and hydrogen peroxide (H_2O_2). The biosensors exhibited a good performance on simultaneous analysis of multianalytes without obvious cross-interference and the detection limits of H_2O_2 , β -amyloid, glutamate and acetylcholine were 1.57 nM, 0.63 nM, 0.55 μ M, and 1.0 μ M, respectively. The developed array biosensors were also applied to determine multianalytes in human serum samples spiked with various concentrations of analytes and showed a good analytical performance with dynamic range of 4 orders of magnitude for β -amyloid. Results obtained in this study clearly demonstrate the possibility of using a sol-gel-derived optical array-based biosensor for simultaneous analysis of multiple samples in the presence of important analytes for Alzheimer's disease.

Keywords: Array-based biosensor; Sol-gel; Alzheimer's disease (AD); Fluorescent dye; β -Amyloid; Multianalytes

Biofertilizer

I. Aviani^{a, b}, Y. Laor^b, Sh. Medina^a, A. Krassnovsky^a and M. Raviv^a. (^a Agricultural Research Organization, Institute of Plant Sciences, Neve Ya'ar Research Center, Ramat Yishay 30095, Israel, ^b Agricultural Research Organization, Institute of Soil, Water and Environmental Sciences, Neve Ya'ar Research Center, Ramat Yishay 30095, Israel). Co-composting of solid and liquid olive mill wastes: Management aspects and the horticultural

value of the resulting composts. Bioresource Technology, Volume 101(17) (2010) : 6699-6706

Successful co-composting of solid and liquid olive mill wastes (OMW) and obtaining a product of horticultural value may increase the viability of this recycling approach. Two composting cycles were performed, in which olive mill solid wastes (OMSW) were used to form five mixtures, wetted either with fresh water or with olive mill wastewater (OMWW). Up to ~ 0.3 m³ of OMWW could be applied to each m³ of the raw materials without negatively affecting the chemical, physical and horticultural properties of the resulted composts. A growing media composed of perlite amended with 25–33% OMW-composts showed higher suppressiveness against *Fusarium oxysporum* f. sp. *melonis* as compared to equivalent perlite:peat moss mixtures. The yields of tomato plants grown in peat moss amended with 20% (v:v) of OMW-composts were not significantly different than plants grown in unamended peat. The viability of co-composting as a treatment approach for OMWW is discussed in the context of management aspects and the horticultural value of the final product.

Keywords: Compost; *Fusarium oxysporum*; Olive mill waste; Phytotoxicity; Suppressiveness

Rahul Kumar^a, Deepshikha Verma^a, Bhanu L. Singh^a, Umesh Kumar^a and Shweta^a. (^a Vermiculture Research Station, D.S. College (Dr. B.R.A. University), Aligarh 202 001, UP, India). **Composting of sugar-cane waste by-products through treatment with microorganisms and subsequent vermicomposting. Bioresource Technology, Volume 101(17) (2010): 6707-6711**

The waste by-products of the sugar-cane industry, bagasse (*b*), pressmud (*p*) and trash (*t*) have been subjected to bioinoculation followed by vermicomposting to shorten stabilization time and improve product quality. Press-mud alone and in combination with other by-products of sugar processing industries was pre-decomposed for 30 days by inoculation with combination of *Pleurotus sajorcaju*, *Trichoderma viridae*, *Aspergillus niger* and *Pseudomonas striatum*. This treatment was followed by vermicomposting for 40 days with the native earthworm, *Drawida willsi*. The combination of both treatments reduced the overall time required for composting to 20 days and accelerated the degradation process of waste by-products of sugar processing industry, thereby producing a nutrient-enriched compost product useful for sustaining high crop yield, minimizing soil depletion and value added disposal of waste materials.

Keywords: Bioinoculants; *Drawida willsi*; Vermicompost; Sugar-cane industries; Integrated system of composting

M. A. Baset Mia² and Z. H. Shamsuddin^{1*}. (¹Department of Land Management, Faculty of Agriculture, University Putra Malaysia, 43400 UPM, Serdang, Selangor DE, Malaysia, ²Department of Crop Botany, Faculty of Agriculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh. *Corresponding author. E-mail: zulsham@agri.upm.edu.my). **Rhizobium as a crop enhancer and biofertilizer for increased cereal production. African Journal of Biotechnology Vol. 9 (37) (2010): 6001-6009**

Greater production of cereals brings forth higher production cost and pollutes the soil environment due to excessive use of chemical fertilizers. Therefore, crop scientists are exploring an alternative source namely biofertilizers which are cost effective and environment friendly. In

the biofertilizer technology, *Rhizobium*-legume is most common and widely used in different countries. Recently, it is also found that rhizobia can make an association with graminaceous plants such as rice, wheat, maize, barley millets and other cereals some time as endophytic without forming any nodule-like structure or causing any disease symptoms. Increasing the ability of rhizobia in biofertilizer, crop enhancing activity in non-legumes especially cereal grains would be a useful technology for increased crop yields among resource-poor farmers. Recent findings showed both more crop enhancing and biofertilizer attributes in cereal crops due to rhizobial inoculation. In addition, plant nutrients like P, K, Ca, Mg and even Fe accumulation were also observed. Therefore, further research in this area will be able to develop a sustainable biofertilizer technology for greater and environment friendly cereal production system.

Keywords: Biofertilizer, cereal, crop enhancer, *Rhizobium*, yield.

Abbreviations: BNF, Biological nitrogen fixation; LCO, lipo-chito-oligosaccharide; N₂, nitrogen; IAA, indole acetic acid; PGPB, plant growth promoting bacteria; GC-MS, gas chromatography- mass spectrometer.

Biocomposting

Manuel J. Fernández-Gómez a, Esperanza Romeroa and Rogelio Nogalesa. (^a Department of Environmental Protection, Estación Experimental del Zaidín (EEZ), CSIC, Profesor Albareda 1, 18008 Granada, Spain). **Feasibility of vermicomposting for vegetable greenhouse waste recycling. Bioresource Technology, Volume 101(24) (2010): 9654-9660**

This study was conducted in order to evaluate the feasibility of *Eisenia andrei* for vermicomposting heterogeneous-plant (HP), tomato-plant (P), and damaged tomato-fruit (T) greenhouse vegetable wastes. Earthworm growth and reproduction were monitored over a 12-week period, and variations in chemical parameters, enzyme activity, phytotoxicity test, and genetic fingerprinting of bacterial communities were evaluated. While high rates of salinity prevented earthworm survival in HP and P (>10 dS m⁻¹), T was vermicomposted recording an adequate earthworm growth and cocoon production. The latter waste was successfully stabilized, as indicated by the significant decrease in its TOC content (~13–26%) and C:N ratio (~16–36%) and its high germination indices (~39–72%). The similar enzyme activities levels and bacterial community fingerprintings recorded in diverse vermicomposts obtained from T waste indicate that this type of waste favoured the existence of analogous bacterial communities responsible for the high degree of stabilization and maturity detected.

Keywords: *Eisenia andrei*; Enzyme activity; Phytotoxicity test; Genetic fingerprinting; PCR-DGGE

Robert Sidelko^a, Beata Janowska^a, Bartosz Walendzik^a and Izabela Siebielska^a. (^a Technical University of Koszalin, ul. Sniadeckich 2, 75-453 Koszalin, Poland). **Two composting phases running in different process conditions timing relationship. Bioresource Technology, Volume 101(17) (2010): 6692-6698**

This research work pertained to establishment of timing relationship for two sludge composting phases proceeding in different conditions. The first phase was performed in a dynamical reactor and the second – in periodically turned windrows. The research was carried out in three independent repetitions with five test series cycles in each varying in the hot phase timing. The compost windrows ripening phase was monitored for 5 months. The determined physical and chemical indicators (independent variables) made a basis for calculation of compost ripeness indices. To perform the numerical analysis an artificial neural network performing the generalised regression task was used. Using specific values of particular indices, as the ripeness criterion, a $t_d = f(t_p)$ model was created, in form of decreasing linear function, interpreting the relationship between time of compost keeping in the reactor and the time of compost windrow ripening. The obtained results suggest that keeping the batch in reactor for a period in excess of 18 days is groundless, therefore, ineffective as it does not shorten significantly the compost ripening phase.

Keywords: Composting time; Ripeness indices; Sewage sludge; Neural network

Ferial M. Rashad^a, , Walid D. Saleh^a and Mohamed A. Moselhy^a. (^a Dept. of Microbiology, Faculty of Agriculture, Cairo Univ., Giza 12613, Egypt). Bioconversion of rice straw and certain agro-industrial wastes to amendments for organic farming systems: 1. Composting, quality, stability and maturity indices. *Bioresource Technology*, Volume 101(15) (2010): 5952-5960

The microbiological and physicochemical parameters were monitored for 12 weeks during composting of five piles ($1.5 \times 1.0 \times 0.80 \text{ m}^3$) containing mainly rice straw, soybean residue and enriched with rock phosphate. Two treatments were inoculated with buffalo's manure or composite inoculum, two were supplemented with vinasse and inoculated with either the composite inoculum or with both, the last one was served as a control. Four typical phases of composting were observed during the bioprocess: short initial mesophilic phase followed by, thermophilic, cooling and maturation phases. Physicochemical changes confirmed the succession of microbial populations depending on the temperature of each phase in all treatments. Intense microbial activities led to organic matter mineralization and simultaneously narrow C/N ratios. Inoculation of composting mixtures enhanced the biodegradation of recalcitrant substances. The duration of exposure to a temperature above 55 °C for at least 16 consecutive days was quite enough to sanitize the produced composts. After 84 days, all composts reached maturity as indicated by various parameters.

Keywords: Composting; Rice straw; Rock phosphate; Microbial population; Maturity indices

Biopesticides

Michelle E. DePrenger-Levin^a, Thomas A. Grant III^b and Carol Dawson^c. (^a Denver Botanic Gardens, Research and Conservation Department, USA, ^b Colorado State University, Graduate Degree Program in Ecology (GDPE) and the Department of Forestry, Rangeland and Watershed Science, USA, ^c US Bureau of Land Management (BLM) – Colorado State Office, USA). Impacts of the introduced biocontrol agent, *Rhinocyllus conicus* (Coleoptera: Curculionidae), on the seed production and population dynamics of

***Cirsium ownbeyi* (Asteraceae), a rare, native thistle. Biological Control, Volume 55(2) (2010): 79-84**

The release of non-native insects to control noxious weeds is commonly used to combat invasions without disturbing the environment through chemical or mechanical methods. However, introduced biological control agents can have unintended effects. This study was initiated to evaluate potential non-target effects of the flowerhead weevil, *Rhinocyllus conicus* Frölich, on *Cirsium ownbeyi* S.L. Welsh, a rare, native and short-lived perennial thistle in northwestern Colorado, northeastern Utah, and southwestern Wyoming. *C. ownbeyi* represents one of 22 known native hosts on which this introduced weevil has naturalized. The study population remained stable over the eight years of the study despite floral damage by the biocontrol beetle. The growth rate (λ) from a count-based population viability analysis of the population was 1.03; however, large inter-year variation indicates this rare species is still vulnerable to local extirpation. The biocontrol weevil consistently damaged the developing seeds over the course of the study independent of changes in overall population size and variation in the number of flowering individuals. The target species, *Carduus nutans* L. (musk thistle) is generally absent near the study plots, which may limit the population levels of *R. conicus* that can be sustained in this area. Although *R. conicus* utilizes *C. ownbeyi* as a host plant, the late flowering period of this native thistle and the small size of the flower heads may limit the demographic impact of *R. conicus* on *C. ownbeyi*.

Keywords: *Carduus nutans*; *Cirsium ownbeyi*; Colorado; Native plants; Non-target effects; *Rhinocyllus conicus*

Saki Shimoi^a, Kanako Inoue^a, Hiroko Kitagawa^a, Masanori Yamasaki^b, Seiya Tsushima^c, Pyoyun Park^a and Kenichi Ikeda^a. (^a Laboratory of Stress Cytology, Graduate School of Agricultural Science, Kobe University, Nada, Kobe 657-8501, Japan, ^b Food Resources Education and Research Center, Graduate School of Agricultural Science, Kobe University, 1348 Uzurano, Kasai, Hyogo 675-2103, Japan, ^c Natural Resources Inventory Center, National Institute for Agro-Environmental Sciences, 3-1-3, Kannondai, Tsukuba, Ibaraki 305-8604, Japan). **Biological control for rice blast disease by employing detachment action with gelatinolytic bacteria. Biological Control, Volume 55(2) (2010): 85-91**

We screened collagenolytic/gelatinolytic bacteria from rice leaves and soil which degraded the fungal extracellular matrix to establish a novel biological control measure inhibiting germling adhesion on the host plant surface against airborne fungal diseases such as rice blast disease *Magnaporthe oryzae* B. Coch. Two different screening methods, i.e., screening from the leaf-associated bacterial library and direct screening from leaf and soil with or without collagen incubation, were conducted. Screening from the collagen treated material resulted in a higher number of gelatinolytic isolates than without collagen treatment. The selected bacteria were identified as *Acidovorax*, *Sphingomonas*, *Chryseobacterium*, and *Pseudomonas* genera by 16S rDNA sequence. Based on treatment with EDTA and addition of divalent cations, four of the five screened isolates tested produced a metalloproteinase. Furthermore, the enzymes produced by *Acidovorax* and *Sphingomonas* sp. were categorized as calcium-dependent metalloproteinases, and the enzymes produced by *Chryseobacterium* sp. were categorized as calcium/zinc-dependent metalloproteinases. The screened bacterial culture showed inhibitory

effects on spore adhesion on the plastic cover glass, and disease protective effects on rice. This study suggests that bacteria inhibiting germling adhesion by phytopathogenic fungi may have promise as a biological agent.

Keywords: Biological control; *Magnaporthe oryzae*; Adhesion; Extracellular matrix; Metalloproteinase

P. Chitrampalam^{a, 1}, C.A. Cox^a, T.A. Turini^b and B.M. Pryor^a. (^a Division of Plant Pathology and Microbiology, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA, ^b University of California Cooperative Extension, Fresno County, CA 93702, USA). **Efficacy of *Coniothyrium minitans* on lettuce drop caused by *Sclerotinia minor* in desert agroecosystem. *Biological Control*, Volume 55(2) (2010): 92-96**

Field experiments were conducted over 2 years in Yuma County, Arizona and Imperial County, California, to evaluate if increased application rates of a commercial formulation of *Coniothyrium minitans* (Contans) were effective against lettuce drop caused by *Sclerotinia minor*. The efficacy of *C. minitans* at varied application rates were compared to two field isolates of *Paenibacillus polymyxa* and the chemical fungicide Boscolid (Endura). Two applications of manufacture recommended rate of Contans (2.2 kg/ha) did not significantly reduce the incidence of lettuce drop caused by *S. minor*, even though applications at this rate have been shown to be very effective in controlling lettuce drop caused by *Sclerotinia sclerotiorum*. However, two applications of high rates of Contans (6.6, 8.8, or 11 kg/ha), one at planting and one at post-thinning, significantly reduced the incidence of lettuce drop in most trials. Two isolates of *P. polymyxa* each applied at a rate of 9.4 L/ha (10⁹ cfu/ml) were not effective in reducing the incidence of lettuce drop. Two applications of Endura, one at thinning and one at 4 weeks post-thinning, significantly reduced the incidence of lettuce drop in Yuma County, AZ, but not in Imperial County, CA. In summary, successful management of lettuce drop caused by *S. minor* in desert ecosystem could best be achieved with high application rates of *C. minitans*.

Keywords: Lettuce drop; *Sclerotinia minor*; Biological control; *Coniothyrium minitans*; *Paenibacillus polymyxa*; Fungicide; Boscolid

Menelaos C. Stavrinides^{a, b}, Jesús R. Lara^c and Nicholas J. Mills^a. (^a Department of Environmental Science, Policy and Management, Mulford Hall, University of California, Berkeley, CA 94720-3114, USA, ^b Agricultural Research Institute (ARI), 1516 Nicosia, Cyprus, ^c Department of Entomology, University of California, Riverside, USA). **Comparative influence of temperature on development and biological control of two common vineyard pests (Acari: Tetranychidae). *Biological Control*, Volume 55(2) (2010): 126-131**

We studied the effects of temperatures from 10 to 40 °C on development of the Pacific spider mite, *Tetranychus pacificus* (McGregor), and the Willamette spider mite, *Eotetranychus willamettei* McGregor (Acari: Tetranychidae), feeding on grape foliage. In addition, we investigated the influence of temperatures from 10 to 37 °C on development of the western predatory mite, *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae), feeding on *T. pacificus* and evaluated the suitability of *E. willamettei* as prey for the predatory mite at 28 °C. Using a non-linear development rate model we estimated that the lower threshold for development of the three mites lay around 10 °C. *T. pacificus* was the most heat-resistant, with its upper threshold for development at 40.3 °C, followed by *G. occidentalis* at 37.1 °C and *E.*

willamettei at 31.0 °C. *T. pacificus* developed significantly more rapidly than *E. willamettei* above 22.8 °C, whereas *G. occidentalis* developed significantly faster than either spider mite from approximately 11 to 36 °C. *G. occidentalis* developed 5% faster when feeding on *E. willamettei* than *T. pacificus* at 28 °C. These results confirm field observations linking *E. willamettei* damage to cooler, coastal vineyards and early in the season in inland vineyards, and *T. pacificus* infestations to hot vineyards in inland and coastal areas. Although *T. pacificus* seems to be more heat tolerant than *G. occidentalis*, additional information on temperature effects on adult life history details of the two mites is needed to fully evaluate *G. occidentalis* performance at high temperatures.

Keywords: *Tetranychus pacificus*; *Eotetranychus willamettei*; *Galendromus occidentalis*; Development rate; Temperature; Grapes; Biological control

L.A. Sandham^a, T.H. Carroll^a and F.P. Retief^a. (^a Environmental Assessment Research Group, School of Environmental Sciences, North-West University, Private Bag X6001, Potchefstroom, South Africa). The contribution of Environmental Impact Assessment (EIA) to decision making for biological pest control in South Africa – The case of *Lantana camara*. *Biological Control*, Volume 55(2) (2010): 141-149

In many countries authorization for the release of biological control agents for invasive species such as *Lantana camara* requires the consideration and evaluation of environmental impact and risk assessment reports. In South Africa the release of biocontrol agents is subject mainly to Environmental Impact Assessment, in terms of which various agents have been authorized for release into the environment for the control of *L. camara*. However the quality of the EIA reports that form the basis for decision making has not been evaluated. An EIA review package adapted to the requirements of biological control agents was used to review the quality of six approved EIA reports to gauge the contribution of EIA to decision making on the release of these biological control agents. The main finding is that the quality of the reports is poor measured against international best practice. The main weaknesses relate to the crux of EIA which is impact identification, impact evaluation, scoping, mitigation measures and monitoring programs. The value of EIA as a tool for Integrated Environmental Management of complex and potentially high impact activities such as the release of biological control agents is found to be less than required.

Keywords: Environmental Impact Assessment (EIA); Environmental Impact Assessment report quality; Biological control; Decision making; *Lantana camara*

Gang Fu^{a, b}, Siliang Huang^c, Yunfeng Ye^a, Yongguan Wu^a, Zhenlu Cen^b and Shanhai Lin^a. (^a College of Agriculture, Guangxi University, Nanning 530005, PR China, ^b Microbiology Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, PR China, ^c College of Life Science and Technology, Nanyang Normal University, Nanyang 473061, PR China). Characterization of a bacterial biocontrol strain B106 and its efficacies on controlling banana leaf spot and post-harvest anthracnose diseases. *Biological Control*, Volume 55(1) (2010): 1-10

An antagonistic bacterial strain B106 was isolated from the rhizospheric soil of a banana plant in Nanning city, Guangxi, China, and identified as *Bacillus subtilis* based on its 16S rDNA

sequence homology with the related bacteria from GenBank as well as physiological and biochemical characters. The cultural conditions were optimized for enhancing the efficacy of the antagonist against banana leaf spot caused by *Pseudocercospora musae* (teleomorph: *Mycosphaerella musicola*) and post-harvest anthracnose by *Colletotrichum musae*. The optimized cultural condition for strain B106 to express higher antagonistic activity against *P. musae* was the combination of 31 °C, pH 6.0, EM medium and 5-day-incubation. However, the optimized cultural condition for the bacterium to produce higher biomass was the combination of 31–34 °C, pH 6.5, EM medium and 3-day-incubation. The results based on greenhouse tests showed that 72.3% efficacy of the antagonist on controlling the banana leaf spot disease was obtained 10 days after pathogen inoculation. The efficacies of strain B106 (1×10^8 CFU ml⁻¹) on controlling both the banana leaf spot diseases in the field and the anthracnose disease at post-harvest stage were 48.3% and 48.6%, respectively, under the optimized cultural condition for the strain to express higher antagonistic activity. The experimental data indicated that the antagonistic strain was a promising biocontrol agent against the banana diseases.

Research highlights

► A biocontrol strain (B106) of *Bacillus subtilis* with stronger antagonistic ability against multiple pathogenic fungi causing banana leaf spot and post-harvest anthracnose was characterized. ► The strain B106 was approved to be a promising biocontrol agent against the banana diseases based on the greenhouse and field trials. ► Successive applications of the antagonist led to an escalation of control efficacy under field conditions. ► Optimization of cultural conditions of the antagonist led to an enhanced efficacy in controlling both banana leaf spot and post-harvest anthracnose diseases.

Keywords: *Bacillus subtilis*; Biological character; *Musa*; Banana leaf spot; *Pseudocercospora musae*; Banana anthracnose; *Colletotrichum musae*

Pampapathy Gurulingappa^a, Gregory A. Sword^a, Gregory Murdoch^b and Peter A. McGee^a. (^a School of Biological Sciences, The University of Sydney, NSW 2006, Australia, ^b Faculty of Agriculture, Food and Natural Resources, The University of Sydney, NSW 2006, Australia). Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when *in planta*. *Biological Control*, Volume 55(1) (2010): 34-41

Fungal entomopathogens can directly regulate populations of various insects. The entomopathogen *Beauveria bassiana* can also endophytically colonize various plants. Endophytic colonization by entomopathogens might be more widespread than currently realized and may provide a source of indirect interactions between fungi and insects. We tested whether some common entomopathogens could colonize six crop plants. We also assessed whether the performance of two insects, *Aphis gossypii* and *Chortoicetes terminifera*, was affected by entomopathogens in plants. The entomopathogens *B. bassiana*, *Lecanicillium lecanii* and *Aspergillus parasiticus* individually colonized the leaves of all six crop plants when inoculated as conidia. *L. lecanii* also readily colonized five different cultivars of cotton. When the entomopathogens were present in the soil in which either cotton or wheat seedlings were grown, *A. parasiticus* was subsequently isolated from the leaves, stem and roots of both plants and *B. bassiana* from the leaves, stem and root of wheat only, whereas *L. lecanii* failed to colonize either plant through the soil. Of the three entomopathogens tested, endophytic presence of *A. parasiticus* reduced growth of cotton, but none reduced growth of wheat. Feeding by *A. gossypii* on cotton leaves colonized by either *B. bassiana* or *L. lecanii* slowed aphid reproduction, and

consumption of wheat leaves colonized by either *B. bassiana* or *A. parasiticus* slowed the growth of *C. terminifera* nymphs. The life cycle of at least three entomopathogens potentially includes plants. The presence of entomopathogens as endophytes can influence growth and fecundity of insect herbivores, suggesting a possible role for endophytic entomopathogens in the regulation of insect populations.

Keywords: *Aphis gossypii*; *Aspergillus parasiticus*; *Beauveria bassiana*; *Chortoicetes terminifera*; *Lecanicillium lecanii*; Biological control; Endophyte; Entomopathogen; Fungal ecology; Interactions

Juan Sorribas^a and Ferran Garcia-Mari^a. (^a Instituto Agroforestal Mediterráneo, Univ. Politécnic de Valencia, Camino de Vera s/n, 46022 Valencia, Spain,). **Comparative efficacy of different combinations of natural enemies for the biological control of California red scale in citrus groves. Biological Control, Volume 55(1) (2010): 42-48**

The California red scale (CRS), *Aonidiella aurantii*, a worldwide citrus pest, has the ectoparasitoid *Aphytis melinus* as its main natural control agent. Other natural enemies can complement *A. melinus* in the pest control. For 3 years we studied the incidence of the parasitoids and predators on CRS populations of 19 citrus groves divided in four groups according to its parasitoid composition to determine which combination of natural enemies reaches higher effectiveness in the biological control of CRS in field conditions. The parasitoids species preferred different scale instars and different periods of the year for parasitism. In the groves where *A. melinus* was present in high numbers, either alone or coexisting with *Aphytis chrysomphali*, parasitism rates reached 22.4% on average, whereas parasitism rates were much lower (11.5%) when *A. chrysomphali* was the dominant parasitoid. The best parasitism level was achieved when these ectoparasitoids occurred together with the endoparasitoid *Encarsia perniciosi*. The average annual mortality rate of *A. aurantii* was 73.6%. The analysis of dead scales killed by *Aphytis* confirms the results obtained in parasitism of live scales. The more abundant predators were *Lestodiplosis aonidiellae* and *Rhyzobius lophantae*. The incidence of predators on the CRS populations was comparatively much lower.

Keywords: *Aonidiella aurantii*; *Aphytis melinus*; *Aphytis chrysomphali*; *Encarsia perniciosi*; Parasitism; Parasitoid coexistence; Scale predators

Yasser Shabana^a, Daljit Singh^b ¹, Loretta M. Ortiz-Ribbing^c and Steven G. Hallett^d. (^aDepartment of Plant Pathology, University of Florida, Gainesville, FL 32611, USA, ^b Monsanto Company, 1512 NC 55 West, Mount Olive, NC 27709, USA, ^c University of Illinois Extension, Macomb Extension Center, Macomb, IL 61455, USA, ^d Department Botany & Plant Pathology, Purdue University, West Lafayette, IN 47907, USA). **Production and formulation of high quality conidia of *Microsphaeropsis amaranthi* for the biological control of weedy *Amaranthus* species. Biological Control, Volume 55(1) (2010): 49-57**

The fungal pathogen, *Microsphaeropsis amaranthi*, is under consideration as a bioherbicide for the control of weeds in the genus *Amaranthus*. This organism has been shown to be virulent against a number of important *Amaranthus* species but has not yet demonstrated sufficient aggressiveness or reliability in the field to be commercially developed. We report a sequence of

experiments evaluating a range of solid media for the production of high quality inoculum, methodologies for the enhancement of aggressiveness in culture, and the effects of a variety of spray adjuvants. Additionally, we report preliminary results testing the efficacy of the bioherbicide applied as granules, prior to weed emergence. The fungus grew well and sporulated profusely on a range of different solid substrates. Conidia produced on corn stover were larger than those produced on other substrates, had thicker cell walls, and exhibited particularly high aggressiveness. Repeated inoculation and re-isolation of the fungus from *Amaranthus tuberculatus* plants increased its aggressiveness. In addition, application in Sunspray oils resulted in improved disease impact under low moisture conditions. Preemergence application of granular formulations, especially where the fungus was grown and applied in ground barley grains, resulted in good control of emerging *A. tuberculatus*. Further progress has been made in improving the *M. amaranthi* bioherbicide by refining the conditions for the production and deployment of quality inoculum.

Keywords: *Microsphaeropsis amaranthi* (Ell. & Barth.); *Amaranthus tuberculatus* (Moq.) J. D. Sauer, waterhemp; Bioherbicide; Integrated weed management

K. Dhileepan^a, M. Treviño^a, D. Bayliss^a, M. Saunders^{a, b}, M. Shortus^a, J. McCarthy^a, E.L. Snow^a and G.H. Walter^b. (^a Department of Employment, Economic Development & Innovation, Biosecurity Queensland, Alan Fletcher Research Station, Sherwood, Queensland 4075, Australia, ^b School of Integrative Biology, University of Queensland, Brisbane, Australia). Introduction and establishment of *Carvalhotingis visenda* (Hemiptera: Tingidae) as a biological control agent for cat's claw creeper *Macfadyena unguis-cati* (Bignoniaceae) in Australia. *Biological Control*, Volume 55(1) (2010): 58-62

Carvalhotingis visenda (Hemiptera: Tingidae) is the first biological control agent approved for release against cat's claw creeper *Macfadyena unguis-cati* (Bignoniaceae) in Australia. The mass-rearing and field releases of *C. visenda* commenced in May 2007 and since then more than half a million individuals have been released at 72 sites in Queensland and New South Wales. In addition, community groups have released over 11,000 tingid-infested potted cat's claw creeper plants at 63 sites in Queensland. Establishment of *C. visenda* was evident at 80% of the release sites after three years. The tingid established on the two morphologically distinct 'long-pod' and 'short-pod' cat's claw creeper varieties present in Australia. Establishment was more at sites that received three or more field releases (83%) than at sites that received two or less releases (73%); and also at sites that received more than 5000 individuals (82%) than at sites that received less than 5000 individuals (68%). In the field, the tingid spread slowly (5.4 m per year), and the maximum distance of *C. visenda* incidence away from the initial release points ranged from 6 m to approximately 1 km.

Keywords: Tingidae; Mass-rearing; Field release; Community groups; Establishment; Weed; Biological control; Cat's claw creeper; Australia

Yasser M. Shabana^{a, 1}, R. Charudattan^a, Ayman H. Abou Tabl^{a, 1}, J. Pablo Morales-Payan^b, Erin N. Rosskopf^c and Waldemar Klassen^d. (^a Department of Plant Pathology, University of Florida, P.O. Box 110680, Gainesville, FL 3261, USA, ^b Department of Crops and Agro-Environmental Sciences, University of Puerto Rico – Mayaguez Campus, P.O. Box 9030, Mayaguez, PR 00681, Puerto Rico, ^c USDA, ARS, USHRL, 2001 South Rock Road, Fort Pierce, FL 34945, USA, ^d Tropical Research and Education Center, 18905 SW 280 St., P.O. Box 111569, Homestead, FL 33031, USA). Production and application of the

bioherbicide agent *Dactylaria higginsii* on organic solid substrates. Biological Control, Volume 54(3) (2010): 159-165

Purple nutsedge and yellow nutsedge are serious weeds in Florida and in many parts of the world. *Dactylaria higginsii* is a promising bioherbicide candidate for these weeds. This fungus does not sporulate in liquid culture. Thus, for mass production of *D. higginsii*, 19 solid substrates were tested, including dried, cut culms or foliage of 14 different plants prepared as hays, sugarcane bagasse, a commercial peat-based microbe carrier (BioAPT), and two recycled papers (kraft paper and newspaper). These substrates were tested alone or amended either with 0.01% indole butyric acid (IBA), which enhances growth and/or sporulation of *D. higginsii*, potato dextrose broth (PDB) for nutrient amendment, or PDB + IBA. Yield of *D. higginsii* conidia produced on these substrates was measured 4 and 12 weeks after inoculation. Conidia were tested for virulence on purple and yellow nutsedges in the greenhouse. Hays without amendments or amended with PDB yielded more conidia which were also more virulent than those produced on hays amended with IBA. Conidial yields were highest when the fungus was grown on purple nutsedge hay without amendments for 4 weeks but conidia produced on this medium had low virulence. Conidia produced on sorghum × sudangrass and cogongrass hays were slightly larger and thicker walled than those produced on other substrates. Conidia produced on sorghum × sudangrass were the most virulent on nutsedge seedlings. Purple nutsedge was more susceptible to *D. higginsii* than yellow nutsedge. Cogongrass and sorghum × sudangrass hay substrates offer a suitable and easy medium for large-scale production of *D. higginsii*.

Keywords: Spore production; Organic substrates; Solid substrates; *Dactylaria higginsii*; Bioherbicides; Nutsedge

Z. Ipek Ekmen^{a, b}, Selcuk Hazir^b, Ibrahim Cakmak^c, Nurdan Ozer^a, Mehmet Karagoz^c and Harry K. Kaya^d. (^a Hacettepe University, Faculty of Science, Department of Biology, 06800 Beytepe-Ankara, Turkey, ^b Adnan Menderes University, Faculty of Arts and Science, Department of Biology, 09010 Aydin, Turkey, ^c Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, 09010 Aydin, Turkey, ^d Department of Nematology, University of California, One Shields Avenue, Davis, CA 95616, USA). **Potential negative effects on biological control by *Sancassania polyphyllae* (Acari: Acaridae) on an entomopathogenic nematode species. Biological Control, Volume 54(3) (2010): 166-171**

Sancassania polyphyllae (Acari: Acaridae) is associated with larvae of the white grub, *Polyphylla fullo* (Coleoptera: Scarabaeidae), and will feed on the infective juveniles of entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae which are important biological control agents of soil insect pests. We conducted laboratory studies to determine the potential negative effects this mite species might have on biological control of soil insect pests. Our objectives in this study were to (1) determine the response of *S. polyphyllae* adult mites to a nematode-killed insects on agar, (2) evaluate the predation by mites on *Steinernema feltiae* infective juveniles from nematode-killed insects on agar and in soil, and (3) assess predation efficiency of the mite on the infective juveniles in the soil. On agar, we found (1) significantly more adult female mites near or on a nematode-killed *Ceratitis capitata* (Diptera: Tephritidae) larva than near or on the freeze-killed larva or a bamboo mimic suggesting

that a chemical or an odor from the nematode-killed larva attracted the mites, and (2) 10 mites consumed 96% of infective juveniles that emerged from an insect cadaver. In soil with a nematode-killed insect, the average number of infective juveniles recovered was <30 when mites were present, whereas the average number of infective juveniles recovered was >375 when the mites were absent. When the infective juveniles alone were placed in different depths in relation to the mites in the soil column for 4 and 10 days, *S. polyphyllae* was not as efficient at finding the infective juveniles when they were separated from each other in the soil lending support to the idea that the mites were cueing in on the cadaver as a food resource. Our data suggest that emerging infective juveniles from an insect cadaver in the soil in the presence of *S. polyphyllae* can adversely affect biological control because of nematode consumption by the mites.

Keywords: Insect-parasitic nematode; Nematophagous mite; *Polyphylla fullo*; *Steinernema feltiae*; White grub

Josep A. Jacas^a, Bryan J. Ulmer^b, Rita E. Duncan^c and Jorge E. Peña^c. (^a Universitat Jaume I, Departament de Ciències Agràries i del Medi Natural, Campus del Riu Sec, E-12071 Castelló de la Plana, Spain, ^b Syngenta Crop Protection, 408 Herzog Drive, Watertown, SD 57201, USA, ^c University of Florida, Department of Entomology and Nematology, Tropical Research and Education Center, 18905 SW 280th Street, Homestead, FL 33031, USA). The role of *Brachyufens osborni* (Hymenoptera: Trichogrammatidae) in the classical biological control program against *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in Florida. *Biological Control*, Volume 54(3) (2010): 213-220

Brachyufens osborni is an indigenous egg parasitoid of weevil species laying concealed eggs in Florida. *Diaprepes abbreviatus*, an exotic weevil pest of different crops in the southern USA, has been reported as a host for *B. osborni*. In this study we investigated the interaction between *B. osborni* and *D. abbreviatus* as well as other hosts including *Pachnaeus litus* and *Artipus floridanus*. The thermal requirements of *B. osborni* and the interaction between this species and three other weevil parasitoids introduced in a classical biological control program against *D. abbreviatus* (*Aprostocetus vaquitarum*, *Haeckeliana sperata* and *Quadrastichus haitiensis*) were also examined in laboratory studies. Although *B. osborni* attacked eggs of all weevil species tested, it could not complete development on *D. abbreviatus*. The three exotic parasitoids did successfully develop on both *P. litus* and *D. abbreviatus* eggs, though our results showed that *P. litus* should not be considered as an alternative host for the introduced parasitoids during winter in central Florida. Because *B. osborni* cannot complete development on *D. abbreviatus*, it has never been recovered from field collected hosts, and its effect on *D. abbreviatus* in the field remains ignored. This study demonstrates that although *B. osborni* could not successfully develop in *D. abbreviatus*, it can inflict important mortality to it and suggests that *B. osborni* could impact the establishment of introduced *D. abbreviatus* egg parasitoids.

Keywords: *Pachnaeus litus*; *Artipus floridanus*; *Aprostocetus vaquitarum*; *Haeckeliana sperata*; *Quadrastichus haitiensis*; Citrus IPM; Alternative hosts; Indigenous parasitoid; Development

Ulrike Krauss^{a1}, Eduardo Hidalgo^a, Roy Bateman^{a, 2}, Valex Adonijah^a, Claudio Arroyo^a, Johnny García^a, Jayne Crozier^a, Neil A. Brown^{a, b}, G. Martijn ten Hoopen^a and Keith A. Holmes^{c, 1}. (^a CABI Caribbean and Latin America (CLA)-Costa Rica, c/o Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), 7170 Turrialba, Costa Rica, ^b Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2

7AY, UK, ^c CABI E-UK, Silwood Park, Buckhurst Rd., Ascot, Berks. SL5 7TA, UK). **Improving the formulation and timing of application of endophytic biocontrol and chemical agents against frosty pod rot (*Moniliophthora roreri*) in cocoa (*Theobroma cacao*). *Biological Control*, Volume 54(3) (2010): 230-240**

Frosty pod rot (FPR), caused by *Moniliophthora roreri*, reduces yields of cocoa (*Theobroma cacao*) by over 80% within a few years of disease outbreak. Both biological and chemical control approaches are being developed to supplement cultural management. Our objectives were to optimize the agent(s), their formulation and application regime for FPR control in Costa Rica. In order to economize scarce resources for field research, we opted for an iterative optimization of treatments. We aimed to (partly) replace copper hydroxide of proven efficacy with two systemic agents, the coevolved endophyte *Trichoderma ovalisporum* and the oxathiin flutolanil, and assessed four stickers for improving the agents' performance. Over 3 years, we selected effective agents and their combinations: eight of 18 treatments increased yields. Orthogonal contrasts extracted three general trends: (1) Copper should be applied in water, whereas the systemic agents were best formulated with a sticker. There was no difference between the stickers BreakThru and NP-7. (2) If the first rule was observed, a switch from systemic agent to the contact fungicide in mid season was beneficial. The optimum timing for this change requires further definition. (3) *T. ovalisporum*-containing agents controlled black pod better than flutolanil-based ones; both systemic agents were equally effective in controlling the more severe FPR. We discuss potential benefits of switching agents in the context of organic agriculture and argue that coevolved endophytes may be particularly suited for the classical biocontrol of *Moniliophthora* spp. in cocoa planted outside its South American centre of origin.

Keywords: Biological control; Classical biocontrol; Cocoa; Delivery system; Endophytes; Formulation; Fungicides; *Moniliophthora roreri*; *Theobroma cacao*; *Trichoderma ovalisporum*

R. Loughner^a, K. Wentworth^a, G. Loeb^a and J. Nyrop^a. (^a Cornell University, New York State Agricultural Experiment Station, Department of Entomology, Barton Lab, 630 W. North St., Geneva, NY 14456, USA). Influence of leaf trichomes on predatory mite density and distribution in plant assemblages and implications for biological control. *Biological Control*, Volume 54(3) (2010): 255-262

Non-glandular trichomes on leaves or stems are strongly positively correlated with the abundance of many predatory phytoseiid mite species. Some perennial fruit crops have few or no trichomes and overcoming this limitation of leaf habitat for natural enemy mites could improve biological control of pest mites in agricultural crops. This study evaluated whether juxtaposing plants with and without trichomes will promote more abundant natural enemies throughout an ensemble of plants. We first asked whether the pattern of increased phytoseiid retention with higher trichome densities on a single leaf “scales up” to larger plantings. For three patch sizes (1, 4 or 16 plants) of two grape varieties inoculated with phytoseiids, no strong effect of patch size on *Typhlodromus pyri* Scheuten abundance was observed in the trichome-rich Baco plantings or in the trichome-free Dechaunac plantings. No predator populations established in Dechaunac plantings of any size. Second, we asked whether the addition of a plant with leaf trichomes within a row (“array”) of plants lacking leaf trichomes can increase phytoseiid density throughout arrays. In both a greenhouse and a field experiment, predatory mites moved onto and established on a neighboring plant only if that plant had trichomes; phytoseiids rapidly dispersed

from trichome-free plants even when the plant was the one inoculated with predators. The overall number of phytoseiids decreased over time in ensembles that included both trichome-rich and trichome-free plants in the greenhouse and the field experiments, clearly indicating that for biological control programs to be successful, all plants must express trichomes.

Keywords: Non-glandular trichomes; Phytoseiid; Domatia; Habitat complexity; Leaf hairs; *Typhlodromus pyri*

Melissa R. Martin^a, Philip W. Tipping^b, K.R. Reddy^a, Samira H. Daroub^c and Katrina M. Roberts^{a, 1}. (^a University of Florida, Department of Soil and Water Science, 106 Newell Hall, P.O. Box 110510, Gainesville, Florida 32611, USA, ^b United States Department of Agriculture, Agricultural Research Service, Invasive Plant Research Laboratory, 3225 College Avenue, Fort Lauderdale, Florida 33314, USA, ^c University of Florida, Everglades Research and Education Center, 3200 East Palm Beach Road, Belle Glade, Florida 33430, USA). **Interactions of biological and herbicidal management of *Melaleuca quinquenervia* with fire: Consequences for ecosystem services. *Biological Control*, Volume 54(3) (2010): 307-315**

Exotic plant species invasion can alter ecosystem nutrient dynamics and natural disturbance patterns. The Australian tree *Melaleuca quinquenervia* has extensively invaded the Florida Everglades and is currently being suppressed using mechanical, herbicidal, and biological control management strategies. While these methods have been evaluated based on reductions in density and abundance of the target weed, other factors should be considered including consequences for ecosystem nutrient storages and interactions with natural disturbances such as fire. We hypothesized that the choice of management tactics, namely herbicidal or biological control, would differentially influence the quantity and availability of soil nutrients before and after a seasonal fire. The management of *M. quinquenervia* with a herbicide reduced the above- and belowground storage of nutrients both before and after a fire compared to a non-invaded area, while biological control increased storage. There were no differences in nitrogen availability between sites (non-invaded, herbicide, biological control) in the 0–5 cm or 5–15 cm soil depths before or after the fire. Pre-fire phosphorus availability was highest in the non-invaded site in the 0–5 cm soil depth and in the biological control site in the 5–15 cm soil depth. However, phosphorus availability was highest at both depths in the herbicide site post-fire. Biological control of *M. quinquenervia* using insect herbivores has proven to be effective at controlling plant growth and reproduction. The results of this study suggest that this method may have less of an impact on nutrient storage and cycling than herbicides.

Keywords: Fire; Invasion; Exotic; Nutrient; *Melaleuca quinquenervia*

Yuanyuan Zong^{a, b}, Jia Liu^{a, b}, Boqiang Li^a, Guozheng Qin^a and Shiping Tian^a. (^a Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China, ^b Graduate School of Chinese Academy of Sciences, Beijing 100049, China). **Effects of yeast antagonists in combination with hot water treatment on postharvest diseases of tomato fruit. *Biological Control*, Volume 54(3) (2010): 316-321**

Hot water treatment (HWT) and two yeast antagonists, *Candida guilliermondii* and *Pichia membranaefaciens* were investigated separately and together for controlling *Botrytis cinerea*, and natural infection in tomato fruit stored at 20 °C. Applied separately, both HWT and

antagonists inhibited decay caused by *B. cinerea*, and natural infection. The combination of antagonists and HWT showed better control efficacy. Application of HWT did not affect the growth of *C. guilliermondii* and *P. membranaefaciens* in tomato wounds, while HWT induced significant increase in the activities of phenylalanine ammonia-lyase (PAL), chitinase (CHI) and β -1,3-glucanase in fruit. The mechanism by which HWT enhanced the biocontrol efficacy of the antagonistic yeasts may be related to the elicitation of biochemical defense responses in tomato fruit. The combination of antagonistic yeasts and HWT could be a promising method for the control of postharvest diseases of tomato fruit.

Keywords: Hot water treatment; Tomato fruit; *Botrytis cinerea*; *Candida guilliermondii*; *Pichia membranaefaciens*

Han Qiao Hu^a, Xin Shen Li^{b,1}, and Hong He^a. (^a Department of Biotechnology, Agricultural College, Guangdong Ocean University, Zhanjiang, Guangdong Province 524088, China, ^b Department of Plant Pathology, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510640, China). **Characterization of an antimicrobial material from a newly isolated *Bacillus amyloliquefaciens* from mangrove for biocontrol of Capsicum bacterial wilt. Biological Control, Volume 54(3) (2010): 359-365**

Understanding the mechanisms of the antagonistic endophytic bacteria is helpful in controlling plant diseases. An endophytic bacterium, Bg-C31, from mangrove was found to be antagonistic to some fungal and bacterial pathogens of plants and to be effective in the biocontrol of Capsicum bacterial wilt in pot and field trials. Bg-C31 was identified as *Bacillus amyloliquefaciens* by biochemical and physiological tests as well as sequences of 16S rDNA and the *LCI* gene. The antimicrobial substance produced by Bg-C31 was identified as a protein, which is resistant to protease k and heat, by ammonium sulfate precipitation and butanol extraction. The antagonistic gene was located in the chromosome by plasmid curing. A 29 kDa fusion protein of the *LCI* gene was expressed. Antimicrobial activity of the fusion protein to *Ralstonia solanacearum* was detected on gels *in situ*, indicating that the *LCI* gene could potentially be used to produce transgenic plants that are resistant to bacterial infection.

Keywords: Endophytic bacteria; Heterologous expression; Mangrove; *Ralstonia solanacearum*

Shenandoah R. Marr^a, Steve A. Johnson^{b, c}, Arnold H. Hara^a and Monica E. McGarrity^c. (^aBeaumont Agricultural Research Center, College of Tropical Agriculture and Human Resources, University of Hawaii at Mānoa, 875 Komohana Street, Hilo, HI 96720-4037, USA, ^b Department of Wildlife Ecology, University of Florida Institute of Food and Agricultural Sciences, 110 Newins-Ziegler Hall, P.O. Box 110430, Gainesville, FL 32611-0430, USA, ^c Gulf Coast Research and Education Center, University of Florida Institute of Food and Agricultural Sciences, 1200 North Park Road, Plant City, FL 33563-1540, USA). **Preliminary evaluation of the potential of the helminth parasite *Rhabdias elegans* as a biological control agent for invasive Puerto Rican coquí (*Eleutherodactylus coqui*) in Hawaii. Biological Control, Volume 54(2) (2010): 69-74**

In Hawaii, the extremely loud mating calls of invasive Puerto Rican coquí (*Eleutherodactylus coqui* Thomas) depress real estate values in infested areas and reduce tourist appeal, with significant economic repercussions. Measures required to prevent inter-island transport of frogs

also result in substantial costs for the floriculture industry. Classical biological control has been used successfully for over 100 years to combat invasive insects in Hawaii, and there is considerable interest in developing similar controls for coquís. Since Hawaii lacks native amphibian and reptile faunas (with the exception of marine species), the risk of unintended effects of a biological control agent on non-target species would be minimal. We identified *Rhabdias elegans* Gutierrez, a helminth parasite of native Puerto Rican populations of *E. coqui* that is not found in introduced Hawaiian populations, as a prime candidate for investigation as a potential biological control. We conducted laboratory experiments to evaluate overt effects of *R. elegans* on growth, survival, and locomotory performance of *E. coqui* from Hawaii. Experimental infection with *R. elegans* did not directly affect growth, survival, or endurance of *E. coqui* maintained under optimal laboratory conditions, but significantly reduced initial locomotory burst performance. We suggest that *R. elegans* holds limited potential as a biological control agent for eradication of *E. coqui*, but warrants additional investigation under more natural conditions of its potential for use as a management tool.

Keywords: *Eleutherodactylus coqui*; *Rhabdias elegans*; Anuran; Invasive; Biological control; Lung nematode; Growth; Survival; Locomotory performance

George N. Mbata^a and David I. Shapiro-Ilan^b. (^a Department of Biology, Fort Valley State University, 1005 University Drive, Fort Valley, GA 31030, USA, ^b USDA, Agricultural Research Service, Southeastern Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008, USA). **Compatibility of *Heterorhabditis indica* (Rhabditida: Heterorhabditidae) and *Habrobracon hebetor* (Hymenoptera: Braconidae) for biological control of *Plodia interpunctella* (Lepidoptera: Pyralidae). *Biological Control*, Volume 54(2) (2010): 75-82**

The potential for integrating the application of *Heterorhabditis indica* Poinar, Karunakar, and David (Homl strain) and release of *Habrobracon hebetor* (Say) in the management of the Indianmeal moth, *Plodia interpunctella* (Hübner), was investigated in the laboratory. A combination of the nematode and the parasitoid was observed to increase the mortality of *P. interpunctella*. The interaction between the nematodes and parasitoids was not antagonistic but could possibly be additive or synergistic. Release of parasitoids or application of nematodes alone generated between 62.25% and 71.25% mortality of the *P. interpunctella* larvae whereas combination of the two resulted in 98.0–99.25% mortality. The nematode was found to be virulent to the larvae of the parasitoid but not to the pupae and the adults. Adult female parasitoids that were exposed to both uninfected and nematode-infected *P. interpunctella* larvae in a free-choice arena were unable to distinguish between the two. In contrast, infective juvenile nematodes preferentially infected parasitized host larvae compared with healthy non-parasitized host larvae. Nematode reproduction was not significantly different in parasitized and non-parasitized host larvae. The combined application of *H. indica* and *H. hebetor* for the control of *P. interpunctella* may be beneficial if the detrimental effects of the nematode on the parasitoid can be minimized through optimum timing.

Keyword: Biological control; Entomopathogenic nematodes; *Heterorhabditis indica*; *Plodia interpunctella*; *Habrobracon hebetor*; Stored-products

R. Manikandan^a, D. Saravanakumar^a, L. Rajendran^a, T. Raguchander^a and R. Samiyappan^a. (^a Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641 003, India). **Standardization of liquid**

formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against *Fusarium* wilt of tomato. Biological Control, Volume 54(2) (2010): 83-89

Pseudomonas fluorescens strain Pf1 is studied as an effective biocontrol agent for the management of plant diseases and plant growth-promoting bacteria. Previous findings from our research group demonstrated that talc-based *P. fluorescens* Pf1 formulation effectively reduced several plant diseases in addition to promoting plant growth. The modernization of agro-techniques necessitates the development of a new formulation where liquid inoculants can play a significant role. Different chemicals such as trehalose, polyvinylpyrrolidone and glycerol were tested for the development of liquid formulation. Among these, glycerol amendment maintained the greater population level of *P. fluorescens* Pf1 up to 6 months of storage. Further, a study was conducted to standardize the dose of liquid-based formulation of Pf1 for seed treatment and seedling dip. An application of 10 ml kg⁻¹ of seeds and 150 ml ha⁻¹ of seedlings was found to be optimum for seed treatment and seedling root dip, respectively. The growth-promoting and antagonistic activities of Pf1 cultures of different ages were found to be greater up to 180 days of storage without much loss in viability of cells. The combination of seed treatment, seedling dip and soil drenching of liquid formulation recorded the minimum disease incidence of *Fusarium* wilt on tomato under glasshouse (17.33%) and field (4.81%) conditions. In addition, the liquid formulation increased the tomato fruit yield compared to untreated control under glasshouse and field conditions. Thus, this study offered successful technology for development of a liquid-based bioformulation *P. fluorescens* Pf1.

Keywords: *Fusarium*; Liquid formulation; *Pseudomonas fluorescens*; Population; Survival ability

David O. Simelane^{a, b}. (^a Weeds Research, Agricultural Research Council-Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa, ^b Department of Zoology, University of Cape Town, P/Bag Rondebosch, 7701 Cape Town, South Africa). Potential impact of an introduced root-feeding flea beetle, *Longitarsus bethae*, on the growth and reproduction of an invasive weed, *Lantana camara*. Biological Control, Volume 54(2) (2010): 114-118

A root-feeding flea beetle, *Longitarsus bethae* Savini & Escalona (Chrysomelidae: Alticinae), was introduced into South Africa to complement the impact of the above-ground insect agents already established on *Lantana camara* L. (Verbenaceae). To estimate the potential effectiveness of *L. bethae* for biological control of *L. camara*, growth of various plant components were measured under different levels of abundance of the insect under quarantine glasshouse and semi-field conditions. Populations of *L. bethae* larvae that developed in previously-sterilized soil from initial cohorts of 200 and 300 eggs per plant significantly stunted the growth of *L. camara*, causing reductions in the number of leaves, stem height, stem diameter, above-ground dry-mass, and below-ground dry-mass, of up to 54%, 21%, 31%, 34% and 40%, respectively. Compared with the controls, the larval population resulting from an inoculum of 100 eggs per plant usually had no statistically significant effect on plant growth. The degree of root damage caused by the larvae was directly proportional to the number of eggs added and to the number of adults that emerged. Under semi-field conditions, cumulative herbivory by *L. bethae* adults and larvae during a six-month period caused severe leaf and root damage, resulting in a cumulative decline of 148% in flower production. Overall, the ability of *L. bethae* to both directly suppress root

growth and indirectly suppress leaf production, stem growth and flower production of *L. camara*, indicates that this flea beetle has the potential to make a considerable impact on the weed's invasiveness in South Africa.

Keywords: Root damage; Impact assessment; Biological control; Chrysomelidae; Alticinae; Verbenaceae

T. Tuovinen^a and I. Lindqvist^a. (^a MTT Agrifood Research Finland, Plant Production Research, FI-31600 Jokioinen, Finland). Maintenance of predatory phytoseiid mites for preventive control of strawberry tarsonemid mite *Phytonemus pallidus* in strawberry plant propagation. *Biological Control*, Volume 54(2) (2010): 119-125

Effective means for the control of the strawberry tarsonemid mite, *Phytonemus pallidus*, are needed for organic strawberry plant production. To study the effect of predatory mites as a preventive control tool of *P. pallidus*, four species of predatory mites were released on mite-free strawberry plants in a greenhouse experiment. Phytoseiid mites were fed by scattering cattail pollen on leaves until the artificial infestation of the strawberry tarsonemid mite took place 6 weeks later. Population dynamics of predatory and phytophagous mites and thrips were monitored every second week by sampling leaves, runners and whole plants. The predatory mite species *Anthoseius rhenanus*, *Euseius finlandicus* and *Neoseiulus cucumeris* successfully reproduced and remained on plants when fed with pollen, whereas *Neoseiulus barkeri* failed to reproduce before the introduction of *P. pallidus*. After the artificial infestation of *P. pallidus* and the termination of pollen feeding, *A. rhenanus*, *N. cucumeris* and *N. barkeri* were able to control the build-up of tarsonemid mite populations for a period of 3 months. *A. rhenanus* and *N. cucumeris* were found to be the most promising preventive predators against *P. pallidus*. At the end of the study *N. cucumeris* and *N. barkeri* replaced *E. finlandicus* and *A. rhenanus*, and were able to form mixed populations when prey mites were richly available. However, the phytoseiid mite species used in this study could not prevent the development of the natural infestation of the two-spotted spider mite, *Tetranychus urticae*, after the initial study was terminated.

Keywords: *Anthoseius rhenanus*; *Euseius finlandicus*; *Neoseiulus barkeri*; *Neoseiulus cucumeris*; Biological control; Organic production

Junjiao Lu^a, Longlong Zhao^a, Ruiyan Ma^a, Pingping Zhang^a, Renjun Fan^b and Jintong Zhang^c. (^a College of Agriculture, Shanxi Agricultural University, Taigu Shanxi 030801, PR China, ^b Institute of Plant Protection, Shanxi Academy of Agricultural Sciences, Taiyuan, Shanxi 030031, PR China, ^c College of Arts and Sciences, Shanxi Agricultural University, Taigu Shanxi 030801, PR China). Performance of the biological control agent flea beetle *Agasicles hygrophila* (Coleoptera: Chrysomelidae), on two plant species *Alternanthera philoxeroides* (alligatorweed) and *A. sessilis* (joyweed). *Biological Control*, Volume 54(1) (2010): 9-13

Agasicles hygrophila has been used as a biological control agent on invasive alligatorweed, *Alternanthera philoxeroides* in China. We conducted tests to determine the impact of *A. hygrophila* on the non-target species *A. sessilis* and examined the developmental differences of *A. hygrophila* raised on these two plant species to assess the ecological and environmental safety of the introduced *A. hygrophila* in China. No significant differences were detected in the developmental timing of *A. hygrophila* from larvae to adults, in larval hatching, and in pupal fresh weight in three treatments: *A. hygrophila* reared on *A. philoxeroides* through all life stages;

A. hygrophila larvae reared on *A. sessilis* but pupated in the stems of *A. philoxeroides*; and *A. hygrophila* reared on *A. sessilis* through all life stages. However, when *A. hygrophila* larvae were reared on *A. sessilis* but pupated in the stems of *A. philoxeroides*, they had a significantly longer preoviposition period, lower pupation rate, and lower fecundity than those in other two treatments. The demographic parameters examined indicated that lower fecundity (GRR, R_0) and intrinsic rate of natural increase ($r_m = 0.06$), but longer doubling time (T_d) were found in *A. hygrophila* larvae reared on *A. sessilis* but pupated in the stems of *A. philoxeroides* compared to the other two treatments ($r_m = 0.11$). Ultimately, *A. hygrophila* has limited effects on the non-target *A. sessilis* and is considered safe to the ecosystem. Moreover, *A. sessilis* would provide a marginally suitable host for *A. hygrophila* in regions where the two species intermingle.

Keywords: *Agasicles hygrophila*; *Alternanthera philoxeroides*; *Alternanthera sessilis*; Non-target plant; Life history; Biological control; Biosafety

Pushpa G. Soti^a and John C. Volin^b. (^a Department of Biological Sciences, Florida Atlantic University, Davie, FL 33314, USA, ^b Department of Natural Resources and the Environment, University of Connecticut, Storrs, CT 06269, USA). **Does water hyacinth (*Eichhornia crassipes*) compensate for simulated defoliation? Implications for effective biocontrol. *Biological Control*, Volume 54(1) (2010): 35-40**

Biocontrol agents of water hyacinth (*Eichhornia crassipes*), one of the most ubiquitous invasive aquatic species in the world, were introduced in the waterways of Florida, USA, more than 30 years ago but have not been as successful as expected. The high nutrient, high light, warm year-round temperatures and lack of natural predators provide an optimal growth environment for the plant. The current study was designed to test if a compensatory response by the water hyacinth plants to low levels of biomass removal was one of the reasons for the ineffectiveness of biocontrol agents in the successful control of water hyacinth. The plants were exposed to two levels of nutrient (high and low) and three levels (0%, 10% and 80%) of simulated herbivory treatment. The effect of the nutrient and repeated (i.e., chronic) defoliation treatments was determined after 6 weeks. Plants with 10% defoliation did not show any significant difference from control plants in biomass allocation or relative growth rate (RGR) in either nutrient concentration, while 80% defoliation caused a significant decrease in the final RGR under high and low nutrient treatments. High nutrient treatment resulted in higher RGR and allocation to asexual reproduction resulting in higher biomass accumulation compared to the low nutrient treatment, which had higher root growth and allocation to sexual reproduction. Results from this study indicate that water hyacinth can fully compensate for low levels of continuous defoliation regardless of the nutrient concentration, which has implications and important considerations for biocontrol strategies.

Keywords: *Eichhornia crassipes*; Water hyacinth; Invasive species; Simulated herbivory; Relative growth rate; Photosynthesis; Allocation; Specific leaf area; Leaf mass ratio; Compensation; *Neochetina eichhorniae*; *Neochetina bruchi*

Louela A. Castrillo^a, Michael H. Griggs^b, Houping Liu^{c, 1}, Leah S. Bauer^{c, d} and John D. Vandenberg^b. (^a Department of Entomology, Cornell University, Ithaca, NY 14853, USA, ^b USDA ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853, USA, ^c Department of Entomology, Michigan State University, East Lansing, MI 48824, USA, ^d

USDA Forest Service, Northern Research Station, East Lansing MI 48823, USA). Assessing deposition and persistence of *Beauveria bassiana* GHA (Ascomycota: Hypocreales) applied for control of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), in a commercial tree nursery. *Biological Control*, Volume 54(1) (2010): 61-67

Determining the deposition and field persistence of mycoinsecticides is essential in the development of effective and economical application strategies, including specifically the timing and frequency of spray applications. In this study we used three methods to evaluate the persistence of *Beauveria bassiana* strain GHA applied for control of the emerald ash borer (EAB), *Agrilus planipennis*, an invasive pest attacking ash trees, *Fraxinus* spp., in North America. Fungal inocula present on ash bark and leaves, collected at 1, 7 and 14 days after spraying, were quantified by use of molecular (real-time PCR assay) and culture-based methods (semi-selective wheat germ dodecane agar). We also assayed fungus-sprayed leaves and bark against adult beetles to determine whether the level of inocula persisting in the field was sufficient to affect beetle survival. Our data quantified deposition of *B. bassiana* and documented fungal persistence. We observed significant decline in recovery of colony-forming units of *B. bassiana* within 1 week of application. The decline was more pronounced on leaves than on bark, and was also evident in loss of virulence of treated substrates to adult EAB with increasing time after application. However, sufficient inocula persisted on bark and leaves for 7--14 days to cause 40--57% and 17--38% mortality, respectively, making pre-emergent sprays a practical means to target adults during emergence, pre-oviposition feeding, or oviposition.

Keywords: *Fraxinus* spp.; Invasive insect; Entomopathogenic fungus; Microbial control; Real-time PCR

Q, M. Grodowitz^r, T.A. Heardⁱ, M.P. Hill^l, J.H. Hoffmann^s, J. Huber^t, M. Julienⁱ, M.T.K. Kairo^u, M. Kenis^v, P. Mason^w, J. Medal^m, R. Messing^x, R. Miller^y, A. Moore^v, P. Neuenschwander^z, R. Newman^{aa}, H. Norambuena^{ab}, W.A. Palmer^{ac}, R. Pemberton^c, A. Perez Panduro^{ad}, P.D. Pratt^c, M. Rayamajhi^c, S. Salom^{ae}, D. Sandsⁱ, S. Schoolerⁱ, M. Schwarzländer^{ag}, A. Sheppard^f, R. Shaw^{af}, P.W. Tipping^c and R.D. van Klinkenⁱ.
(^aPSIS/Entomology, University of Massachusetts, Fernald Hall, Amherst, MA 01003, USA, ^b USDA-ARS, Exotic and Invasive Weeds Research Unit, Albany, CA 94710, USA, ^c Invasive Plant Research Laboratory, ARS, USDA, 3225 College Avenue, Fort Lauderdale, FL 33314, USA, ^d Department of Entomology, University of California, Riverside, CA 92521, USA, ^e Entomology & Wildlife Ecology, University of Delaware, Newark, DE 19716, USA, ^f CSIRO Entomology, G.P.O. Box 1700, Canberra, ACT 2601, Australia, ^g Center for Conservation and Biodiversity, University of Connecticut, Storrs, CT 06269-3043, USA, ^h Department of Natural Resources, Fernow Hall, Cornell University, Ithaca, NY 14853, USA, ⁱ CSIRO Entomology, 120 Meiers Road, Indooroopilly, Qld 4068, Australia, ^j Department of Plant Sciences, University of Rhode Island, Kingston, RI 02881, USA, ^k Fundación Charles Darwin, Puerto Ayora, Santa Cruz, Galapagos Islands, Ecuador, ^l Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa, ^m Department of Entomology & Nematology, University of Florida, Gainesville, FL 32611-0620, USA, ⁿ Invasion Ecology and Biocontrol Lab, Wuhan Botanical Garden/Institute, Chinese Academy of Sciences, Moshan, Wuhan, Hubei Province 430074, China, ^o Landcare Research, P.O. Box 40, Lincoln 7640, New Zealand, ^p USDA-ARS, Beneficial Insects Introduction Res., 501 S. Chapel St., Newark, DE 19713, USA, ^q USDA-ARS, Beneficial Insects Res. Unit, 2413 E. Hwy. 83, Weslaco, TX 78596, USA, ^r US Army Engineer Research and Development Center, Vicksburg, MS 39180, USA,

^s Zoology Department, University of Cape Town, Rondebosch 7700, South Africa, ^t Natural Resources Canada, c/o AAFC, K.W. Neatby Building, 960 Carling Avenue, Ottawa, Ont., Canada K1A 0C6, ^u Center for Biological Control, CESTA, Florida A&M University, Tallahassee, FL 32307, USA, ^v CABI Europe-Switzerland, 1, Rue des Grillons, 2800 Delémont, Switzerland, ^w Agriculture and Agri-Food Canada, Research Centre, K.W. Neatby Building, 960 Carling Avenue, Ottawa, Ont., Canada K1A 0C6, ^x University of Hawaii at Manoa, Kauai Agricultural Research Center, 7370 Kuamoo Road, Kapaa, HI 96746, USA, ^y Western Pacific Tropical Research Center, University of Guam, Mangilao, GU, USA, ^z International Institute of Tropical Agriculture, IITA-Benin 08 BP 0932 Cotonou, Benin, ^{aa} Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, MN 55108, USA, ^{ab} Instituto de Investigaciones Agropecuarias, INIA Carillanca, Camino, Cajón-Vilcún, Km 10, Casilla 58-D, Temuco, Chile, ^{ac} Biosecurity Queensland, Department of Employment, Economic Development & Innovation, Alan Fletcher Research Station, P.O. Box 36, Sherwood, Qld 4075, Australia, ^{ad} Colegio de Postgraduados, Carr. México – Texcoco Km 36.5, 56230 Montecillo, Edo de México, Mexico, ^{ae} Department of Entomology, Virginia Tech, Blacksburg, VA 24061-0319, USA, ^{af} CABI E-UK, Bakeham Lane, Egham, Surrey TW20 9TY, England, UK, ^{ag} Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID 83844, USA). **Classical biological control for the protection of natural ecosystems** [★] **Biological Control, Volume 54(1) (2010): S2-S33**

Of the 70 cases of classical biological control for the protection of nature found in our review, there were fewer projects against insect targets (21) than against invasive plants (49), in part, because many insect biological control projects were carried out against agricultural pests, while nearly all projects against plants targeted invasive plants in natural ecosystems. Of 21 insect projects, 81% (17) provided benefits to protection of biodiversity, while 48% (10) protected products harvested from natural systems, and 5% (1) preserved ecosystem services, with many projects contributing to more than one goal. In contrast, of the 49 projects against invasive plants, 98% (48) provided benefits to protection of biodiversity, while 47% (23) protected products, and 25% (12) preserved ecosystem services, again with many projects contributing to several goals. We classified projects into complete control (pest generally no longer important), partial control (control in some areas but not others), and “in progress,” for projects in development for which outcomes do not yet exist. For insects, of the 21 projects discussed, 62% (13) achieved complete control of the target pest, 19% (4) provided partial control, and 43% (9) are still in progress. By comparison, of the 49 invasive plant projects considered, 27% (13) achieved complete control, while 33% (16) provided partial control, and 49% (24) are still in progress. For both categories of pests, some projects’ success ratings were scored twice when results varied by region. We found approximately twice as many projects directed against invasive plants than insects and that protection of biodiversity was the most frequent benefit of both insect and plant projects. Ecosystem service protection was provided in the fewest cases by either insect or plant biological control agents, but was more likely to be provided by projects directed against invasive plants, likely because of the strong effects plants exert on landscapes. Rates of complete success appeared to be higher for insect than plant targets (62% vs 27%), perhaps because most often herbivores gradually weaken, rather than outright kill, their hosts, which is not the case for natural enemies directed against pest insects. For both insect and plant biological control, nearly half of all projects reviewed were listed as currently in progress,

suggesting that the use of biological control for the protection of wildlands is currently very active.

Keywords: Invasive species; Ecosystem function; Insect pests; Invasive plants; Ecological restoration; Biological control; Natural ecosystems

Milan Řezáč, Stano Pekár and Jitka Stará. The negative effect of some selective insecticides on the functional response of a potential biological control agent, the spider *Philodromus cespitum*. *BioControl*, Volume 55(4) (2010): 503-510

The impact of five selective insecticides on the functional response of a potential biological control agent, the spider *Philodromus cespitum* (Walckenaer) (Araneae: Philodromidae), was studied in the laboratory. This spider is the most abundant beneficial arthropod on trees in commercial orchards in central Europe. We expected that selective insecticides applied at the recommended doses would have no effect or a negligible effect on the spiders' performance. Our results showed that the mortality of spiders resulting from residual uptake of the chemicals differed among insecticides. Dimilin, NeemAzal, Mospilan, and Integro caused mortality of less than 10%, while SpinTor caused mortality of 17%. All five preparations can be considered harmless in terms of mortality in comparison with Decis, which caused 80% mortality. Exposure to residues of NeemAzal, SpinTor, and Dimilin resulted in a significantly lower predation rate than the control. The lowest predation rate was observed in spiders treated with SpinTor. These results imply that the natural pest control provided by *P. cespitum* spiders can be weakened by the application of SpinTor, NeemAzal, and Dimilin. On the other hand, the functional response was not significantly affected by the application of Integro and Mospilan. Therefore, these two insecticides are recommended for use in the integrated pest management (IPM) of orchards.

Keywords: Acetamiprid - Azadirachtin - Biological control - Deltamethrin - Diflubenzuron - Methoxyfenozide - Philodromidae - *Philodromus cespitum* - Spinosad

M. Hultberg, T. Bengtsson and E. Liljeroth. Late blight on potato is suppressed by the biosurfactant-producing strain *Pseudomonas koreensis* 2.74 and its biosurfactant. *BioControl*, Volume 55(4) (2010): 543-550

Potato late blight disease caused by the zoospore-producing pathogen *Phytophthora infestans* (Mont.) de Bary is one of the most destructive plant diseases world-wide and currently its management mainly relies on the frequent use of fungicides. This study investigated the possibility of reducing potato late blight by biocontrol with the biosurfactant-producing strain *Pseudomonas koreensis* 2.74. Significant disease reduction with the biosurfactant-producing strain and its biosurfactant was observed in greenhouse trials using a detached-leaf assay. A direct effect of the biosurfactant on zoospores of *P. infestans* was also observed, whereas the biosurfactant only caused a minor reduction in mycelial growth rate and had no effect on the rate of sporangia production in pure culture.

Keywords Biocontrol - Biosurfactants - *Phytophthora infestans* - Potato late blight

Yangzhou Wang, Kai Wu and Jianqing Ding. Host specificity of *Euops chinensis*, a potential biological control agent of *Fallopia japonica*, an invasive plant in Europe and North America. *BioControl*, Volume 55(4) (2010): 551-559

Fallopia japonica (Houttuyn) Ronse Decraene (Polygonaceae) is a serious invasive weed in North American and Europe. In its native China, a leaf-rolling weevil, *Euops chinesis* (Coleoptera: Attelabidae) was found attacking *F. japonica* in the field. No-choice tests, multiple-choice tests, open field tests and field surveys were conducted as a measure of its host specificity. Forty-six plant species were selected from 17 families for host range testing, among which, six species, *F. multiflora*, *F. japonica*, *Persicaria perfoliata*, *Rumex acetosa*, *R. japonicus* and *R. aquaticus*, were exposed to adults in no-choice tests. However, larvae could only develop successfully on *F. japonica*, and this plant appeared to be the only host in the field, suggesting the weevil is host-specific. As larval development appears to depend on a fungus in the leaf rolls, the insect–fungus mutualism and risks including host specificity of the fungus should be evaluated before the insect’s introduction.

Keywords: Host-specificity - Leaf-rolling weevil - Japanese knotweed - *Euops chinesis* - *Fallopia japonica* - Mutualism

Andrea L. Joyce, Martin Aluja, John Sivinski, S. Bradleigh Vinson, Ricardo Ramirez-Romero, Julio S. Bernal and Larissa Guillen. Effect of continuous rearing on courtship acoustics of five braconid parasitoids, candidates for augmentative biological control of *Anastrepha* species. *BioControl*, Volume 55(5) (2010): 573-582

The courtship acoustics of five species of parasitoid wasps (Hymenoptera: Braconidae), potential candidates for augmentative biological control of *Anastrepha* (Schiner) species (Diptera: Tephritidae), were compared between recently colonized individuals and those continuously reared 70–148 generations. During courtship, males of these parasitoid species fan their wings and produce a series of low amplitude pulses. The first series of 15 or more continuous courtship pulses was used to measure the pulse duration, frequency, and interpulse interval (IPI) from the beginning, middle, and end of the pulse series. Each parameter was compared between young and old colonies, and among species. Several differences in courtship acoustics were detected in colonies that had been continuously reared. The pulse duration at the end of the pulse series was longer in old colonies for *Doryctobracon crawfordi* (Viereck) (Hymenoptera: Braconidae), but shorter for old colonies of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). The IPI of the middle pulse was shorter in old colonies of *Opius hirtus* (Fischer) (Hymenoptera: Braconidae), and was also shorter at the last pulse for old colonies of both *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae) and *D. longicaudata*. The duration of the middle pulse distinguished the three native species, and separated the two introduced species from each other. We discuss our findings in light of their biological and applied implications, particularly those dealing with quality control of mass-reared parasitoids.

Keywords: *Anastrepha ludens* - *Doryctobracon crawfordi* - *Opius hirtus* - *Utetes anastrephae* - *Diachasmimorpha longicaudata* - *Diachasmimorpha tryoni* - Mass rearing - Laboratory selection - Quality - Rearing substrate - Courtship vibration

Ning Ling, Chao Xue, Qiwei Huang, Xingming Yang, Yangchun Xu and Qirong Shen. Development of a mode of application of bioorganic fertilizer for improving the biocontrol efficacy to *Fusarium* wilt. *BioControl*, Volume 55(5) (2010): 673-683

More effective ways of applying biocontrol products should be developed based both on the characteristics of the biocontrol agents and the normal practices of the agricultural producer. A new system was developed to improve the biocontrol efficacy of *Fusarium* wilt for watermelon production, and this system was tested in pot and field experiments. Biocontrol was achieved by applying a novel bioorganic fertilizer product (BIO) to *Fusarium*-infested soil. The best biocontrol was obtained by application of a bioorganic fertilizer, BIO, into soil during the nursery phase of watermelon seedling followed by a second application to *Fusarium*-infested soil when watermelon seedlings were transplanted. In comparison with the controls, the incidence of the disease was reduced by 60–100% in the pot experiment and by 59–73% in the field experiment when the BIO was applied during the nursery stage. After application of BIO during the nursery stage, the number of colony-forming units of *Fusarium oxysporum* in rhizospheric soil was significantly ($P < 0.05$) inhibited compared to the controls. An in vitro experiment showed that the antagonist *Paenibacillus polymyxa* in the BIO could effectively colonize the rhizosphere of watermelon and proliferate along the extending plant roots. This inhibited growth of *Fusarium oxysporum* in the rhizosphere of watermelon and protected the watermelon roots from attack by the pathogens. The method used for biocontrol *Fusarium* wilt disease in watermelon should be a useful strategy to improve field efficacy of other biocontrol agents.

Keywords: Bioorganic fertilizer (BIO) - Nursery application - *Fusarium* wilt - Watermelon - *Paenibacillus polymyxa*

X. Liu, J. Jia, S. Atkinson, M. Cámara, K. Gao, H. Li and J. Cao. Biocontrol potential of an endophytic *Serratia* sp. G3 and its mode of action. World Journal of Microbiology and Biotechnology, Volume 26(8) (2010): 1465-1471

Biological control is being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture. An endophytic strain G3 with potential as a biocontrol agent was isolated from the stems of *Triticum aestivum* L. It was classified by 16S rDNA sequencing as a member of *Serratia*. Strain G3 displayed a broad spectrum of antifungal activity in vitro against a number of phytopathogens such as *Botrytis cinerea*, *Cryphonectria parasitica*, *Rhizoctonia cerealis* and *Valsa sordida*. Molecular mechanisms involved in biocontrol by *Serratia* sp. G3 was investigated for its potential application to plant health management. The results showed that G3 produces an array of antimicrobial exoproducts, including chitinase, protease, antibiotic pyrrolnitrin, and siderophores for iron competition. Moreover, it also produced the plant growth hormone indole-3-acetic acid, suggesting that multiple mechanisms and their synergistic effects may be involved in biocontrol of plant diseases. Additionally, strain G3 can produce at least ten *N*-acyl homoserine lactones (AHLs) signal molecules for cell to cell communication, including unsubstituted, 3-oxo, and 3-hydroxy at the C3 position through liquid chromatography-tandem mass spectrometry (LC-MS/MS), which is different from the previously reported *Serratia* species. For the first time, *N*-3-oxo-heptanoyl-homoserine lactone, one of the main molecules was reported in the genus *Serratia*. The role of AHL-dependent quorum sensing system in the interactions between the endophytic strain G3 and host plants and its potential application in improving biocontrol efficacy will be further explored.

Keywords: Endophytic *Serratia* - Biocontrol - Indole-3-acetic acid - Quorum sensing signal molecules

Archana Gajbhiye, Alok R. Rai, Sudhir U. Meshram and A. B. Dongre. Isolation, evaluation and characterization of *Bacillus subtilis* from cotton rhizospheric soil with biocontrol activity against *Fusarium oxysporum*. World Journal of Microbiology and Biotechnology, Volume 26(7) (2010): 1187-1194

Present investigation is based on the isolation of *Bacillus subtilis* from cotton rhizosphere and their evaluation as biocontrol agent against *Fusarium oxysporum*. The production of extracellular hydrolytic enzyme was studied for determining the antagonism. 43% of 21 isolates were identified under the *B. subtilis* group on the basis of biochemical characterization. 38% isolates showed competitive activity against *Fusarium oxysporum* and exhibit more than 50% mycelial inhibition in dual culture bioassay. The pot assay of cotton by seed treatment and soil amendment technique under green house condition showed the competent activity of the isolates in preventing the wilting of cotton seedlings due to *F. oxysporum* infection. SVI values of 30 day old seedlings indicated that the soil inoculation with *B. subtilis* BP-2 and seed treatment with *B. subtilis* BP-9 significantly promoted the growth of cotton seedlings. RAPD profiling revealed the diversity in the *Bacillus subtilis* group, ranging from 10 to 32%. The discriminative pattern among the isolates belonging to the same species was validated by 16S rDNA partial sequencing which identified them into four different strains of *B. subtilis*.

Keywords: *Bacillus subtilis* - *Fusarium oxysporum* - Biocontrol agent - RAPD - 16S rDNA

K. R. N. Reddy^{1*}, C. R. Raghavender², B. N. Reddy² and B. Salleh¹. (¹School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia, ²Department of Botany, Osmania University, Hyderabad, India. *Corresponding author. E-mail: drkrnreddy@gmail.com. Tel: +604-6534001. Fax: +604- 6565125). **Biological control of *Aspergillus flavus* growth and subsequent aflatoxin B₁ production in sorghum grains. African Journal of Biotechnology, Vol. 9 (27) (2010): 4247-4250**

Sorghum grains suffer from severe infection and colonization by several toxigenic fungi and subsequent production of mycotoxins, posing a threat to human and animal health. Among all the mycotoxins, aflatoxins represent one of the most important toxicants considered as important constraint of grain quality and sorghum production. Various physical and chemical methods of reducing mycotoxins have been recommended, but only few have been accepted for practical use. Biodegradation of aflatoxins, deploying microbes is an attractive strategy for mycotoxin management. This study, therefore explored the potential use of certain biocontrol agents for the reduction of growth of toxigenic *Aspergillus flavus* and subsequent aflatoxin B₁ (AFB₁) production in sorghum. Among all the biocontrol agents tested, culture filtrate of *Rhodococcus erythropolis* completely inhibited the *A. flavus* growth and AFB₁ production at 25 mlkg⁻¹ concentration. The other biocontrol agents, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma viride* showed 72, 74 and 65% inhibition of *A. flavus* growth whereas 54, 62.6 and 39% reduction of AFB₁ at 200 mlkg⁻¹ of sorghum grains, respectively.

Key words: Sorghum, *Aspergillus flavus*, AFB₁, biological control.

Abbreviations: AFB₁, Aflatoxin B₁; ELISA, enzyme-linked immunosorbent assay; IgG-ALP, immunoglobulin G -alkaline phosphatase; BSA, bovine serum albumin; PBST, phosphate buffered saline tween-20.

Biodegradation

Alfredo Ruiz-Arias, Cleotilde Juárez-Ramírez, Daniel de los Cobos-Vasconcelos, Nora Ruiz-Ordaz, Angélica Salmerón-Alcocer, Deifilia Ahuatz-Chacón and Juvencio Galíndez-Mayer. Aerobic Biodegradation of a Sulfonated Phenylazonaphthol Dye by a Bacterial Community Immobilized in a Multistage Packed-Bed BAC Reactor. APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, Volume 162(6) (2010): 1689-1707

A microbial community able to aerobically degrade the azo dye Acid Orange 7 was selected from riparian or lacustrine sediments collected at sites receiving textile wastewaters. Three bacterial strains, pertaining to the genera *Pseudomonas*, *Arthrobacter*, and *Rhizobium*, constitute the selected community. The biodegradation of AO7 was carried out in batch-suspended cell culture and in a continuously operated multistage packed-bed BAC reactor. The rapid decolorization observed in batch culture, joined to a delay of about 24 h in COD removal and cell growth, suggests that enzymes involved in biodegradation of the aromatic amines generated after AO7 azo-bond cleavage (1-amino-2-naphthol [1-A2N] and 4-aminobenzenesulfonic acid [4-ABS]), are inducible in this microbial consortium. After this presumptive induction period, the accumulated byproducts, measured through COD, were partially metabolized and transformed in cell mass. At all azo dye loading rates used, complete removal of AO7 and 1-A2N was obtained in the multistage packed-bed BAC reactor (PBR).; however, the overall COD (η COD) and 4-ABS (η ABS) removal efficiencies obtained in steady state continuous culture were about 90%. Considering the toxicity of 1-A2N, its complete removal has particular relevance. In the first stages of the packed-bed BAC reactor (Fig. 4a–c), major removal was observed. In the last stage, only a slight removal of COD and 4-ABS was obtained. Comparing to several reported studies, the continuously operated multistage packed-bed BAC reactor showed similar or superior results. In addition, the operation of large-packed-bed BAC reactors could be improved by using several shallow BAC bed stages, because the pressure drop caused by bed compaction of a support material constituted by small and fragile particles can be reduced.

Keywords: Acid Orange 7 - Sulfanilic acid - 4-aminobenzenesulfonic acid - 1-amino-2-naphthol - Packed-bed reactor - Biofilm - Biodegradation - *Arthrobacter* - *Rhizobium*

Fuying Ma^a, Na Yang^a, Chunyan Xu^a, Hongbo Yu^a, Jianguo Wu^a and Xiaoyu Zhang^a. (^a Key Laboratory of Molecular Biophysics of MOE, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, PR China). Combination of biological pretreatment with mild acid pretreatment for enzymatic hydrolysis and ethanol production from water hyacinth. Bioresource Technology, Volume 101(24) (2010): 9600-9604

The mild acid pretreatment and the combination of biological pretreatment by a white rot fungus *Echinodontium taxodii* or a brown rot fungus *Antrodia* sp. 5898 with mild acid pretreatment were evaluated under different pretreatment conditions for enzymatic hydrolysis and ethanol production from water hyacinth. The combined pretreatment with *E. taxodii* (10 days) and 0.25% H₂SO₄ was proved to be more effective than the sole acid pretreatment. The reducing sugar yield from enzymatic hydrolysis of co-treated water

hyacinth increased 1.13–2.11 fold than that of acid-treated water hyacinth at the same conditions. The following study on separate hydrolysis and fermentation with *Saccharomyces cerevisiae* indicated that the ethanol yield from co-treated water hyacinth achieved 0.192 g/g of dry matter, which increased 1.34-fold than that from acid-treated water hyacinth (0.146 g/g of dry matter). This suggested that the combination of biological and mild acid pretreatment is a promising method to improve enzymatic hydrolysis and ethanol production from water hyacinth with low lignin content.

Keywords: Water hyacinth; Biological pretreatment; Mild acid pretreatment; Saccharification; Ethanol fermentation

Sandipta Ghosh^a, Gautam Sen^a, U. Jha^a and Sagar Pal^b. (^a Department of Applied Chemistry, Birla Institute of Technology, Mesra, Ranchi 835 215, Jharkhand, India, ^b Department of Applied Chemistry, Indian School of Mines, Dhanbad 826 004, Jharkhand, India). **Novel biodegradable polymeric flocculant based on polyacrylamide-grafted tamarind kernel polysaccharide. Bioresource Technology, Volume 101(24) (2010): 9638-9644**

Novel biodegradable polymeric flocculants were produced by conventional redox grafting, microwave-initiated and microwave-assisted grafting of acrylamide to tamarind kernel polysaccharide (TKP). The graft copolymers were characterized by viscometry, elemental analysis, molecular weight determination using SLS analysis, and NMR spectroscopy. The flocculation efficiency of the grafting products in kaolin suspension, municipal sewage wastewater and textile industry wastewater was primarily dependent on the length of the grafted polyacrylamide chain. The flocculant obtained by microwave-assisted grafting method was superior to TKP and polyacrylamide-based commercial flocculant (Rishfloc 226 LV) in flocculation tests.

Keywords: Flocculation; Graft copolymer; Tamarind kernel polysaccharide; Turbidity; Wastewater

Sonia Arriaga^a, Julia A. Acosta-Munguía^{a, b}, Ana S. Pérez-Martínez^{b, 1}, Antonio De León-Rodríguez^b and Ana P. Barba de la Rosa^b. (^a Institute for Scientific and Technological Research at San Luis Potosí, Environmental Science Division, Camino a la Presa San José 2055, Lomas 4a Sección, CP 78216 San Luis Potosí, Mexico, ^b Institute for Scientific and Technological Research at San Luis Potosí, Molecular Biology Division, Camino a la Presa San José 2055, Lomas 4a Sección, CP 78216 San Luis Potosí, Mexico). **Coupling aerobic biodegradation of methanol vapors with heterologous protein expression of endochitinase Ech42 from *Trichoderma atroviride* in *Pichia pastoris*. Bioresource Technology, Volume 101(24) (2010): 9661-9665**

Methanol is included among the most hazardous air pollutants, and an effort of vapors biofiltration by using microbial consortiums has been reported. The aim of this work was to couple the methanol vapors biodegradation with the production of recombinant endochitinase (*ech42*) from *Trichoderma atroviride* in *Pichia pastoris* transformed with the pPIC-*ech42* plasmid. After carrying out batch experiments at 0.5% (w/v) of methanol concentration, the recombinant *P. pastoris* Mut⁺ strain was selected because it showed methanol

biodegradation rates similar to those of wild type GS115 strain (39 g/m³ h), but 15% higher than the transformed Mut^S strain. In addition, the recombinant Ech42 protein production was higher in Mut⁺ than Mut^S. After various methanol vapor concentrations were evaluated, the maximum recombinant protein recovery was 317 mg/l and the volumetric methanol consumption rate was 88.7 g/m³ h at 0.5% (w/v) of methanol concentration. This research underlines the promising application of linking methanol vapors biodegradation with the production of recombinant protein with high biotechnological interests.

Keywords: Endochitinase-42; Methanol; Microcosms; Recombinant proteins; Zimograms

Yi-Rui Wu^a, Zhu-Hua Luo^{a,b} and L.L.P. Vrijmoed^a. (^a Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong SAR, China, ^b Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, 178 Daxue Road, Xiamen 361005, China). **Biodegradation of anthracene and benz[a]anthracene by two *Fusarium solani* strains isolated from mangrove sediments. *Bioresource Technology*, Volume 101(24) (2010): 9666-9672**

An investigation was undertaken on the biodegradation of two kinds of polycyclic aromatic hydrocarbons (PAHs), anthracene (ANT) and benz[a]anthracene (BAA), by fungi isolated from PAH-contaminated mangrove sediments environment in Ma Wan, Hong Kong. ANT (50 mg l⁻¹) and BAA (20 mg l⁻¹), respectively, were added to mineral salt medium initially for screening of PAH-degrading fungi, and finally two fungal species capable of using ANT or BAA as the sole carbon source were isolated and identified as *Fusarium solani* species. Removal of ANT and BAA reached 40% and 60% of the added amount, respectively, after 40 days of incubation. A total of six metabolites were isolated and characterized by solid phase microextraction (SPME) combined with gas chromatography–mass spectrometry (GC/MS), which indicate that *F. solani* degraded both ANT and BAA via their respective quinone molecules to generate phthalic acid. Free extracellular laccase was detected during the degradation process without detectable lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP), suggesting that laccase might play an important role in the transformation of PAHs compounds.

Keywords: PAHs; SPME; Laccase; Non-white-rot fungi; Lignin-degrading enzymes

Zilian Zhang^a, Sathaporn Srichuwong^a, Tooru Kobayashi^b, Mitsuhiro Arakane^a, Jeung-yil Park^a and Ken Tokuyasu^a. (^a Food Resource Division, National Food Research Institute, National Agriculture and Food Research Organization (NARO), 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642, Japan, ^b Research Team for Biomass Recycling System, Upland Farming Research Station, National Agricultural Research Center for Kyushu Okinawa Region, NARO, 6651-2 Yokoichi, Miyakonojo, Miyazaki 885-0091, Japan). **Bioconversion of L-arabinose and other carbohydrates from plant cell walls to α -glucan by a soil bacterium, *Sporosarcina* sp. N52. *Bioresource Technology*, Volume 101(24) (2010): 9734-9741**

A Gram-positive bacterium, N52, that produces intracellular glucan from L-arabinose, was isolated from soil and identified as *Sporosarcina* sp. according to rRNA gene sequence analysis and physiological/biochemical characterizations. Glucan production by N52 increased significantly in the exponential phase of aerobic liquid culture and was maintained at the highest

level during the stationary phase, reaching 37.0% of the cell dry weight. The glucan was also produced from other tested sugars originating from plant cell walls and was composed exclusively of α -1,4- and α -1,6-glucosidic linkages. When distillery waste was treated with N52 for 72 h, the total organic carbon (TOC), chemical oxygen demand and biochemical oxygen demand were reduced by 42.6%, 45.9% and 82.5%, respectively. Bacterial cells accumulated 31.9% of glucan per cell dry weight, fixing 16.0% of the TOC in the soluble fraction. Thus, this strain could provide us with a new process for waste management, including the bioconversion of organic materials to the valuable byproduct, α -glucan.

Keywords: Aerobic treatment; L-Arabinose; Distillery waste; α -Glucan; *Sporosarcina*

S.O. Kulkarni^a, P.P. Kanekar^a, S.S. Nilegaonkar^a, S.S. Sarnaik^a and J.P. Jog^b. (^a Microbial Sciences Division, MACS-Agharkar Research Institute, G.G. Agarkar Road, Pune 411 004, India, ^b National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008, India). **Production and characterization of a biodegradable poly (hydroxybutyrate-co-hydroxyvalerate) (PHB-co-PHV) copolymer by moderately haloalkalitolerant *Halomonas campisalis* MCM B-1027 isolated from Lonar Lake, India. *Bioresource Technology*, Volume 101(24) (2010): 9765-9771**

Several microorganisms produce polyhydroxyalkanoates (PHA). They are accumulated intracellularly as energy storage compounds. The PHAs are of interest because of their potential in biomedical applications. Halophilic bacteria and archaea are known to produce polyhydroxybutyrate (PHB). This paper describes production of a biodegradable copolymer, PHB-co-PHV by a moderately haloalkalitolerant *Halomonas campisalis*, isolated from Lonar Lake, India.

The production of PHA was in the range of 45–81% on dry cell weight basis when the organism was grown in a production medium containing 1% (w/v) maltose and 0.1% (w/v) yeast extract, at pH ranging from 6 to 9 with an inoculum density of 10^5 – 10^7 cells/ml of medium, for incubation period of 15–30 h and at 37 °C. The polymer produced by the organism is a hydroxyester with molecular weight of 1.3014×10^6 . Its melting temperature was 171 °C. The ¹H NMR analysis revealed that the polymer was a copolymer of PHB-co-PHV. This could be achieved by providing simple carbon source viz. maltose.

Keywords: Polyhydroxyalkanoates; *Halomonas campisalis*; Biodegradable plastics

Yi An^a, Tielong Li^a, Zhaohui Jin^a, Meiyong Dong^a, Hongcai Xia^a and Xue Wang^a. (^a College of Environmental Science and Engineering, Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Ministry of Education Key Laboratory of Pollution Processed and Environmental Criteria, Nankai University, Tianjin 300071, China). **Effect of bimetallic and polymer-coated Fe nanoparticles on biological denitrification. *Bioresource Technology*, Volume 101(24) (2010): 9825-9828**

Bimetallic nanoparticles (nano Fe–Ni, nano Fe–Cu) and coated iron nanoparticles (chitosan–Fe⁰, sodium oleate–Fe⁰) were utilized to support autotrophic denitrification. In comparison to nanoscale zero-valent iron (NZVI) particles, Ni-containing nanoparticles resulted in faster nitrate removal, but generated 17% more ammonium. The nano Fe–Cu integrated system, required two

days less than the unmodified NZVI integrated system to remove all the nitrate and decrease ammonium by 13%, but a large amount of nitrite remained in the system. Compared to uncoated NZVI particles, chitosan-coated nanoparticles allowed the same nitrate removal time but 23% more ammonium production. The sodium oleate-Fe⁰ nanoparticles did not only decrease the generation of ammonium by 17%, but also reduced the toxicity of the nanoparticles to bacteria. Therefore, sodium oleate-Fe⁰ nanoparticles may be an appropriate substitute for NZVI particles to support autotrophic denitrification provided that additional time (two days) is allowed for complete nitrate removal.

Keywords: Fe⁰ nanoparticles; Catalyst; Coating; Autotrophic denitrification; Nitrate

Zhongshun Yuan^a, Shuna Cheng^b, Mathew Leitch^b and Chunbao (Charles) Xu^a. (^a Department of Chemical Engineering, Lakehead University, Thunder Bay, ON, Canada P7B 5E1, ^b Faculty of Forestry and Forest Environment, Lakehead University, Thunder Bay, ON, Canada P7B 5E1). **Hydrolytic degradation of alkaline lignin in hot-compressed water and ethanol. Bioresource Technology, Volume 101(23) (2010): 9308-9313**

Alkaline lignin of a very high molecular weight was successfully degraded into oligomers in a hot-compressed water-ethanol medium with NaOH as the catalyst and phenol as the capping agent at 220–300 °C. Under the optimal reaction conditions, i.e., 260 °C, 1 h, with the lignin/phenol ratio of 1:1 (w/w), almost complete degradation was achieved, producing <1% solid residue and negligible gas products. The obtained degraded lignin had a number-average molecular weight M_n and weight-average molecular weight M_w of 450 and 1000 g/mol respectively, significantly lower than the M_n and M_w of 10,000 and 60,000 g/mol of the original lignin. A higher temperature and a longer reaction time favoured phenol combination, but increased the formation of solid residue due to the condensation reactions of the degradation intermediates/products. The degraded lignin products were soluble in organic solvents (such as THF), and were characterized by HPLC/GPC, IR and NMR. A possible mechanism for lignin hydrolytic degradation was also proposed in this study.

Keywords: Alkaline lignin; Hydrolytic degradation; De-polymerization; Phenol; Hot-compressed water and ethanol

Niraj Sheth^a and Shailesh Dave^b. (^a Department of Microbiology, M. D. Gramseva Mahavidyalaya, Gujarat Vidyapeeth, Sadra 382 320, India, ^b Department of Microbiology, School of Sciences, Gujarat University, Ahmedabad 380 009, India). **Enhanced biodegradation of Reactive Violet 5R manufacturing wastewater using down flow fixed film bioreactor. Bioresource Technology, Volume 101(22) (2010): 8627-8631**

The present study emphasizes on the development of bioprocess for biodegradation and bioremediation of Reactive Violet 5R (RV5) manufacturing industry's wastewater in laboratory scale indigenously designed down flow fixed film bioreactor (DFFR). Process was investigated in DFFR, packed with furnace charcoal as a support material. During the batch and continuous operation of DFFR more than 95% degradation, 88% COD reduction and 99% copper remediation was obtained in less than 8 h of contact time. Continuous mode treatment gave degradation of more than 2500 mg dye in only 1 h of contact time. Addition of 0.25% peptone enhanced biodegradation rate more than three-fold. The biologically treated wastewater was found to stimulate the growth of selected soil bacteria. Degradation of major

components of the wastewater was confirmed by HPTLC. DFFR was operated successfully for 750 d continuously, during which 716 cycles of batch treatment were operated.

Keywords: Degradation; Reactive Violet 5R; Fixed film bioreactor; HPTLC; *Pseudomonas* sp.

Özlem Karahan^a, Tugba Olmez-Hanci^a, Idil Arslan-Alaton^a and Derin Orhon^a. (^a Istanbul Technical University, Faculty of Civil Engineering, Department of Environmental Engineering, 34469 Maslak, Istanbul, Turkey). **Modelling biodegradation of nonylphenol ethoxylate in acclimated and non-acclimated microbial cultures. *Bioresource Technology*, Volume 101(21) (2010): 8058-8066**

The biodegradation and inhibition kinetics of a commercial nonylphenol ethoxylate formulation were modelled for an activated sludge system fed with a synthetic substrate and nonylphenol ethoxylate mixture. Kinetic and stoichiometric coefficients of the proposed activated sludge model were obtained by employing on-line respirometry. Experimental as well as model results confirmed that nonylphenol ethoxylate exhibited non-competitive inhibition on the hydrolysis process with a coefficient of 150 mg/L on the basis of COD and negatively influenced biomass growth through a competitive inhibition mechanism with a coefficient of 500 mg/L on the basis of COD, when the biomass was not acclimated. Upon acclimation of the activated sludge system, the inhibition concentration for non-competitive inhibition on hydrolysis was increased to 5000 mg/L, practically showing no inhibition, and the coefficient of competitive inhibition increased to 450 mg/L, corresponding to a significant decrease in the inhibitory effects of NPEO on growth.

Keywords: Alkyl phenol ethoxylates; Nonylphenol ethoxylate; Biodegradation kinetics; Inhibition kinetics; Activated sludge modelling

Chun-Hua Li^a, Yuk-Shan Wong^b and Nora Fung-Yee Tam^a. (^a Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong SAR, PR China, ^b Department of Biology, The Hong Kong University of Science and Technology, Hong Kong SAR, PR China). **Anaerobic biodegradation of polycyclic aromatic hydrocarbons with amendment of iron(III) in mangrove sediment slurry. *Bioresource Technology*, Volume 101(21) (2010): 8083-8092**

Mangrove sediment, influenced by tidal cycles, switches between low-oxygen and non-oxygen conditions, and iron is abundant in it. Polycyclic aromatic hydrocarbon (PAH) contamination often occurs in mangrove wetlands. In the present paper, the effects of iron [Fe(III)] amendment on the biodegradation of four mixed PAHs, namely fluorene (Fl), phenanthrene (Phe), fluoranthene (Flua) and pyrene (Pyr), in mangrove sediment slurries, with and without the inoculation of the enriched PAH-degrading bacterial consortia, under low-oxygen ($2 \pm 0.3\%$ O₂) and non-oxygen (0% O₂) conditions were investigated. Under both oxygen conditions and for all four PAHs, the highest PAHs biodegradation was observed in the groups with the inoculation of the enriched PAH-degrading consortia, while the groups without the inoculum and without Fe(III) amendment had the lowest biodegradation. However, the amendment of Fe(III) did not show any significant improvement on the biodegradation of all the four mixed PAHs.

Keywords: Electron acceptor; Mangrove; Oxygen condition; PAH stress; PAH-degrading bacterial consortia

Rajesh Gupta^{1,a} and Y.Y. Lee^a. (^a Department of Chemical Engineering, Auburn University, AL 36849, United States). Investigation of biomass degradation mechanism in pretreatment of switchgrass by aqueous ammonia and sodium hydroxide. *Bioresource Technology*, Volume 101(21) (2010): 8185-8191

Pretreatment of switchgrass using aqueous ammonia or NaOH was investigated as a means to enhance the enzymatic digestibility. To increase the effectiveness of pretreatment, H₂O₂ was supplemented with the alkaline reagents. Since H₂O₂ is unstable at high temperature, low-to-high step-change of temperature was applied, and this scheme was found to be effective in the case of NH₃/H₂O₂ treatment. The composition of pretreatment liquid indicates that hemicellulose solubilized during alkaline treatment exists either in the form of oligomers or as lignin-carbohydrate complex (LCC). LCC formation was prominent in ammonia treatment and in NaOH/H₂O₂ treatment, and that LCC formation protects the hemicellulose sugars from degradation. Lignin in the pretreatment liquor was precipitated and subjected to TGA and FTIR analyses. TGA data indicate that NaOH-lignin has more uniform structure and higher O/C ratio than ammonia-lignin. FTIR analysis indicates that NaOH-lignin has lower aromatic content but higher guaiacyl type structure than ammonia-lignin.

Keywords: Switchgrass; Pretreatment; Ammonia; NaOH; H₂O₂

Zhengzhi Zhang^{a,b,1}, Lixue Gai^{c,d,1}, Zhaowei Hou^d, Chunyu Yang^b, Cuiqing Ma^b, Zhongguo Wang^d, Baiping Sun^b, Xiaofei He^a, Hongzhi Tang^a and Ping Xu^a. (^a MOE Key Laboratory of Microbial Metabolism and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China, ^b State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, People's Republic of China, ^c Daqing Petroleum Institute, Daqing 163318, People's Republic of China, ^d Daqing Oilfield Co. Ltd., China National Petroleum Corporation, Daqing 163453, People's Republic of China). Characterization and biotechnological potential of petroleum-degrading bacteria isolated from oil-contaminated soils. *Bioresource Technology*, Volume 101(21) (2010): 8452-8456

A collection of 38 bacteria was obtained by enrichment cultivation from oil-contaminated soils of an oil field in Daqing, China. Twenty-two strains could utilize diesel oil as the sole source of carbon and energy, and 11 strains could degrade the total petroleum hydrocarbons (TPHs) of diesel oil by more than 70% in 7 d. Phylogenetically, 19 of the bacteria related to *Bacillus* species. About 87.5% TPHs of crude oil were degraded by a consortium of seven strains. Denaturing gradient gel electrophoresis analysis suggested that five of the strains persisted throughout the degradation process. The collection of isolated bacteria might be a useful resource for bioremediation of oil-contaminated soils and biotreatment of oil wastewater.

Keywords: Petroleum biodegradation; Microbial consortium; Denaturing gradient gel electrophoresis; Phylogenetic analysis

Ming W. Lau^a and Bruce E. Dale^a. (^a Department of Chemical Engineering and Materials Science, DOE Great Lakes Bioenergy Research Center, Michigan State University, 3900 Collins Rd., Lansing, MI 48910, United States). Effect of primary degradation-reaction

products from Ammonia Fiber Expansion (AFEX)-treated corn stover on the growth and fermentation of *Escherichia coli* KO11. *Bioresource Technology*, Volume 101(20) (2010): 7849-7855

The primary degradation–reaction products (DRP) identified in Ammonia Fiber Expansion (AFEX)-pretreated corn stover are acetate, lactate, 4-hydroxybenzaldehyde (4HBD) and acetamide. The effects of these products at a broad concentration range were tested on *Escherichia coli* KO11, a strain engineered for cellulosic ethanol production. Fermentations using glucose or xylose as the sole carbohydrate source and a sugar mixture of glucose and xylose were conducted to determine how these products and sugar selection affected fermentation performance. Co-fermentation of the sugar mixture exhibited the lowest overall ethanol productivity compared to single-sugar fermentations and was more susceptible to inhibition. Metabolic ethanol yield increased with the increasing initial concentration of acetate. Although these degradation–reaction products (with exception of acetamide) are generally perceived to be inhibitory, organic acids and 4-hydroxybenzaldehyde at low levels stimulated fermentation. Adaptation of cells to these products prior to fermentation increased overall fermentation rate.

Keywords: Cellulosic ethanol; Fermentation; AFEX pretreatment; *Escherichia coli*; Acetate

Abbreviations: AFEX, Ammonia Fiber Expansion; CS, corn stover; DRP, degradation–reaction product

C. García-Balboa^a, I. Chion Bedoya^b, F. González^a, M.L. Blázquez^a, J.A. Muñoz^a and A. Ballester^a. (^aDepartamento de Ciencia de los Materiales e Ingeniería Metalúrgica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain, ^bFacultad de Ciencias y Filosofía, Departamento de Química, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, Urb. Ingeniería-San Martín de Porres, Lima 31, Peru). **Bio-reduction of Fe(III) ores using three pure strains of *Aeromonas hydrophila*, *Serratia fonticola* and *Clostridium celerecrescens* and a natural consortium. *Bioresource Technology*, Volume 101(20) (2010): 7864-7871**

The present work describes a research approach to the anaerobic bioleaching of Fe(III) ores. Three strains (*Serratia fonticola*, *Aeromonas hydrophila* and *Clostridium celerecrescens*) isolated from an acidic abandoned mine were selected to test their ability to reduce dissimilatory Fe(III). Total iron bio-reduction was achieved after 48 h using either the consortium or the *Aeromonas* cultures. In the latter case, there was no evidence of precipitates and Fe(II) remained in solution at neutral pH through complex formation with citrate. None of the other cultures tested (mixed culture and the two isolates) exhibited this behaviour. Biotechnologically, this is a very promising result since it obviates the problem associated with undesirable precipitation of iron compounds in Fe(III)-reducing bacterial cultures. The performance of the *Aeromonas* culture was improved progressively by adaptation to moderately acidic pH values (up to 4.5) and to three different Fe(III)-oxyhydroxides as the sole source of iron: ferrihydrite, hematite and jarosite, commonly found as weathering compounds at mine sites. Dissimilatory Fe(III)-reducers for iron extraction from ores is therefore especially attractive in that acidification of the surrounding area can be minimized.

Keywords: Microbial; Iron-ores; Anaerobic reduction

M. Srikanth Reddy^a, B. Naresh^a, T. Leela^a, M. Prashanthi^a, N.Ch. Madhusudhan^a, G. Dhanasri^a and Prathibha Devi^a. (^a Biotechnology Laboratory, Department of Botany, Osmania University, Hyderabad 500007, India). **Biodegradation of phenanthrene with biosurfactant production by a new strain of *Brevibacillus* sp. Bioresource Technology, Volume 101(20) (2010): 7980-7983**

In this work, a phenanthrene-degrading bacterial strain was isolated by enrichment method from hydrocarbon contaminated sludge samples and identified as *Brevibacillus* sp. PDM-3 based on morphological, biochemical, chemotaxonomic (FAMEs analysis) and molecular (16S rDNA sequencing) analysis. Growth parameters for efficient degradation of phenanthrene such as nutrient medium, pH, temperature, rpm and inoculum size were standardized and 93% of phenanthrene was degraded in 6 days as analysed by HPLC. The bacterial strain PDM-3 also has the ability to produce biosurfactant during phenanthrene degradation as detected by the surface tension measurements of the culture supernatant and the emulsification index (EI₂₄). The biosurfactant was identified by its functional groups through FT-IR spectroscopy. Phenanthrene degradation and biosurfactant production are associated with each other and can be used in environmental biotechnology. Further, the strain has the ability to degrade other PAHs such as anthracene and fluorene by utilizing them as sole carbon and energy source.

Keywords: Biodegradation; Phenanthrene; Biosurfactant; *Brevibacillus* sp.

Heartwin A. Pushpadass^a, Robert W. Weber^a, Joseph J. Dumais^b and Milford A. Hanna^a. (^a Industrial Agricultural Products Center, 211 L.W. Chase Hall, University of Nebraska, Lincoln, NE 68583-0730, USA, ^b Department of Chemistry, Hamilton Hall, University of Nebraska-Lincoln, Lincoln, NE, USA). **Biodegradation characteristics of starch-polystyrene loose-fill foams in a composting medium[†]. Bioresource Technology, Volume 101(19) (2010): 7258-7264**

The structures and biodegradabilities of loose-fill foams, containing starch and polystyrene at ratios of 70:30 and 80:20, were evaluated using a laboratory composting system. Each formulation was foamed (extrusion expanded) using either 0.2% azodicarbonamide or 0.25% citric acid as the chemical blowing agent. Biodegradability, a measure of the quantity of material mineralized, was expressed as the percentage of CO₂ in the exhaust gas eluted from the individual chambers. The CO₂ generation peaked after about 15 days of composting, and then decreased. The rate and amount of CO₂ eluted depended on the starch content in the foams. Similarly, there were significant differences in the rates and quantities of CO₂ emissions for the foams blown with azodicarbonamide versus citric acid. At the end of the composting tests, the remaining foam material had fibrous and crumbly textures, presumably consisting primarily of polystyrene. FTIR and NMR spectra of the foams, taken after 39 days of composting, did not reveal the spectral features of starch, thereby confirming the decomposition of the starch.

Keywords: Biodegradation; Carbon dioxide; FTIR; NMR; Polystyrene

A.L. Rodrigues^a, M.A. Pereira^a, P. Janknecht^b, A.G. Brito^a and R. Nogueira^a. (^a IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal, ^b Stadtwerke

Düsseldorf AG – Wasserwerke, Himmelgeister Landstrasse 1, 40589 Düsseldorf, Germany). Biofilms formed on humic substances: Response to flow conditions and carbon concentrations. *Bioresource Technology*, Volume 101(18) (2010): 6888-6894

Stream biofilms are exposed to dynamic conditions of flow velocity and organic carbon availability. Thus, the aim of this study was to investigate the response of biofilms formed with and without humic substances (HSs) to an increase in flow velocity ($0.04\text{--}0.10\text{ m s}^{-1}$) and HSs concentration (9.7 ± 1.0 to $19.8 \pm 0.4\text{ mg L}^{-1}\text{ C}$). The highest amount of biofilm, measured as volatile suspended solids and total countable cells, was observed at 0.10 m s^{-1} without HSs. The bacterial community composition of the biofilm with HSs was characterized by sequences with high similarities ($\geq 97\%$) to the genus *Dokdonella* and to the genera *Comamonas*, *Cupriavidus* and *Ralstonia*. Sequences retrieved from the biofilm without HSs presented high similarities ($\geq 97\%$) to the genus *Sphingomonas* and the genus *Nitrosospira*. Experimental results suggested that the presence of HSs under different concentrations and flow velocities did not significantly enhance the cell density of biofilms but influenced its microbial composition.

Keywords: Humic substances; Biofilm; Flow velocity; Community composition; DGGE

Weihong Zhong^a, Chenjing Zhu^a, Ming Shu^b, Kedan Sun^a, Lei Zhao^a, Chang Wang^a, Zhijuan Ye^c and Jianmeng Chen^a. (^a College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, PR China, ^b Technology Center, China Tobacco Zhejiang Industrial Co. Ltd., Hangzhou 310009, PR China, ^c Technology Center, Hangzhou Liquor Environment Protecting Paper Co. Ltd., Hangzhou 310018, PR China). Degradation of nicotine in tobacco waste extract by newly isolated *Pseudomonas* sp. ZUTSKD. *Bioresource Technology*, Volume 101(18) (2010): 6935-6941

The newly isolated *Pseudomonas* sp. ZUTSKD was evaluated for its ability to degrade nicotine in tobacco waste extract (TWE). The strain degraded nicotine completely when the concentration of reducing sugar in TWE was lower than 8 g L^{-1} . Yeast extract and phosphate additions improved nicotine degradation in 5% TWE. At $30\text{ }^{\circ}\text{C}$ and pH 7.0, with additional $15\text{ g L}^{-1}\text{ Na}_2\text{HPO}_4\cdot 6\text{H}_2\text{O}$ and $6\text{ g L}^{-1}\text{ KH}_2\text{PO}_4$ in 5% TWE, *Pseudomonas* sp. ZUTSKD could degrade 97% of nicotine (1.6 g L^{-1}) in 12 h. The data showed that strain ZUTSKD could be useful to control the nicotine content in TWE.

Keywords: Nicotine; Degradation; *Pseudomonas* sp.; Tobacco waste extract

Lorena Lima de Oliveira^a, Rachel Biancalana Costa^a, Dagoberto Yukio Okada^a, Daniele Vital Vich^a, Iolanda Cristina Silveira Duarte^b, Edson Luiz Silva^c and Maria Bernadete Amâncio Varesche^a. (^a Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo, Av. Trabalhador São-carlense, 400, 13566-590 São Carlos, SP, Brazil, ^b Federal University of São Carlos, Rod. João Leme dos Santos, Km 110, SP 264, 18052-780 Sorocaba, SP, Brazil, ^c Department of Chemical Engineering, Federal University of São Carlos, Rod. Washington Luiz, Km 235, SP 310, 13565-905 São Carlos, SP, Brazil). Anaerobic degradation of linear alkylbenzene sulfonate (LAS) in

fluidized bed reactor by microbial consortia in different support materials. *Bioresource Technology*, Volume 101(14) (2010): 5112-5122

Four anaerobic fluidized bed reactors filled with activated carbon (R1), expanded clay (R2), glass beads (R3) and sand (R4) were tested for anaerobic degradation of LAS. All reactors were inoculated with sludge from a UASB reactor treating swine wastewater and were fed with a synthetic substrate supplemented with approximately 20 mg l⁻¹ of LAS, on average. To 560 mg l⁻¹ COD influent, the maximum COD and LAS removal efficiencies were mean values of 97 ± 2% and 99 ± 2%, respectively, to all reactors demonstrating the potential applicability of this reactor configuration for treating LAS. The reactors were kept at 30 °C and operated with a hydraulic retention time (HRT) of 18 h. The use of glass beads and sand appear attractive because they favor the development of biofilms capable of supporting LAS degradation. Subsequent 16S rRNA gene sequencing and phylogenetic analysis of samples from reactors R3 and R4 revealed that these reactors gave rise to broad microbial diversity, with microorganisms belonging to the phyla Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria, indicating the role of microbial consortia in degrading the surfactant LAS.

Keywords: Fluidized bed reactor; Immobilized biomass; Support material; Surfactant degradation; 16S rRNA

Ignacio de Godos^{a, b, 1}, Virginia A. Vargas^{c, 2}, Saúl Blanco^{c, 4}, María C. García González^{d, 3}, Roberto Soto^{c, 2}, Pedro A. García-Encina^a, Eloy Becares^{b, 1} and Raúl Muñoz^a. (^a Department of Chemical Engineering and Environmental Technology, University of Valladolid, Paseo del Prado de la Magdalena s/n, 47011 Valladolid, Spain, ^b Department of Biodiversity and Environmental Management, University of León, Campus Vegazana, 24071 León, Spain, ^c Center of Biotechnology, San Simon Mayor University of Cochabamba, Campus Universitario, s/n Cochabamba, Bolivia, ^d Institute of Agriculture Technology of Castilla y León (ITACyL), Ctra. Burgos, Km 119, 47071 Valladolid, Spain, ^e Institute of Environmental Sciences, University of León, C/La Serna 58, 24071 León, Spain). **A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. *Bioresource Technology*, Volume 101(14) (2010): 5150-5158**

Two green microalgae (*Scenedesmus obliquus* and *Chlorella sorokiniana*), one cyanobacterium (*Spirulina platensis*), one euglenophyt (*Euglena viridis*) and two microalgae consortia were evaluated for their ability to support carbon, nitrogen and phosphorous removal in symbiosis with activated sludge bacteria during the biodegradation of four and eight times diluted piggery wastewater in batch tests. *C. sorokiniana* and *E. viridis* were capable of supporting the biodegradation of four and eight times diluted wastewater. On the other hand, while *S. obliquus* and the consortia isolated from a swine manure stabilization pond were only able to grow in eight times diluted wastewater, *S. platensis* and the consortium isolated from a high rate algal pond treating swine manure were totally inhibited regardless of the dilution applied. TOC removal efficiencies (RE) ranging from 42% to 55% and NH₄⁺-RE from 21% to 39% were recorded in the tests exhibiting photosynthetic oxygenation. The similar oxygen production rates exhibited by the tested microalgae under autotrophic conditions (from 116 to 133 mg O₂ L⁻¹ d⁻¹) suggested that factors other than the photosynthetic oxygenation potential governed piggery wastewater biodegradation. Microalgal tolerance towards NH₃ was hypothesized as the key selection criterion. Further studies in a continuous algal–bacterial photobioreactor inoculated

with *C. sorokiniana*, *S. obliquus* and *S. platensis* showed that *C. sorokiniana*, the species showing the highest NH_3 -tolerance, rapidly outcompeted the rest of the microalgae during the biodegradation of eight times diluted wastewater, achieving TOC and NH_4^+ -RE comparable to those recorded in the batch biodegradation tests.

Keywords: Bioremediation; Microalgae selection; Nutrients removal; Photosynthetic oxygenation; Piggery wastewater

Qiao Lin^{a,b}, Wen Donghui^c and Wang Jianlong^a. (^a Laboratory of Environmental Technology, INET, Tsinghua University, Beijing 100084, PR China, ^b Beijing Wei Ming Kai Tuo Agriculture Biotechnology Co. Ltd., Beijing 100085, PR China, ^c College of Environmental Sciences and Engineering, Peking University, Beijing 100871, PR China). **Biodegradation of pyridine by *Paracoccus* sp. KT-5 immobilized on bamboo-based activated carbon. Bioresource Technology, Volume 101(14) (2010): 5229-5234**

The performance of pyridine degradation by *Paracoccus* sp. strain KT-5 immobilized on bamboo-based activated carbon was investigated and compared with that of free cells. The results showed that *Paracoccus* sp. was able to attach well on the surface and pores of bamboo-carbon. Compared with freely suspended cells, the pyridine-degrading rate of the immobilized cells was higher when initial pyridine concentration was in the range of 489–1476 mg/L, suggesting that the efficiency of pyridine degradation by *Paracoccus* sp. strain KT-5 was enhanced significantly by immobilization technique. Additionally, the attached biomass on the bamboo-carbon increased from 391.9 to 430–500 mg/g during pyridine degradation. Furthermore, the semi-continuous degradation of pyridine by immobilized cells were carried out when the pyridine concentration was in the range of 293–1476 mg/L, the results demonstrated the feasibility and reusability of immobilized cells for pyridine degradation.

Keywords: Pyridine; Biodegradation; *Paracoccus* sp.; Immobilization; Activated carbon

Aditi Banerjee^a and Alope K. Ghoshal^b. (^a Center for Environment, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India, ^b Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India). **Phenol degradation by *Bacillus cereus*: Pathway and kinetic modeling. Bioresource Technology, Volume 101(14) (2010): 5501-5507**

The microbial degradation of phenol by pure cultures *Bacillus cereus* MTCC 9817 strain AKG1 and *B. cereus* MTCC 9818 strain AKG2 is studied in batch mode for several initial concentrations of phenol in the range of 100–2000 mg/L with an interval of 100 mg/L. Degradation pathways are investigated at initial phenol concentrations of 100, 500, 1000, 1500, and 2000 mg/L. The bacteria are able to degrade phenol of concentration as high as 2000 mg/L. The maximum degradation rate is obtained at an initial phenol concentration of about 800 mg/L for the strain AKG1 and about 200 mg/L for the strain AKG2. Both the strains degrade phenol via *meta*-cleavage pathway through formation of 2-hydroxymuconic semialdehyde (2-HMSA) as an intermediate product. Modeling of the biodegradation of phenol indicates that the Haldane inhibitory model predicts the experimental data fairly well for both the strains.

Keywords: *Bacillus cereus*; Degradation kinetics; Degradation pathway; Phenol degradation

Nyuk-Min Chong and Jing-Ning Chiou. Biodegradation of BTE in soil by indigenous microbial populations with and without biogenic substrates. World Journal of Microbiology and Biotechnology, Volume 26(9) (2010): 1587-1594

Degradation of benzene, toluene, and ethylbenzene (BTE) by microbial populations indigenous to the soil and populations proliferated from the indigenous using biogenic substrates were compared. The reaction system consisted of aerobic microcosms representing an unsaturated soil. Microcosms supplemented with glucose and citrate, when compared to the unsupplemented microcosms, showed increases in bacterial counts, but the overall degradation rates for B, T, or E were reduced in spite of shorter lag times. Both biogenic substrate supplements were non-beneficial for BTE degradation due largely to the preferential and healthy growth of the indigenous populations on the biogenic substrates, and thus the urgency of developing a favorable amount of BTE degraders was reduced.

Keywords: Bioremediation - BTEX - Soil microcosm - Biogenic substrate - Diauxic growth

Luiz Fernando Ferreira, Mario Aguiar, Georgia Pompeu, Tâmara Guido Messias and Regina Rosim Monteiro. Selection of vinasse degrading microorganisms. World Journal of Microbiology and Biotechnology, Volume 26(9) (2010): 1613-1621

Vinasse is a highly colored effluent with a high pollutant potential when disposed in the environment. Assays for decolorization of vinasse were performed, selecting the fungus *Pleurotus sajor-caju* CCB 020. The discoloration was cocominant with the increase of the activities of laccase, manganese-peroxidase and peroxidases. *P. sajor-caju* demonstrated a rise in biomass production ($1.06 \text{ g } 100 \text{ ml}^{-1}$), and the enzyme activities such as laccase (varying from 400 to 450 IU l^{-1}) reached between the 9th and 10th day of growth and for MnP at the 12th day of cultivation (varying from 60 to 100 IU l^{-1}). It was concluded that the system *P. sajor-caju*/vinasse can be utilized as a bioprocess for color removal and degradation of complex vinasse compounds. It was observed an improvement in the characteristics and detoxification allowing its utilization as reused water, laccase and manganese-peroxidase enzymes production and for fungal biomass production with a high nutritional value.

Keywords: Vinasse - Biodegradation - Decolorization - Enzymes - Lignolytic fungi

Isam A. Mohamed Ahmed, Jiro Arima, Tsuyoshi Ichianagi, Emi Sakuno and Nobuhiro Mori. Isolation and characterization of homocholine-degrading *Pseudomonas* sp. strains A9 and B9b. World Journal of Microbiology and Biotechnology, Volume 26(8) (2010): 1455-1464

Soil isolates, identified as *Pseudomonas* sp. strain A9 and *Pseudomonas* sp. strain B9b (based on the phenotypic features and phylogenetic analysis) were found to degrade homocholine aerobically. Morphological characterization using the optical microscope under light and phase contrast conditions showed that cells of strain A9 formed short rods measuring approximately $0.5\text{--}1 \times 1.5\text{--}2.0 \text{ }\mu\text{m}$ in size while those of B9b formed long rods of $0.5\text{--}1 \times 2.5\text{--}3.0 \text{ }\mu\text{m}$ during the early growth phase on both nutrient broth and basal-homocholine (basal-HC) media. Strain A9 was able to grow on basal-HC medium at a wide range of temperatures ($4\text{--}41^\circ\text{C}$) whereas strain B9b was not able to grow at either 4 or 41°C . Comparative 16S rRNA sequencing studies

indicated that strain A9 fell into the *Pseudomonas putida* subclade whereas strain B9b located in *Pseudomonas fulva* subclade. Washed cells of strains A9 and B9b degraded homocholine completely within 6 h with concomitant formation of several metabolites. Analysis of the metabolites by capillary electrophoresis, fast atom bombardment–mass spectrometry, and gas chromatography–mass spectrometry, showed trimethylamine (TMA) as the major metabolite beside β -alanine betaine and trimethylaminopropionaldehyde. Therefore, the possible degradation pathway of homocholine in the isolated strains is through successive oxidation of the alcohol group (–OH) to aldehyde (–CHO) and acid (–COOH), and thereafter the cleavage of β -alanine betaine C–N bonds yielding trimethylamine and an alkyl chain.

Keywords: Biodegradation - Homocholine - *Pseudomonas* sp. - 3-*N*-Trimethylamino-1-propanol - 16S rRNA gene sequence

Flavio M. Lopes, Karla A. Batista, Gustavo L. A. Batista, Sydnei Mitidieri, Luiz Artur M. Bataus and Kátia F. Fernandes. Biodegradation of epoxyconazole and piraclostrobin fungicides by *Klebsiella* sp. from soil. World Journal of Microbiology and Biotechnology, Volume 26, Number 7, 1155-1161

Three bacterial strains have been isolated from soil in which soybean had been continuously cropped and treated with Opera[®], a fungicide containing epoxyconazole and pyraclostrobin. The three strains (1,805, 2,801 and 3,803), obtained from soil at 80–100 cm depth, were selected on medium containing 0.03% Opera[®]. Morphological examination revealed that the strains were Gram-negative, and two of them (1,805 and 2,801) exhibited polymorphism. The growth profiles demonstrated that 1,805 and 3,803 were more efficient growing in the presence of Opera[®] than 2,801. Maximum growth was reached between 24 and 48 h, however, 2,801 was not able to survive after this period. The total protein content produced by 1,805, 2,801 and 3,803 in liquid selective medium containing Opera[®] were 111.0 ± 0.02 , 80.0 ± 0.05 and 130.5 ± 0.07 $\mu\text{g/ml}$, respectively. According to its biochemical and molecular features, strain 1,805 was identified as *Klebsiella* sp. On the basis of the characteristics presented (facultative anaerobic nature, polymorphic character and capacity of growing in the presence of Opera[®]) strain 1,805 seems to be able to degrade the epoxyconazole and pyraclostrobin.

Keywords: *Klebsiella* sp. - Biodegradation - Fungicides - Opera[®]

Samuel G. Dennison^{a, b, 1}, Parnell O'Brien^{a, 1}, Saumya Gopalkrishnan^{a, 2} and Benjamin C. Stark^a. (^a Biology Division, Department of Biological, Chemical, and Physical Sciences, Illinois Institute of Technology, Chicago, IL 60616, USA, ^b Metropolitan Water Reclamation District of Greater Chicago, Chicago, IL, USA). Enhancement of aerobic degradation of benzoate and 2-chlorobenzoate by adapted activated sludge. Microbiological Research, Volume 165(8) (2010): 687-694

Activated sludge from the Stickney Water Reclamation Plant of the Metropolitan Water Reclamation District of Greater Chicago was adapted in the laboratory to either benzoate or 2-chlorobenzoate as the sole carbon source in sequencing batch reactors with a 48-h feed–aerate–settle–draw cycle and a mean cell residence time (MCRT) of 10 days. Benzoate degradation increased by more than 80-fold after 7 MCRTs compared to unadapted activated sludge. A greater than 15-fold increase in 2-chlorobenzoate metabolism occurred after adaptation for about

5–7 MCRTs. For each substrate the maximum rate measured for adapted cultures was near or above the highest previously reported in the literature. For both adapted and unadapted sludges, benzoate metabolism was considerably faster than that of 2-chlorobenzoate, and for both substrates the rate of metabolism increased incrementally with time of adaptation. As expected, addition of the benzoate-adapted sludge to unadapted sludge enhanced the latter's ability to degrade benzoate.

Keywords: Activated sludge; Adaptation; Benzoate; 2-Chlorobenzoate; Sequencing batch reactor(s)

Christophe Chassard, Eve Delmas, Céline Robert, Annick Bernalier-Donadille. The cellulose-degrading microbial community of the human gut varies according to the presence or absence of methanogens. FEMS Microbiology Ecology. Volume 74(1) (2010): 205–213

Cellulose-degrading microorganisms involved in the breakdown of plant cell wall material in the human gut remain rather unexplored despite their role in intestinal fermentation. Microcrystalline cellulose-degrading bacteria were previously identified in faeces of methane-excreting individuals, whereas these microorganisms were undetectable in faecal samples from non-methane excretors. This suggested that the structure and activity of the cellulose-degrading community differ in methane- and non-methane-excreting individuals. The purpose of this study was to characterize in depth this cellulose-degrading community in individuals of both CH₄ statuses using both culture-dependent and molecular methods. A new real-time PCR analysis was developed to enumerate microcrystalline cellulose-degrading ruminococci and used to confirm the predominance of these hydrolytic ruminococci in methane excretors. Culture-dependent methods using cell wall spinach (CWS) residue revealed the presence of CWS-degrading microorganisms in all individuals. Characterization of CWS-degrading isolates further showed that the main cellulose-degrading bacteria belong essentially to *Bacteroidetes* in non-methane-excreting subjects, while they are predominantly represented by *Firmicutes* in methane-excreting individuals. This taxonomic diversity was associated with functional diversity: the ability to degrade different types of cellulose and to produce H₂ from fermentation differed depending on the species. The structure of the cellulolytic community was shown to vary depending on the presence of methanogens in the human gut.

Keywords: human intestinal microbiota; cellulose degradation; bacterial community structure; metabolic activity; methanogenesis

Ziv Arbeli, Cilia Fuentes. Prevalence of the gene *trzN* and biogeographic patterns among atrazine-degrading bacteria isolated from 13 Colombian agricultural soils. FEMS Microbiology Ecology. Volume 73(3) (2010): 611–623

The following study evaluated the diversity and biogeography of 83 new atrazine-degrading bacteria and the composition of their atrazine degradation genes. These strains were isolated from 13 agricultural soils and grouped according to rep-PCR genomic fingerprinting into 11 major clusters, which showed biogeographic patterns. Three clusters (54 strains) belonged to the genus *Arthrobacter*, seven clusters (28 strains) were similar to the genus *Nocardioides* and only one strain was a gram-negative from the genus *Ancylobacter*. PCR assays for the detection of the genes *atzA*, *B*, *C*, *D*, *E*, *F* and *trzN* conducted with each of the 83 strains revealed that 82 strains (all gram positive) possessed *trzN*, 74 of them possessed the combination of *trzN*, *atzB* and *atzC*,

while only the gram-negative strain had *atzA*. A similar PCR assay for the two analogous genes, *atzA* and *trzN*, responsible for the first step of atrazine degradation, was performed with DNA extracted directly from the enrichment cultures and microcosms spiked with atrazine. In these assays, the gene *trzN* was detected in each culture, while *atzA* was detected in only six out of 13 soils. These results raise an interesting hypothesis on the evolutionary ecology of the two atrazine chlorohydrolase genes (i.e. *atzA* and *trzN*) and about the biogeography of atrazine-degrading bacteria.

Keywords: atrazine; biodegradation; *atzA*; *trzN*; Colombia; Tropic

Joaquim Vila¹, José María Nieto¹, Jelle Mertens², Dirk Springael², Magdalena Grifoll¹. Microbial community structure of a heavy fuel oil-degrading marine consortium: linking microbial dynamics with polycyclic aromatic hydrocarbon utilization. FEMS Microbiology Ecology, Volume 73(2) (2010): 349–362

A marine microbial consortium obtained from a beach contaminated by the *Prestige* oil spill proved highly efficient in removing the different hydrocarbon families present in this heavy fuel oil. Seawater cultures showed a complete removal of all the linear and branched alkanes, an extensive attack on three to five-ring polycyclic aromatic hydrocarbons [PAHs; including anthracene, fluoranthene, pyrene, benzo(*a*)anthracene, chrysene, and benzo(*a*)pyrene] (30–100%), and a considerable depletion of their alkyl derivatives. Community dynamics analysis revealed that *Alcanivorax* species, known alkane degraders, predominated in the initial stages. This was followed by an increase in *Alphaproteobacteria* (i.e. *Maricaulis*, *Roseovarius*), which coincided with the depletion of low molecular PAHs. Finally, these were succeeded by *Gammaproteobacteria* (mainly *Marinobacter* and *Methylophaga*), which were involved in the degradation of the high molecular-weight PAHs. The role of these populations in the removal of the specific components was confirmed by the analysis of subcultures established using the aliphatic or the aromatic fraction of the fuel oil, or single PAHs, as carbon sources. The genus *Marinobacter* seemed to play a major role in the degradation of a variety of hydrocarbons, as several members of this group were isolated from the different enrichment cultures and grew on plates with hexadecane or single PAHs as sole carbon sources.

Keywords: microbial diversity; PAHs; oil-degrading consortium; DGGE; biodegradation

Lorraine Lillis, Nicholas Clipson, Evelyn Doyle. Quantification of catechol dioxygenase gene expression in soil during degradation of 2,4-dichlorophenol. FEMS Microbiology Ecology. Volume 73(2) (2010): 363–369

The *tfdC* and *C23O* genes encode two catechol dioxygenases that catalyse *ortho* and *meta* cleavage of a key metabolite (chlorocatechol) of 2,4-dichlorophenol (2,4-DCP) metabolism, respectively. Primers were designed and a real-time PCR assay was developed to assess the abundance and expression of both *tfdC* and *C23O* genes in a soil amended with 2,4-DCP over a 21-day period. *tfdC*, the gene encoding the *ortho* cleaving dioxygenase, was significantly more abundant than the *meta* cleaving dioxygenase gene (*C23O*) throughout the experiment. The highest levels of *tfdC* were observed 2 days after amendment of soil with 2,4-DCP, at which stage the rate of 2,4-DCP degradation was at its maximum. In contrast, *C23O* copy numbers declined initially and peaked when degradation had slowed considerably. mRNA of the two

chlorocatechol dioxygenase genes was not detected on day 0, but both genes were expressed after this time point. *tfdC* was expressed at a significantly higher level than *C23O* in 2,4-DCP-amended soil throughout the course of the microcosm, indicating the dominance of the *ortho* metabolic pathway. Phylogenetic analysis revealed a wide diversity of chlorocatechol dioxygenase genes in the 2,4-DCP-exposed soil examined.

Keywords: 2,4-DCP; biodegradation; gene expression; catechol dioxygenase

Li Wang, Yang Wen, Xinqing Guo, Guangli Wang, Shunpeng Li and Jiandong Jiang. Degradation of methamidophos by *Hyphomicrobium* species MAP-1 and the biochemical degradation pathway. *Biodegradation*, Volume 21(4) (2010): 513-523

Methamidophos is one of the most widely used organophosphorus insecticides usually detectable in the environment. A facultative methylotroph, *Hyphomicrobium* sp. MAP-1, capable of high efficiently degrading methamidophos, was isolated from methamidophos-contaminated soil in China. It was found that the addition of methanol significantly promoted the growth of strain MAP-1 and enhanced its degradation of methamidophos. Further, this strain could utilize methamidophos as its sole carbon, nitrogen and phosphorus source for growth and could completely degrade 3,000 mg l⁻¹ methamidophos in 84 h under optimal conditions (pH 7.0, 30°C). The enzyme responsible for methamidophos degradation was mainly located on the cell inner membrane (90.4%). During methamidophos degradation, three metabolites were detected and identified based on tandem mass spectrometry (MS/MS) and gas chromatography-mass spectrometry (GC-MS) analysis. Using this information, a biochemical degradation pathway of methamidophos by *Hyphomicrobium* sp. MAP-1 was proposed for the first time. Methamidophos is first cleaved at the P-N bond to form *O,S*-dimethyl hydrogen thiophosphate and NH₃. Subsequently, *O,S*-dimethyl hydrogen thiophosphate is hydrolyzed at the P-O bond to release -OCH₃ and form *S*-methyl dihydrogen thiophosphate. *O,S*-dimethyl hydrogen thiophosphate can also be hydrolyzed at the P-S bond to release -SCH₃ and form methyl dihydrogen phosphate. Finally, *S*-methyl dihydrogen thiophosphate and methyl dihydrogen phosphate are likely transformed into phosphoric acid.

Keywords: Biodegradation pathway - Facultative methylotroph - *Hyphomicrobium* sp. MAP-1 - Methamidophos

Kazunari Sei, Takashi Kakinoki, Daisuke Inoue, Satoshi Soda, Masanori Fujita and Michihiko Ike. Evaluation of the biodegradation potential of 1,4-dioxane in river, soil and activated sludge samples. *Biodegradation*, Volume 21(4) (2010): 585-591

To evaluate the biodegradation potential of 1,4-dioxane in natural environments, a total of 20 environmental samples including river water, activated sludge, soil from the drainage area of a chemical factory and garden soil were subjected to a 1,4-dioxane degradation test. The five soil samples from the drainage area of the chemical factory were capable of reducing 100 mg l⁻¹ of 1,4-dioxane to below the detection limit (0.8 mg l⁻¹) within 33 days. In one activated sludge sample, 100 mg l⁻¹ of 1,4-dioxane decreased by 69% within 14 days via cometabolic degradation in the presence of 100 mg l⁻¹ of tetrahydrofuran (THF). The ability of all samples to degrade 1,4-dioxane degradation with or without THF increased after repeated enrichment, except for one soil sample from the drainage area of the chemical factory that was no longer able to degrade 1,4-dioxane after the third cycle of enrichment. However, most of the samples (14/20) were not

able to degrade 1,4-dioxane degradation. Thus, it can be concluded that the potential for 1,4-dioxane degradation is not ubiquitously distributed in natural environment.

Keywords: 1,4-Dioxane - Biodegradation potential - Environmental samples - Tetrahydrofuran (THF) - Enrichment culture

Rutwik Ravindra Mandakini Thengodkar and S. Sivakami. Degradation of Chlorpyrifos by an alkaline phosphatase from the cyanobacterium *Spirulina platensis*. Biodegradation, Volume 21(4) (2010): 637-644

Spirulina is a photosynthetic, filamentous, spiral-shaped, multicellular, blue-green microalga. The two most important species are *Spirulina maxima* and *Spirulina platensis*. *Spirulina* is considered an excellent food, lacking toxicity and having corrective properties against viral attacks, anemia, tumor growth and malnutrition. We have observed that cultures of *Spirulina platensis* grow in media containing up to 80 ppm of the organophosphorous pesticide, Chlorpyrifos. It was found to be due to an alkaline phosphatase (ALP) activity that was detected in cell free extracts of *Spirulina platensis*. This activity was purified from the cell free extracts using ammonium sulphate precipitation and gel filtration and shown to belong to the class of EC 3.1.3.1 ALP. The purified enzyme degrades 100 ppm Chlorpyrifos to 20 ppm in 1 h transforming it into its primary metabolite 3, 5, 6-trichloro-2-pyridinol. This is the first report of degradation of Chlorpyrifos by *Spirulina platensis* whose enzymic mechanism has been clearly identified. These findings have immense potential for harnessing *Spirulina platensis* in bioremediation of polluted ecosystems.

Keywords: Alkaline phosphatase - Chlorpyrifos - Cyanobacteria - *Spirulina platensis* - Bioremediation

Ram Naresh Bharagava and Ram Chandra. Biodegradation of the major color containing compounds in distillery wastewater by an aerobic bacterial culture and characterization of their metabolites. Biodegradation, Volume 21(5) (2010): 703-711

This study deals the biodegradation of the major color containing compounds extracted from distillery wastewater (DWW) by an aerobic bacterial consortium comprising *Bacillus licheniformis* (DQ79010), *Bacillus* sp. (DQ779011) and *Alcaligenes* sp. (DQ779012) and characterization of metabolic products. The degradation of color containing compounds by bacteria was studied by using the different carbon and nitrogen sources at different environmental conditions. Results revealed that the bacterial consortium was efficient for 70% color removal in presence of glucose (1.0%) and peptone (0.1%) at pH 7.0 and temperature 37°C. The HPLC analysis of control and bacterial degraded samples has shown the reduction in peak area as well as shifting of peaks compared to control indicating the bacterial degradation as well as transformation of color containing compounds from DWW. The comparative LC-MS-MS and other spectrophotometric analysis has shown the presence of dihydroxyconiferyl alcohol, 2, 2'-bifuran-5-carboxylic acid, 2-nitroacetophenone, p-chloroanisole, 2, 3-dimethylpyrazine, 2-methylhexane, methylbenzene, 2, 3-dihydro-5-methylfuran, 3-pyrroline, and acetic acid in control samples that were biodegraded and biotransformed into 2-nitroacetophenone, p-chloroanisole, 2, 2'-bifuran, indole, 2-methylhexane, and 2, 3-dihydro-5-methylfuran by bacterial consortium. In this study, it was observed that most of the compounds detected in control

samples were diminished from the bacterial degraded samples and compounds 2, 2'-bifuran and indole with molecular weight 134 and 117 were produced as new metabolites during the bacterial degradation of color containing compounds from DWW.

Keywords: Distillery wastewater - Melanoidins - Biodegradation - Aerobic bacteria - Metabolites

K. S. Rajini, Ch. Sasikala and Ch. V. Ramana. Reductive degradation of pyrazine-2-carboxylate by a newly isolated *Stenotrophomonas* sp. HCU1. Biodegradation, Volume 21(5) (2010): 801-813

A bacterium growing on pyrazine-2-carboxylate broth was isolated, purified and identified as a strain of *Stenotrophomonas* sp. based on polyphasic taxonomic analyses and designated as strain HCU1. 16S rRNA gene sequence of strain HCU1 showed 98.7% sequence similarity with the type strain of *Stenotrophomonas maltophilia* belonging to *Gammaproteobacteria*. Growth of strain HCU1 was demonstrated when pyrazine-2-carboxylate was used as a sole source of nitrogen. Ring reduction of pyrazine-2-carboxylate was shown as increase in absorbance at 268 nm and the reduced product was confirmed as 1,2,5,6-tetrahydropyrazine-2-carboxylate, while a ring opened product, 2-amino-2-hydroxy-3-(methylamino) propanoic acid (with a loss in carbon atom), indicated a reductive degradation of pyrazine-2-carboxylate by strain HCU1.

Keywords: Biodegradation - Pyrazine-2-carboxylate - *Stenotrophomonas*

Shaker A. Hassan², Khasraw M. Hassan¹ and Amera Al-Rubeii². (¹Department of Animal Production, College of Agriculture, University of Sulaimani, Iraq, ²Department of Animal Research, College of Agriculture, University of Baghdad, Iraq. *Corresponding author. E-mail: xasraw57@yahoo.com). Carcass characteristics of Karadi lambs as affect by different levels of dietary supplement of rumen degradable nitrogen fed with *Nigella sativa*. African Journal of Biotechnology Vol. 9 (27) (2010): 4295-4299

The objective of the work described here was to investigate the effect of two levels of *Nigella sativa* (0 and 7.5 g NS / kg DM) supplementation to rations of Karadi lambs fed three levels of rumen degradable nitrogen (1, 1.3 and 1.6 g RDN / MJ of ME) on carcass characteristics, using 2 x 3 factorial experiment. Twenty four individual Karadi male lambs were used (four lambs for each treatment). They were weighing approximately 30 kg live weight and 7 months old. The diets were formulated to be given at 40% NaOH-treated barley straw DM to 60% concentrates DM. At the end of feeding trial, all the lambs were slaughtered after over night with feeding straw. Live weight gain was improved ($P < 0.05$) in lambs fed diets supplemented with NS as compared with those lambs fed diet without NS. However, live weight gain was not affected by levels of RDN. Changes in slaughter weight, Killing out proportion, leg cuts tissue, the wholesale cuts weight and fat-tail weight were not significantly affected by both NS supplementation and levels of RDN. However, the heavier slaughter weights, cold carcass and fat tail weights were associated with lambs fed highest level of RDN supplemented with NS ($P < 0.05$). Moreover, there is a linear increase in fat-tail weight associated with lambs fed increasing levels of RDN supplemented with NS.

Keyword: Karadi, Lambs, Carcass characteristics, Rumen degradable nitrogen, *Nigella Sativa*.

Abbreviations: RDN, Rumen degradable nitrogen; NS, *Nigella sativa*; SBM, soybean meal; FTSBM, formaldehyde- treated soybean meal; UDN, undegradable nitrogen; CRD, completely randomized design; FCR, feed conversion ratio; LWG, live weight gain; CCW, cold carcass weight; TN, total nitrogen; MJ, metabolizable energy.

Zewen Dai, Zhilong Wang, Jian-He Xu and Hanshi Qi. Assessing bioavailability of the solubilization of organic compound in nonionic surfactant micelles by dose–response analysis. Applied Microbiology and Biotechnology, Volume 88(1) (2010): 327-339

It is uncertain in some extent that organic compounds solubilized in micelles of a nonionic surfactant aqueous solution are bioavailable directly by the microbes in an extractive microbial transformation or biodegradation process. In this work, a dose–response method, where a bioequivalence concept is introduced to evaluate the synergic toxicity of the nonionic surfactants and the organic compounds, was applied to analyze the inhibition effect of organic compounds (naphthalene, phenyl ether, 2-phenylethanol, and 1-butanol) in nonionic surfactant Triton X-100 micelle aqueous solutions and Triton X-114 in aqueous solutions forming cloud point systems. Based on the result, a mole solubilization ratio of organic compounds in micelle was also determined, which consisted very well with those of classic semi-equilibrium dialysis experiments. The results exhibit that bioavailability of organic compounds solubilized in micelles to microbial cells is negligible, which provides a guideline for application of nonionic surfactant micelle aqueous solutions or cloud point systems as novel media for microbial transformations or biodegradations.

Keywords: Bioavailability - Dose–response analysis - Micelle - Nonionic surfactant - Cloud point system

C. Mirande¹, E. Kadlecikova¹, M. Matulova², P. Capek², A. Bernalier-Donadille¹, E. Forano¹, C. Béra-Maillet¹. Dietary fibre degradation and fermentation by two xylanolytic bacteria *Bacteroides xylanisolvens* XB1A^T and *Roseburia intestinalis* XB6B4 from the human intestine. Journal of Applied Microbiology, Volume 109(2) (2010): 451–460

AimsZ: To characterize fibre degradation, colonization and fermentation, and xylanase activity of two xylanolytic bacteria *Bacteroides xylanisolvens* XB1A^T and *Roseburia intestinalis* XB6B4 from the human colon.

Methods and Results: The bacteria grew well on all the substrates chosen to represent dietary fibres: wheat and corn bran, pea, cabbage and leek fibres, and also on purified xylans. *Roseburia intestinalis* colonized the substrates more efficiently than *Bact. xylanisolvens*. For the two bacteria, 80–99% of the total xylanase activity was associated with the cells whatever the substrate and time of growth. Optimal specific activities of cells were obtained on oat spelt xylan; they were higher than those previously measured for xylanolytic bacteria from the human gut. *Roseburia intestinalis* produced high molecular mass xylanases (100–70 kDa), while *Bact. xylanisolvens* produced lower molecular mass enzymes, including a cell-associated xylanase of 37 kDa.

Conclusions: The two bacteria display very high xylanolytic activity on the different substrates. Differences were observed on substrate attachment and enzyme systems, suggesting that the two species occupy different niches within the gut microbiota.

Significance and Impact of the Study: This study characterizes xylan degradation by two major species of the human intestine.

Keywords: *Bacteroides xylanisolvens*; fibre; human gut microbiota; *Roseburia intestinalis*; xylanase

S. Yousaf¹, K. Ripka¹, T.G. Reichenauer², V. Andria¹, M. Afzal¹, A. Sessitsch¹. Hydrocarbon degradation and plant colonization by selected bacterial strains isolated from Italian ryegrass and birdsfoot trefoil. *Journal of Applied Microbiology*, Volume 109(4) (2010): 1389–1401

Aims: To assess the degradation potential and plant colonization capacity of four alkane-degrading strains (ITSII10, ITRI15, ITRH76 and BTRH79) in combination with birdsfoot trefoil and Italian ryegrass and to evaluate the diversity of indigenous alkane-degrading soil bacteria in the rhizo- and endosphere.

Methods and Results: Contaminated soil was prepared by spiking agricultural soil with 10 g diesel fuel per kg soil. Italian ryegrass (*Lolium multiflorum* var. Taurus) and birdsfoot trefoil (*Lotus corniculatus* var. Leo) were inoculated with four alkane-degrading strains. Hydrocarbon degradation (up to 57%) was observed in all inoculated treatments of vegetated and unvegetated samples. Italian ryegrass in combination with compost and BTRH79 showed highest degradation, while birdsfoot trefoil performed best with compost and strain ITSII10. Cultivation-based as well as cultivation-independent analysis showed that both strains were competitive colonizers.

Conclusions: The combination between vegetation, inoculation with well-performing degrading bacteria and compost amendment was an efficient approach to reduce hydrocarbon contamination. Two *Pantoea* sp. strains, ITSII10 and BTRH79, established well in the plant environment despite the presence of a variety of other, indigenous alkane-degrading bacteria.

Significance and Impact of the Study: This study suggests that the application of degrading bacterial strains, which are able to compete with the native microflora and to tightly associate with plants, are promising candidates to be used for phytoremediation applications.

Keywords: bioremediation; degradation; rhizosphere

Agostina Chiavola, Renato Baciocchi and Federico Barducci. 3-Chlorophenol Biodegradation in a Sequencing Batch Reactor: Kinetic Study and Effect of the Filling Time. *Water, Air, & Soil Pollution*, Volume 212(1-4_ (2010): 219-229

Biodegradation kinetics of 3-chlorophenol (3-CP) were studied in two identical lab scale sequencing batch reactors (SBR) fed with the compound as the sole energy and carbon source and operated at different filling time (1 h for SBR1 and 2 h for SBR2). High removal efficiency was always obtained in both SBRs in the range of feed concentration of 300–960 mg L⁻¹. Increased feed load to 1,200 mg L⁻¹ 3-CP could also be removed in SBR1 despite the presence of inhibition, whereas determined failure of SBR2. Long filling time and high biomass

concentration were shown to have beneficial effect on process kinetics since they allowed to avoid substrate concentration peaks at the end of the fill phase. However, longer filling time (in the present case higher than 1 h) did not allow to select and enrich robust microbial population. The Haldane equation well fitted the kinetic test data measured in the presence of inhibition, i.e., at 960 and 1,200 mg L⁻¹ 3-CP in SBR1.

Keywords: 3-chlorophenol - Filling time - Haldane equation - Kinetics - Sequencing batch reactor

Hsien-Yi Lee, Chien-Li Lee and Chih-Ju G. Jou. Comparison Degradation of Pentachlorophenol Using Microwave-Induced Nanoscale Fe⁰ and Activated Carbon. Water, Air, & Soil Pollution, Volume 211(1-4) (2010): 17-24

Microwave (MW) is applied to enhance pentachlorophenol (PCP) removal using zerovalent iron (ZVI, Fe⁰) or granular activated carbon (GAC) as the dielectric media. Applying MW energy at 700 W for 20 s, the results show that Fe⁰ is capable of enhancing the CB removing 2.7 times (91% vs. 34 %) than GAC. Because Fe⁰ has higher dielectric loss (39.5 F/m vs. 8.3 F/m), it absorbs more MW energy to speed up the oxidation rate resulting in a faster temperature rise than GAC. Thus, in the presence of MW, Fe⁰ is superior to GAC for PCP removal. Additionally, excessive MW exposure will damage the surface structure of either Fe⁰ or GAC causing excessive electric charges to accumulate in the media that brings about the phenomenon of sparks.

Keywords: Microwave (MW) - Zerovalent iron - Granular activated carbon - Pentachlorophenol (PCP)

Mônica L. Adam, Rodrigo A. Torres, Graziela Sponchiado, Thalita S. Motta, Cíntia M. R. Oliveira, Marco A. Carvalho-Filho and M. T. S. Correia. Environmental Degradation at a Public Park in Southern Brazil as Revealed Through a Genotoxicity Test (MN) on Peripheral Blood Cells from *Poecilia vivipara* (Teleostei). Water, Air, & Soil Pollution, Volume 211(1-4) (2010): 61-68

The effects of anthropogenic activities on water, environment, and consequently quality of life can be evaluated using genetic, biochemical, and microbiological parameters. Regarding genetic parameters, the micronucleus test is a fast, efficient, inexpensive method for detecting alterations in genetic material induced by a variety of genotoxic agents. In the present study, blood cells from *Poecilia vivipara* from the Belém River in the city of Curitiba, Paraná, Brazil were evaluated for genotoxic effects stemming from human-produced pollution, as expressed by the micronucleus. The water in the river was evaluated with regard to physiochemical and microbiological parameters as well as for heavy metals. The analysis revealed the presence of copper, zinc, and nickel, with high concentrations of copper. The micronucleus analysis revealed significant differences in relation to the groups (study and control), suggesting a positive relation between the water quality of the Belém River and micronucleus expression as a result of the pollution to which this river is subjected.

Keywords: Micronuclei - Genotoxicity - Public parks - Environmental degradation - *Poecilia vivipara*

Amauri G. Souza, Leticia M. Costa, Rodinei Augusti and Zenilda L. Cardeal. Degradation of Prototype Pesticides Submitted to Conventional Water Treatment Conditions: The Influence of Major Parameters. *Water, Air, & Soil Pollution*, Volume 211(1-4) (2010): 427-434

The behavior of several pesticides in aqueous solution, namely bifenthrin, amethrin (pyrethroid insecticides), endosulfan and endosulfan sulfate (organochlorine pesticides), disulfoton, methyl pyrimiphos, and phorate (organophosphorus pesticides), submitted to the conditions typically employed in water treatment stations was investigated. Continuous pesticide depletion was monitored by solid-phase microextraction sampling followed by gas chromatography–mass spectrometry analysis. The influence of major parameters (sodium hypochlorite concentration, solution pH, and exposure time to ultraviolet (UV) light) was, thus, adequately established via two complementary approaches: factorial (2^3 , three variables—two levels) and Doehlert designs. Hence, the sodium hypochlorite concentration and the solution pH produced distinct effects depending on the pesticide evaluated (for instance, acidic and basic media caused increasing rates of degradation for the organophosphorus/pyrethroid and organochlorine pesticides, respectively). Conversely, higher rates of degradation were achieved for all of the pesticides investigated when increased exposure times to UV radiation were employed. Finally, the exposure time to UV radiation that lead to complete degradation of disulfoton and endosulfan sulfate (organophosphorus and organochlorine pesticides, respectively) in aqueous media under ordinary conditions employed in water treatment stations was established; disulfoton and endosulfan sulfate were completely degraded after 10 and 40 h, respectively.

Keywords: Organochlorine - Organophosphorus - Pyrethroid pesticides - Degradation - Sodium hypochlorite - pH - UV irradiation

Natalie R. Couling^a, Marcie G. Towell^a and Kirk T. Semple^a. (^a Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, United Kingdom). Biodegradation of PAHs in soil: Influence of chemical structure, concentration and multiple amendment. *Environmental Pollution*, Volume 158(11) (2010): 411-3420

The influence of PAH chemical structure and concentration, added in either single (75 or 300 mg kg⁻¹) or multiple (2 × 75, 2 × 150 or 4 × 75 mg kg⁻¹) applications as single- or multiple-contaminant systems, on the development of PAH biodegradation in a pristine soil was investigated. Development in microbial catabolic ability was assessed at 0, 28, 56 and 84 d by monitoring ¹⁴C-naphthalene, ¹⁴C-phenanthrene and ¹⁴C-pyrene mineralisation over 14 d in respirometric assays. The presence of other contaminants influenced the ability of the indigenous microflora to mineralise structurally different contaminants over time. ¹⁴C-Naphthalene mineralisation was inhibited by the presence of other contaminants; whereas the presence of naphthalene significantly enhanced rates of mineralisation in multiple-contaminant systems containing ¹⁴C-phenanthrene and ¹⁴C-pyrene. Generally, increasing the number of contaminant applications has implications for catabolic activity of soil microbes. It is suggested the toxic nature of PAHs retarded mineralisation at increased contaminant concentrations.

The simultaneous effects of PAH concentration, contaminant mixture and repeated application on the development of catabolic activity in soil.

Keywords: Catabolism; Mineralisation; Multiple applications; PAHs; Multiple-contaminant systems

Tineke De Wilde^a , Pieter Spanoghe^a, Jaak Ryckeboer^b, Peter Jaeken^{c, 1} and Dirk Springael^b. (^a Laboratory of Crop Protection Chemistry, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium, ^b Division Soil and Water Management, Faculty of Bioscience Engineering, Catholic University Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium, ^c PCF-Royal Research Station of Gorseme, De Brede Akker 13, 3800 Sint-Truiden, Belgium). Transport and degradation of pesticides in a biopurification system under variable flux, part I: A microcosm study. *Environmental Pollution*, Volume 158(1) (2010): 3309-3316

The efficiency of a biopurification system, developed to treat pesticide contaminated water, is to a large extent determined by the chemical and hydraulic load. Insight into the behaviour of pesticides under different fluxes is necessary. The behaviour of metalaxyl, bentazone, linuron, isoproturon and metamitron was studied under three different fluxes with or without the presence of pesticide-primed soil in column experiments. Due to the time-dependent sorption process, retention of the pesticides with intermediate mobility was significantly influenced by the flux. The higher the flux, the slower pesticides will be sorbed, which resulted in a lower retention. Degradation of the intermediate mobile pesticides was also submissive to variations in flux. An increase in flux, led to a decrease in retention, which in turn decreased the opportunity time for biodegradation. Finally, the presence of pesticide-primed soil was only beneficial for the degradation of metalaxyl.

Retention and degradation of pesticides in microcosms liable to different fluxes.

Keywords: Biopurification system; Flux; Pesticide; Sorption; Degradation; Transport

Tineke De Wilde^a , Pieter Spanoghe^a, Jaak Ryckeboer^b, Peter Jaeken^{c, 1} and Dirk Springael^b. (^a Laboratory of Crop Protection Chemistry, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium, ^b Division Soil and Water Management, Faculty of Bioscience Engineering, K.U. Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium, ^c PCF-Royal Research Station of Gorseme, De Brede Akker 13, 3800 Sint-Truiden, Belgium). Transport and degradation of pesticides in a biopurification system under variable flux Part II: A macrocosm study. *Environmental Pollution*, Volume 158(10) (2010): 3317-3322

Transport of bentazone, isoproturon, linuron, metamitron and metalaxyl were studied under three different flows in macrocosms. The aim was to verify the observations from Part I of the accompanying paper, with an increase in column volume and decrease in chemical and hydraulic load. Very limited breakthrough occurred in the macrocosms for all pesticides, except bentazone, at all flows.

From batch degradation experiments, it was observed that the lag time of metamitron and linuron decreased drastically in time for all flows, indicating a growth in the pesticide degrading population. This in contrast to isoproturon and metalaxyl, where an increase in lag time could be observed in time for all flows. From the batch degradation experiments, it could be concluded that the influence of flow on the lag time was minimal and that the inoculation of the pesticide-primed soil had a little surplus value on degradation.

Retention and degradation of pesticides in macrocosms liable to different fluxes.

Keywords: Macrocosm; Pesticide; Biopurification system; Flux; Sorption; Degradation

Nele Weyens^a, Sascha Truyens^a, Joke Dupae^a, Lee Newman^b, Safiyh Taghavi^b, Daniel van der Lelie^b, Robert Carleer^a and Jaco Vangronsveld^a. (^a Hasselt University, Centre for Environmental Sciences, Agoralaan Building D, B-3590 Diepenbeek, Belgium, ^b Brookhaven National Laboratory (BNL), Biology Department, Building 463, Upton NY 11973, USA). **Potential of the TCE-degrading endophyte *Pseudomonas putida* W619-TCE to improve plant growth and reduce TCE phytotoxicity and evapotranspiration in poplar cuttings. *Environmental Pollution*, Volume 158(9) (2010): 2915-2919**

The TCE-degrading poplar endophyte *Pseudomonas putida* W619-TCE was inoculated in poplar cuttings, exposed to 0, 200 and 400 mg l⁻¹ TCE, that were grown in two different experimental setups. During a short-term experiment, plants were grown hydroponically in half strength Hoagland nutrient solution and exposed to TCE for 3 days. Inoculation with *P. putida* W619-TCE promoted plant growth, reduced TCE phytotoxicity and reduced the amount of TCE present in the leaves. During a mid-term experiment, plants were grown in potting soil and exposed to TCE for 3 weeks. Here, inoculation with *P. putida* W619-TCE had a less pronounced positive effect on plant growth and TCE phytotoxicity, but resulted in strongly reduced amounts of TCE in leaves and roots of plants exposed to 400 mg l⁻¹ TCE, accompanied by a lowered evapotranspiration of TCE. Dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA), which are known intermediates of TCE degradation, were not detected.

The endophyte *P. putida* W619-TCE degrades TCE during its transport through the xylem, leading to reduced TCE concentrations in poplar, and decreased TCE evapotranspiration.

Keywords: *Pseudomonas putida* W619-TCE; Endophyte; Trichloroethylene; Phytoremediation; Poplar

Michael Fahrbach^a, Martin Krauss^a, Alfred Preiss^b, Hans-Peter E. Kohler^a and Juliane Hollender^a. (^a Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, P.O. Box 611, CH-8600 Dübendorf, Switzerland, ^b Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM), Nikolai-Fuchs-Strasse 1, D-30625 Hannover, Germany). **Anaerobic testosterone degradation in *Steroidobacter denitrificans* – Identification of transformation products. *Environmental Pollution*, Volume 158(8) (2010): 2572-2581**

The transformation of the androgenic steroid testosterone by gammaproteobacterium *Steroidobacter denitrificans* was studied under denitrifying conditions. For the first time, growth experiments showed that testosterone was mineralized under consumption of nitrate and concurrent biomass production. Experiments with cell suspensions using [4-¹⁴C]-testosterone revealed the intermediate production of several transformation products (TPs). Characterisation of ten TPs was carried out by means of HPLC coupled to high resolution mass spectrometry with atmospheric pressure chemical ionization as well as ¹H and ¹³C NMR spectroscopy. 3β-hydroxy-5α-androstan-17-one (trans-androsterone) was formed in the highest amount followed by 5α-androstan-3,17-dione. The data suggests that several dehydrogenation and hydrogenation processes take place concurrently in ring A and D because no consistent time-resolved pattern of TP peaks was observed and assays using 2 TPs as substrates resulted in essentially the same TPs.

The further transformation of testosterone in *S. denitrificans* seems to be very efficient and fast without formation of detectable intermediates.

Testosterone is completely mineralized by *Steroidobacter denitrificans* under denitrifying conditions with initial formation of several reduced and oxidized transformation products.

Keywords: Testosterone; Steroid; Biodegradation; Denitrification; Transformation product

Angela Yu-Chen Lin^a, Chih-Ann Lin^a, Hsin-Hsin Tung^a and N. Sridhara Chary^a. (^aGraduate Institute of Environmental Engineering, National Taiwan University, 71, Chou-Shan Rd., Taipei 106, Taiwan). **Potential for biodegradation and sorption of acetaminophen, caffeine, propranolol and acebutolol in lab-scale aqueous environments. Journal of Hazardous Materials, Volume 183(1-3) (2010): 242-250**

Sorption and combined sorption-biodegradation experiments were conducted in laboratory batch studies with 100 g soil/sediments and 500 mL water to investigate the fates in aqueous environments of acetaminophen, caffeine, propranolol, and acebutolol, four frequently used and often-detected pharmaceuticals. All four compounds have demonstrated significant potential for degradation and sorption in natural aqueous systems. For acetaminophen, biodegradation was found to be a primary mechanism for degradation, with a half-life ($t_{1/2}$) for combined sorption-biodegradation of 2.1 days; in contrast, sorption alone was responsible only for a 30% loss of aqueous-phase acetaminophen after 15 days. For caffeine, both biodegradation and sorption were important ($t_{1/2}$ for combined sorption-biodegradation was 1.5 days). However, for propranolol and acebutolol, sorption was found to be the most significant removal mechanism and was not affected by biodegradation. Desorption experiments revealed that the sorption process was mostly irreversible. High values were found for K_d for caffeine, propranolol, and acebutolol, ranging from 250 to 1900 L kg⁻¹, which explained their greater tendency for sorption onto sediments, compared to the more hydrophilic acetaminophen. Experimentally derived values for log K_{oc} differed markedly from values calculated from correlation equations. This discrepancy was attributed to the fact that these equations are well suited for hydrophobic interactions but may fail to predict the sorption of polar and ionic compounds. These results suggest that mechanisms other than hydrophobic interactions played an important role in the sorption process.

Keywords: Pharmaceuticals; Emerging contaminants; Sorption; Biodegradation; Distribution coefficient (K_d)

Liming Wang^a, Yong Li^b, Ping Yu^a, Zhixiong Xie^b, Yunbai Luo^a and Yanwen Lin^a. (^aCollege of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, PR China, ^b College of Life Sciences, Wuhan University, Wuhan 430072, PR China). **Biodegradation of phenol at high concentration by a novel fungal strain *Paecilomyces variotii* JH6. Journal of Hazardous Materials, Volume 183(1-3) (2010): 366-371**

A novel phenol-degrading filamentous fungus, strain JH6, was isolated from activated sludge and identified as a member of *Paecilomyces variotii* based on standard morphological and phylogenetic analysis. The degradation assays suggested that the strain was able to utilize phenol as the sole source of carbon and energy at concentrations up to 1800 mg/l. The strain exhibited

optimum phenol degradation performance with the addition of 100 mg/l glucose at pH 5, 37 °C. Haldane's model could be fitted to the growth kinetics data well over a wide range of initial phenol concentrations (100–1800 mg/l), with kinetic values $\mu_{\max} = 0.312 \text{ h}^{-1}$, $K_s = 130.4 \text{ mg/l}$, and $K_i = 200 \text{ mg/l}$. The decay coefficient was found to be 0.0073 h^{-1} . Complete phenol degradation by strain JH6 could be achieved in the presence of other toxicants, such as m-cresol and quinoline, which were often found in the real phenol-containing wastewater.

Keywords: Phenol; Biodegradation; Kinetics; *Paecilomyces variotii*

Jun Zeng^{a, b, c}, Xiangui Lin^{a, b}, Jing Zhang^{a, b} and Xuanzhen Li^{a, b, c}. (^a State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Beijing East Road, 71, Nanjing 210008, PR China, ^b Joint Open Laboratory of Soil and the Environment, Hongkong Baptist University & Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, PR China, ^c Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China). Isolation of polycyclic aromatic hydrocarbons (PAHs)-degrading *Mycobacterium* spp. and the degradation in soil. *Journal of Hazardous Materials*, Volume 183(1-3) (2010): 718-723

The goal of this study was to isolate PAHs degraders that can utilize PAHs associated with soil particulates and investigate the biodegradation of PAHs on agar plate, in liquid culture and soil. Two *Mycobacterium* strains (NJS-1 and NJS-P) were isolated from PAHs-contaminated farmland soil using enrichment based on soil slurry. The isolates could degrade five test PAHs including pyrene, phenanthrene, fluoranthene, anthracene and benzo[a]pyrene on plate, but showed different effects in liquid culture, especially for fluoranthene. Isolate NJS-1 was capable of utilizing benzo[a]pyrene as a sole carbon and energy source, and an enhanced degradation was observed when pyrene was supplied as cometabolic substrate. Reintroduction of the isolates into sterile contaminated soil resulted in a significant removal of aged pyrene and fluoranthene (over 40%) in 2-months incubation. In pyrene-spiked soil, the degradation of pyrene and fluoranthene increased to 90% and 50%, respectively. Comparing PAHs degradation on plate, in liquid culture and soil, we can conclude that there was corresponding degradation in different test systems. In addition, the degradation of aged PAHs in soil suggested the potential application of two isolates in further bioremediation.

Keywords: Soil slurry; PAHs; Biodegradation; *Mycobacterium*

Zhang Cai^a, Qixing Zhou^{a, b}, Shengwei Peng^a and Kenan Li^a. (^a Key Laboratory of Pollution Processes and Environmental Criteria at Ministry of Education, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China, ^b Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China). Promoted biodegradation and microbiological effects of petroleum hydrocarbons by *Impatiens balsamina* L. with strong endurance. *Journal of Hazardous Materials*, Volume 183(1-3) (2010): 731-737

Phytoremediation is a promising green technology for cleanup of petroleum hydrocarbons (PHCs) in contaminated environment. Based on the objective of identifying special ornamental plants for the effective biodegradation of PHCs, the efficacy of *Impatiens balsamina* L. to phytoremedy petroleum contaminated soil from the Shengli Oil Field in Dongying City, Shandong Province, China, was further examined in a field plot-culture experiment under greenhouse conditions. After a 4-month culture period, the average degradation rate of total

petroleum hydrocarbons (TPHs) by the plant was up to 18.13–65.03%, greatly higher than that (only 10.20–35.61%) in their corresponding controls by natural degradation. Among petroleum compositions saturated hydrocarbons had the highest degradation. The release of polar metabolic byproducts during phytoremediation of contaminated soils with $\geq 20,000$ mg/kg of PHCs by *I. balsamina* may occur. Some growth indexes of *I. balsamina* indicated that the plant had a good tolerance to contaminated soils with $\leq 10,000$ mg/kg of PHCs. Moreover rhizosphere bacteria and fungi became the dominant microbial population in soils with 5000 and 10,000 mg/kg of PHCs and were probably responsible for TPH degradation. Thus, *I. balsamina* L. could be a potential ornamental plant for effective phytoremediation of contaminated soils with $\leq 10,000$ mg/kg of PHCs.

Keywords: Phytoremediation; Contaminated soil; Total petroleum hydrocarbons (TPHs); Ornamental plant; Microbial population

Lijun Zhao^a Jiti Zhou^b, Yuhong Jia^b and Jinfu Chen^a. (^a School of Chemical Engineering, China University of Petroleum, Fuxue Road 18#, Changping District, Beijing 102249, China, ^b Key Laboratory of Industrial Ecology and Environmental Engineering, MOE, School of Environmental and Biological Science and Technology, Dalian University of Technology, Dalian 116024, China). **Biodecolorization of Acid Red GR by a newly isolated *Dyella ginsengisoli* LA-4 using response surface methodology. Journal of Hazardous Materials, Volume 181(1-3) (2010): 602-608**

Decolorization of Acid Red GR by a newly isolated biphenyl-degrading bacterium, *Dyella ginsengisoli* LA-4 was presented in this paper. The optimal decolorization conditions were determined by response surface methodology (RSM) based on the rotatable central composite design. The results indicated that strain LA-4 possessed the highest decolorizing activity under anaerobic conditions with inoculation amount 6.49%, pH 7.06 and temperature 29 °C. Some ions such as Cu^{2+} , Zn^{2+} could inhibit the decolorization, whereas 1 mmol/L of Mg^{2+} , Ca^{2+} , Co^{2+} , Fe^{3+} , Ni^{2+} and Mn^{2+} had no effects on the process. It was demonstrated that anthraquinone as a redox mediator could significantly accelerate the reduction process of azo dyes.

Keywords: Azo dyes; Decolorization; *Dyella ginsengisoli*; Response surface methodology; Redox mediator

Tamal Mandal^a Dalia Dasgupta^b, Subhasis Mandal^a and Siddhartha Datta^c. (^aDepartment of Chemical Engineering, NIT, Durgapur, Mahatma Gandhi Avenue, Durgapur, West Bengal, India, ^b Department of Biotechnology, NIT, Durgapur, India, ^c Department of Chemical Engineering, Jadavpur University, Raja S.C. Mallik Road, Kolkata 32, India).

Degradation of leather industry wastewater by sole aerobic treatment incorporating *Thiobacillus ferrooxidans*, Fenton's reagents, and combined treatment was investigated in this study. The sole treatment by Fenton's oxidation involving the introduction of 6 g FeSO_4 and 266 g H_2O_2 in a liter of wastewater at pH of 3.5 and 30 °C for 30 min at batch conditions reduced COD, BOD_5 , sulfide, total chromium and color up to 69%, 72%, 88%, 5%, 100% and *T. ferrooxidans* alone showed maximum reduction to an extent of 77, 80, 85, 52, 89, respectively, in 21 d treatment at pH 2.5, FeSO_4 16 g/L and temperature of 30 °C. The combined treatment at batch conditions involving 30 min chemical treatment by Fenton's oxidation followed by 72 h biochemical

treatment by *T. ferrooxidans* at batch conditions gave rise up to 93%, 98%, 72%, 62% and 100% removal efficiencies of COD, BOD, sulfide, chromium and color at pH of 2.5 and 30 °C. Decrease in photo absorption of the Fenton's reagent treated samples, as compared to the banks, at 280, 350 and 470 nm wave lengths was observed. This may be the key factor for stimulating the biodegradation by *T. ferrooxidans*.

Keywords: Leather industrial wastewater; COD; Chromium; Sulfide; *T. ferrooxidans*; Fenton's reagents

Sylwia Różalska^a, Rafał Szewczyk^a and Jerzy Długoński^a. (^a Department of Industrial Microbiology and Biotechnology, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland). Biodegradation of 4-*n*-nonylphenol by the non-ligninolytic filamentous fungus *Gliocephalotrichum simplex*: A proposal of a metabolic pathway. *Journal of Hazardous Materials*, Volume 180(1-3) (2010): 323-331

4-Nonylphenols (NPs) are endocrine disrupting compounds (EDCs) which are known to interfere with the endocrine system of humans and animals. The aim of this study was to test the ability of non-ligninolytic filamentous fungus *Gliocephalotrichum simplex* to biodegrade 4-*n*-NP. The results revealed that during the first 24 h of incubation, 4-*n*-NP at the concentration of 50 mg L⁻¹ was eliminated from the culture medium by 88%, whereas at the concentration of 100 mg L⁻¹ by 50%. In this paper, glucose utilization as a co-substrate during toxic compound degradation was also shown. It was found that the presence of 4-*n*-NP caused sugar metabolism retardation and this inhibition was dependent on NP concentration. The qualitative GC-MS analysis showed the presence of products of *G. simplex* 4-*n*-NP biodegradation. We proposed the metabolic pathway of 4-*n*-NP biodegradation, which is based on subsequent C1 removals from the alkyl chain followed by the aromatic ring cleavage. In further experiments with 4-*n*-NP [ring-¹⁴C(U)] we proved aromatic ring cleavage occurrence. After 72 h of incubation the evolution of ¹⁴CO₂ was observed and the mineralization efficiency was on the level of 29%. The results suggest the existence of a novel mechanism of 4-*n*-NP degradation in fungi.

Keywords: 4-*n*-NP; EDCs; *Gliocephalotrichum simplex*; Biodegradation; Mineralization

Guihua Xu^a, Shaohua Chen^a, Jianwen Shi^a, Shumei Wang^a and Gefu Zhu^a. (^a Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China). Combination treatment of ultrasound and ozone for improving solubilization and anaerobic biodegradability of waste activated sludge. *Journal of Hazardous Materials*, Volume 180(1-3) (2010): 340-346

The hydrolysis is known to be the rate-limiting step of biological sludge anaerobic degradation. The disruptions of sludge flocs and microbial cell walls by ultrasound combined with ozone treatment (US/O₃) were investigated in laboratory-scale experiments. The results showed that temperature, O₃ dose, US energy density and pH had a positive effect on the disintegration of sludge. The organic substrates were released into the liquor, which induced the increases of soluble chemical oxygen demand (COD_S) and turbidity in the aqueous phase. Accordingly, the biodegradability of sludge was improved. The COD_S increased from 1821 to 2513 mg/l after reaction for 30 min when NaHCO₃ was added, which indicated that the ozone molecule played a major role in the disintegration of waste activated sludge. The COD_S was 2483 mg/l after 60 min O₃ treatment followed by 60 min US treatment, and it changed into 3040 mg/l after 60 min US/O₃ treatment, which proved that US/O₃ induced a synergetic effect. The pH-drop of sludge

from 6.8 to 5.21 might be attributed to the increase of volatile fatty acid from 61.35 to 111.96 mg/l during the US/O₃ treatment process.

Keywords: Ultrasound; Ozone; Disintegration; Mechanism; Waste activated sludge

Lionel Ho^{a, b}, Daniel Hoefel^{a, b}, Sebastien Palazot^c, Emma Sawade^a, Gayle Newcombe^a, Christopher P. Saint^{a, b} and Justin D. Brookes^b. (^a Australian Water Quality Centre, SA Water Corporation, 250 Victoria Square, Adelaide, SA 5000, Australia, ^b School of Earth & Environmental Sciences, The University of Adelaide, SA 5005, Australia, ^c École Supérieure de Chimie Physique Électronique de Lyon, 43 Boulevard du 11 Novembre 1918 BP 2077, 69616 Villeurbanne Cedex, France). **Investigations into the biodegradation of microcystin-LR in wastewaters. Journal of Hazardous Materials, Volume 180(1-3) (2010): 628-633**

Microcystins are potent hepatotoxins that can be produced by cyanobacteria. These organisms can proliferate in wastewaters due to a number of factors including high concentrations of nutrients for growth. As treated wastewaters are now being considered as supplementary drinking water sources, in addition to their frequent use for irrigated agriculture, it is imperative that these wastewaters are free of toxins such as microcystins. This study investigated the potential for biodegradation of microcystin-LR (MCLR) in wastewaters through a biological sand filtration experiment and in static batch reactor experiments. MCLR was effectively removed at a range of concentrations and at various temperatures, with degradation attributed to the action of microorganisms indigenous to the wastewaters. No hepatotoxic by-products were detected following the degradation of MCLR as determined by a protein phosphatase inhibition assay. Using TaqMan polymerase chain reaction, the first gene involved in bacterial degradation of MCLR (*mlrA*) was detected and the responsible bacteria shown to increase with the amount of MCLR being degraded. This finding suggested that the degradation of MCLR was dependent upon the abundance of MCLR-degrading organisms present within the wastewater, and that MCLR may provide bacteria with a significant carbon source for proliferation; in turn increasing MCLR removal.

Keywords: Biodegradation; Microcystin; *mlrA* gene; Polymerase chain reaction (PCR); Protein phosphatase 2A (PP2A) assay

Yiming He^a, Ying Wu^c, Tianlu Sheng^b and Xintao Wu^b. (^a College of Mathematics, Physics and Information Engineering, Zhejiang Normal University, Jinhua, 321004, China, ^b State Key Laboratory of Structural Chemistry, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou, 350002, China, ^c Institute of Physical Chemistry, Zhejiang Key Laboratory for Reactive Chemistry on Solid Surfaces, Zhejiang Normal University, Jinhua, 321004, China). **Photodegradation of acetone over V-Gd-O composite catalysts under visible light. Journal of Hazardous Materials, Volume 180(1-3) (2010): 675-682**

A series of visible light active catalysts, V-Gd-O composites, were prepared by the impregnation method. In the photodegradation of acetone, the highest acetone conversion was obtained on V₁Gd₁O_x catalyst under visible light. The physical and photophysical properties of the composite catalyst have been characterized by XRD, FT-IR, Raman, BET surface area, UV-vis diffuse

reflectance spectra, and photoluminescence (PL) spectra. The characterization indicates the V-Gd-O photocatalyst exhibits three phases: Gd_2O_3 , $GdVO_4$ and V_2O_5 . On the basis of the calculated energy band positions and PL spectra, the high activity of the V-Gd-O catalysts could be attributed to the coupling effect between $GdVO_4$ and V_2O_5 in retarding the recombination of electron-hole pairs.

Keywords: Photodegradation; Photocatalyst; V_2O_5 ; $GdVO_4$; Acetone

M.J. Huertas^a, L.P. Sáez^b, M.D. Roldán^b, V.M. Luque-Almagro^b, M. Martínez-Luque^b, R. Blasco^c, F. Castillo^b, C. Moreno-Vivián^b and I. García-García^d. (^a Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla Avda Américo Vespucio, 49, 41092 Sevilla, Spain, ^b Departamento de Bioquímica y Biología Molecular, Edificio Severo Ochoa, 1^a Planta, Campus de Rabanales, Universidad de Córdoba, 14071 Córdoba, Spain, ^c Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Veterinaria, Universidad de Extremadura, 11071 Cáceres, Spain, ^d Departamento de Ingeniería Química, Edificio Marie Curie, Campus de Rabanales, Universidad de Córdoba, 14071 Córdoba, Spain). Alkaline cyanide degradation by *Pseudomonas pseudoalcaligenes* CECT5344 in a batch reactor. Influence of pH. *Journal of Hazardous Materials*, Volume 179(1-3) (2010): 72-78

Water containing cyanide was biologically detoxified with the bacterial strain *Pseudomonas pseudoalcaligenes* CECT5344 in a batch reactor. Volatilization of toxic hydrogen cyanide (HCN) was avoided by using an alkaline medium for the treatment. The operational procedure was optimized to assess cyanide biodegradation at variable pH values and dissolved oxygen concentrations. Using an initial pH of 10 without subsequent adjustment allowed total cyanide to be consumed at a mean rate of approximately $2.81 \text{ mg CN}^- \text{ L}^{-1} \text{ O.D.}^{-1} \text{ h}^{-1}$; however, these conditions posed a high risk of HCN formation. Cyanide consumption was found to be pH-dependent. Thus, no bacterial growth was observed with a controlled pH of 10; on the other hand, pH 9.5 allowed up to $2.31 \text{ mg CN}^- \text{ L}^{-1} \text{ O.D.}^{-1} \text{ h}^{-1}$ to be converted. The combination of a high pH and a low dissolved oxygen saturation (10%) minimized the release of HCN. This study contributes new basic knowledge about this biological treatment, which constitutes an effective alternative to available physico-chemical methods for the purification of wastewater containing cyanide or cyano-metal complexes.

Keywords: Cyanide; *Pseudomonas*; Batch reactor; Biodegradation; Wastewater

Shiying Yang^{a, b}, Ping Wang^b, Xin Yang^b, Liang Shan^b, Wenyi Zhang^b, Xueting Shao^b and Rui Niu^b. (^a Key Laboratory of Marine Environment and Ecology, Ministry of Education, Qingdao 266100, China, ^b College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China). Degradation efficiencies of azo dye Acid Orange 7 by the interaction of heat, UV and anions with common oxidants: Persulfate, peroxymonosulfate and hydrogen peroxide. *Journal of Hazardous Materials*, Volume 179(1-3) (2010): 552-558

In this paper, the degradation of azo dye Acid Orange 7 (AO7) by three common peroxides (persulfate (PS), peroxymonosulfate (PMS) or hydrogen peroxide (H_2O_2)) under various activation conditions, i.e., heat (25–80 °C), UV light (254 nm), or anions (SO_4^{2-} , NO_3^- , CO_3^{2-} , HCO_3^- , HPO_4^{2-} , and Cl^-), was investigated. The order of AO7 degradation efficiencies by heat activation is $PS \gg PMS > H_2O_2$. PS oxidation activated by heat (>50 °C) is an effective

degradation technology, while PMS and H₂O₂ are hardly activated. When assisted by UV, peroxides could all be activated and degrade AO7 quickly. The order is PS > H₂O₂ > PMS. We activated peroxides, for the first time, by using some anions and compared the subsequently degradation efficiencies of AO7. It was found that PMS could be activated by some anions, but PS and H₂O₂ cannot. The activation efficiencies of PMS by SO₄²⁻ and NO₃⁻ are negligible, whereas remarkable by HCO₃⁻, HPO₄²⁻, Cl⁻ and CO₃²⁻. For HCO₃⁻, HPO₄²⁻ and Cl⁻, the activation efficiencies become higher with the increase of anion concentration. For CO₃²⁻, however, the activation efficiency is higher at lower concentration.

Keywords: Azo dye degradation; Activated peroxide oxidation; Heat; UV light; Anions

Biosensor

Meral Yüce^a, Hasan Nazır^b and Gönül Dönmez^c. (^a Ankara University, Institute of Biotechnology, 06500 Beşevler-Ankara, Turkey, ^b Faculty of Science, Department of Chemistry 06500 Beşevler-Ankara, Turkey, ^c Faculty of Science, Department of Biology, 06500 Beşevler-Ankara, Turkey). **An advanced investigation on a new algal sensor determining Pb(II) ions from aqueous media. Biosensors and Bioelectronics, Volume 26(2) (2010): 321-326**

It has been well documented that heavy metal accumulation in environment is harmful for living organisms at even trace levels. A new voltammetric algal sensor based on *Phormidium* sp. modification for Pb(II) determination from aqueous solutions was developed, and selectivity of the biomass to Pb(II) was investigated comprehensively. Many important experimental parameters were performed by using electrochemical techniques, including cyclic voltammetry and differential pulse stripping voltammetry. The preconcentrated ions at open circuit were reduced by scanning the potential from -1.5 to 1.5 V and current values obtained were related to the concentration of Pb(II) in the solutions. The best peak values belonging to Pb(II) were achieved at pH 8.0 with 0.05 M Tris-HCl solution. Preconcentration time was selected as 10 min, and the sensor was found in a linear range from 5.0×10^{-8} M to 2.0×10^{-5} M Pb(II) (0.01–4.0 mg L⁻¹) with a detection limit of 2.5×10^{-8} M. Other analytical properties of the developed microbial biosensor were also investigated.

According to the Fourier transform infrared attenuated total reflectance (FTIR-ATR) analyses, the possible functional groups involved in Pb(II) accumulation in the *Phormidium* sp. were defined as carboxyl, sulphoxide and alcoholic groups. A simple chemical modification by formaldehyde both enhanced Pb(II) determination and content of functional groups involving Pb(II) binding. The proposed usage form of *Phormidium* sp. does not need complicated immobilization procedures and expensive preliminary preparations.

Keywords: Algal sensor; *Phormidium* sp.; Heavy metal; FTIR-ATR; Voltammetry; Environmental control

Paolo Actis^a, Olufisayo Jejelowo^b and Nader Pourmand^a. (^a Department of Biomolecular Engineering, University of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, USA, ^b Department of Biology, Texas Southern University, 3100 Cleburne Street,

Houston, TX 77004, USA). Ultrasensitive mycotoxin detection by STING sensors. Biosensors and Bioelectronics, Volume 26(2) (2010): 333-337

Signal transduction by ion nano-gating (STING) technology is a label-free biosensor capable of identifying DNA and proteins. Based on a functionalized quartz nanopipette, the STING sensor includes specific recognition elements for analyte discrimination based on size, shape and charge density. A key feature of this technology is that it does not require any nanofabrication facility; each nanopipette can be easily, reproducibly, and inexpensively fabricated and tailored at the bench, thus reducing the cost and the turnaround time. Here, we show that STING sensors are capable of the ultrasensitive detection of HT-2 toxin with a detection limit of 100 fg/ml and compare the STING capabilities with respect to conventional sandwich assay techniques.

Keywords: Nanopipette; Label-free; Single-molecule detection; Biosensor; Nanopore; STING

Ying Li^a, Bangwei Liu^a, Xia Li^a and Qingli Wei^a. (^a Key Laboratory of Eco-chemical Engineering, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, PR China). Highly sensitive electrochemical detection of human telomerase activity based on bio-barcode method. Biosensors and Bioelectronics, Volume 25(11) (2010): 2543-2547

In the present study, an electrochemical method for highly sensitive detection of human telomerase activity was developed based on bio-barcode amplification assay. Telomerase was extracted from HeLa cells, then the extract was mixed with telomerase substrate (TS) primer to perform extension reaction. The extension product was hybridized with the capture DNA immobilized on the Au electrode and then reacted with the signal DNA on Au nanoparticles to form a sandwich hybridization mode. Electrochemical signals were generated by chronocoulometric interrogation of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ that quantitatively binds to the DNA on Au nanoparticles *via* electrostatic interaction. This method can detect the telomerase activity from as little as 10 cultured cancer cells without the polymerase chain reaction (PCR) amplification of telomerase extension product.

Keywords: Telomerase; Cancer cell; Chronocoulometry; Au nanoparticle; Bio-barcode

Wenjuan Li^a, Ruo Yuan^a, Yaqin Chai^a and Shihong Chen^a. (^a Chongqing Key Laboratory of Analytical Chemistry, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China). Reagentless amperometric cancer antigen 15-3 immunosensor based on enzyme-mediated direct electrochemistry. Biosensors and Bioelectronics, Volume 25(11) (2010): 2548-2552

A novel strategy was proposed for the construction of reagentless and mediatorless immunosensors based on the direct electrochemistry of glucose oxidase (GOD). Firstly, a composite material containing carbon nanotubes (CNTs) and core-shell organosilica@chitosan nanospheres was successfully prepared and cast on the glassy carbon electrode surface directly. Then, Pt nanoclusters (Pt NCs) as an electron relay were deposited on it to form the interface of biocompatibility and huge surface free energy for the adsorption of the first GOD layer. Subsequently, the second Pt NCs layer was deposited on the surface of GOD to capture CA15-3 antibodies (anti-CA15-3). Finally, GOD, as a blocking reagent instead of bovine serum albumin, was employed to block the possible remaining active sites of the Pt NCs and avoid the nonspecific adsorption. The immunosensor with the double layer GOD membranes as tracer

performed excellent biocompatible and avoided the pollution of mediator molecules. The immobilized GOD showed direct electron transfer with a rate constant of 4.89 s^{-1} and the peak current decreased linearly with increasing logarithm of CA15-3 concentration from 0.1 to 160 U/mL with a relatively low limit of detection of 0.04 U/mL at 3σ . Such a detection of immunointeraction provided a new promising platform for clinical immunoassay.

Keywords: Immunosensor; Organosilica@chitosan nanospheres; Carbon nanotubes; Glucose oxidase; Pt nanoclusters; Direct electrochemistry

Sheng Tang^a, Xizhang Wang^b, Jianping Lei^{a,b}, Zheng Hu^b, Shengyuan Deng^a and Huangxian Ju^a. (^a Key Laboratory of Analytical Chemistry for Life Science (Ministry of Education of China), Department of Chemistry, Nanjing University, Nanjing 210093, PR China, ^b Key Laboratory of Mesoscopic Chemistry (Ministry of Education of China), Department of Chemistry, Nanjing University, Nanjing 210093, PR China). **Pt-dispersed flower-like carbon nanosheet aggregation for low-overpotential electrochemical biosensing. Biosensors and Bioelectronics, Volume 26(2) (2010): 432-436**

A Pt nanoparticle-decorated flower-like carbon nanosheet aggregation (FCNA) was prepared via one-step ethylene glycol method. The aggregation was characterized with scanning electron micrographs, X-ray photoelectron spectra, X-ray diffraction and electrochemical impedance spectra. When the aggregation was immobilized on a glassy carbon electrode, the dense dispersion of Pt nanoparticles (Pt NPs) on the carbon nanosheets of FCNA could combine the good conductivity of FCNA with the excellent catalytic activity of Pt NPs for the electroreduction of oxygen at a low overpotential, which led to a method for electrochemical detection of oxygen from 6.3 to 69.3 μM . Using glucose oxidase (GOx) as a model, the resulting GOx/Pt/FCNA nanocomposite-based amperometric biosensor showed a linear response to glucose ranging from 0.5 to 8.0 mM with a detection limit of 0.3 mM at a S/N ratio of 3. The designed biosensor was of excellent performance with high selectivity, acceptable recovery and good repeatability, and could be successfully applied in the detection of glucose in human serum. The FCNA could be expected as a carrier for the preparation of other metal nanoparticle-dispersed aggregations and biosensing applications.

Keywords: Biosensor; Electrochemistry; Pt nanoparticles; Glucose oxidase; Flower-like carbon nanosheet aggregation

O. Frey^a, T. Holtzman^b, R.M. McNamara^b, D.E.H. Theobald^b, P.D. van der Wal^a, N.F. de Rooij^a, J.W. Dalley^b and M. Koudelka-Hep^a. (^a Ecole Polytechnique Fédérale de Lausanne (EPFL), Institute of Microengineering (IMT), Sensors, Actuators and Microsystems Laboratory (SAMLAB), Rue Jaquet-Droz 1, 2000 Neuchâtel, Switzerland, ^b University of Cambridge, Department of Experimental Psychology, Downing Site, Cambridge, UK). **Enzyme-based choline and L-glutamate biosensor electrodes on silicon microprobe arrays. Biosensors and Bioelectronics, Volume 26(2) (2010): 477-484**

Brain-implantable microprobe arrays, 6.5 mm shaft-length, incorporating several recessed Pt microelectrodes ($50 \mu\text{m} \times 150 \mu\text{m}$) and an integrated Ag/AgCl reference electrode fabricated by silicon micromachining dry etching techniques (DRIE) are described. The microelectrodes are coated by an enzyme membrane and a semi-permeable *m*-phenylenediamine layer for the

selective detection of the neurotransmitters choline and L-glutamate at physiologically relevant concentrations. The functionalisation is based on electrochemically aided adsorption (EAA) combined with chemical co-cross-linking using glutaraldehyde and electrochemical polymerisation, respectively. These deposition methods are fully compatible with the fabricated microprobe arrays for the simultaneous detection of several analytes in different brain target areas. They are spatially controlled and allow fabricating biosensors on several microelectrodes in parallel or providing a cross-talk-free coating of closely spaced microelectrodes with different enzyme membranes. A sensitivity of $132 \pm 20 \mu\text{A mM}^{-1} \text{cm}^{-2}$ for choline and $95 \pm 20 \mu\text{A mM}^{-1} \text{cm}^{-2}$ for L-glutamate with limits of detections below $0.5 \mu\text{M}$ was obtained. The results of *in vitro* and *in vivo* experiments confirm the functional viability of the choline and L-glutamate biosensors.

Keywords: Biosensor; Choline; Glutamate; Microprobe array; Enzyme immobilisation

Wei Chen^{a, 1}, Yu Ding^a, Joshua Akhigbe^b, Christian Brückner^b, Chang Ming Li^c and Yu Lei^a. (^a Department of Chemical, Materials and Biomolecular Engineering, University of Connecticut, Storrs, CT 06269-3222, USA, ^b Department of Chemistry, University of Connecticut, Storrs, CT 06269-3060, USA, ^c School of Chemical and Biomedical Engineering, Nanyang Technological University, 637457, Singapore). **Enhanced electrochemical oxygen reduction-based glucose sensing using glucose oxidase on nanodendritic poly[meso-tetrakis(2-thienyl)porphyrinato]cobalt(II)-SWNTs composite electrodes. Biosensors and Bioelectronics, Volume 26(2) (2010):504-510**

The direct electrochemistry of glucose oxidase immobilized on a nanodendritic poly[meso-tetrakis(2-thienyl)porphyrinato]cobalt(II)-single walled carbon nanotube modified glassy carbon electrode (pCoTTP-SWNTs-Nafion-GOD/GCE) is reported. The immobilized GOD retained its activity and exhibited a surface controlled, reversible two-proton and two-electron transfer reaction with a fast heterogeneous electron transfer rate constant (k_s) of 1.01 s^{-1} . The pCoTTP-SWNTs-Nafion matrix also showed an extremely low peak potential of -0.2 V vs. Ag/AgCl and strong response with respect to oxygen reduction. This forms the basis for the use of the pCoTTP-SWNTs-Nafion-GOD composite as a sensing platform for oxygen reduction-based glucose detection. The apparent Michaelis–Menten constant ($K_{m,\text{app}}$) was estimated to be as low as 0.98 mM . A linear range up to 1 mM glucose with a low detection limit of $5.33 \mu\text{M}$ ($S/N = 3$) and a high sensitivity of $16.57 \mu\text{A mM}^{-1} \text{cm}^{-2}$ was achieved. The biosensor also shows excellent selectivity against 0.2 mM uric acid and ascorbic acid. These results indicate that the pCoTTP-SWNTs-Nafion-GOD/GCE has potential application in sensitive and selective glucose detection.

Keywords: Glucose; Glucose oxidase; Biosensor; Direct electrochemistry; Nanodendritic pCoTTP; SWNTs

Wen-Hui Zhou^{a, b}, Shui-Fen Tang^a, Qiu-Hong Yao^a, Fa-Rong Chen^b, Huang-Hao Yang^{a, b} and Xiao-Ru Wang^b. (^a The Key Lab of Analysis and Detection Technology for Food Safety of the MOE, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou, PR China, ^b The First Institute of Oceanography, SOA, Qingdao, PR China). **A quartz crystal microbalance sensor based on mussel-inspired molecularly imprinted polymer. Biosensors and Bioelectronics, Volume 26(2) (2010): 585-589**

In this work, we describe a simple, inexpensive and fast method for the generation of molecularly imprinted polymer (MIP) film on quartz crystal microbalance (QCM) crystals using mussel-inspired polymer. Commonly known as a neurotransmitter, dopamine is also a small-molecule mimic of the adhesive proteins of mussels. Polymerization of dopamine in the presence of template molecule (1,3,5-pentanetricarboxylic acid, an analogue of domoic acid, in this case) could produce an adherent molecularly imprinted polydopamine film coating on QCM crystals. Advantages, such as high hydrophilicity, high biocompatibility and controllable thickness, make this molecularly imprinted polydopamine film an attractive recognition element for sensors. Selective rebinding of domoic acid on mussel-inspired molecularly imprinted polymer (m-MIP) coated crystal was observed as a frequency shift quantified by piezoelectric microgravimetry with the QCM system. The decreasing frequency shows a good linear relationship with the concentration of domoic acid. The quantitation limit of domoic acid was 5 ppb with the linear range of 0–100 ppb. The QCM sensor has high selectivity and was able to distinguish domoic acid from its analogous *p*-phthalic acid and *o*-phthalic acid owing to the molecular imprinting effect. In addition, the practical analytical performance of the sensor was examined by evaluating the detection of domoic acid in mussel extracts with satisfactory results. It is envisaged that m-MIP could be suitable as recognition element for sensors and the proposed m-MIP QCM sensor could be employed to detect analyte of interest in complex matrices.

Keywords: Molecular imprinting polymers (MIP); Quartz crystal microbalance (QCM); Domoic acid; Mussel; Sensor

Jinghua Yu^a, Fuwei Wan^a, Congcong Zhang^a, Mei Yan^a, Xiaona Zhang^a and Shaowei Wang^a. (^a Shandong Provincial Key Laboratory of Fluorine Chemistry and Chemical Materials, School of Chemistry and Chemical Engineering, University of Jinan, No. 106, Jiwei Road, Jinan 250022, China). **Molecularly imprinted polymeric microspheres for determination of bovine serum albumin based on flow injection chemiluminescence sensor. Biosensors and Bioelectronics, Volume 26(2) (2010): 632-637**

A novel flow injection chemiluminescence (FI-CL) sensor for the determination of bovine serum albumin (BSA) using molecularly imprinted polymeric microspheres (MIPMs) as recognition element is reported. The BSA–MIPM was synthesized by suspension polymerization in toluene, using methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EGDMA) as cross-linker in the bovine serum albumin template molecule. Scanning electron microscope (SEM) was employed to characterize the surface morphology of the resultant imprinted microspheres. Molecular modeling was employed to simulate the possible recognition process of the MIPM. Then the synthesized BSA–MIPM was employed as recognition element by packing into flow cell to establish a novel FI-CL sensor. The chemiluminescence (CL) intensity was correlated linear with the concentration of BSA over the range of 1.0×10^{-8} to 5.0×10^{-6} g mL⁻¹ and the detection limit was 1.5×10^{-9} g mL⁻¹. The relative standard deviation (RSD) for the determination of 1.0×10^{-7} g mL⁻¹ BSA was 1.4% ($n = 11$). The sensor is reusable and has a great improvement in sensitivity and selectivity for CL analysis. As a result, the new MIPM–CL sensor had been successfully applied to the determination of BSA in milk samples.

Keywords: Chemiluminescence; Molecularly imprinted polymeric microspheres (MIPMs); Flow injection; Bovine serum albumin (BSA)

Ki Young Kwon^a, Jongkyu Youn^{a, 1}, Jae Hyun Kim^b, Yongjin Park^b, Chulmin Jeon^b, Byoung Chan Kim^c, Yongchai Kwon^d, Xueyan Zhao^e, Ping Wang^e, Byoung In Sang^f, Jinwoo Lee^g, Hyun Gyu Park^a, Ho Nam Chang^a, Taeghwan Hyeon^h, Su Haⁱ, Hee-Tae Jung^a and Jungbae Kim^b. (^a Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, South Korea, ^b Department of Chemical and Biological Engineering, Korea University, Seoul 136-701, South Korea, ^c Institut Pasteur Korea, Seongnam-si, Gyeonggi-do 463-400, South Korea, ^d Department of Chemical and Environmental Technology, Inha Technical College, Incheon 402-752, South Korea, ^e Bioproducts and Biosystems Engineering, Biotechnology Institute, University of Minnesota, St. Paul, MN 55108, USA, ^f Hazardous Substances Research Center, Korea Institute of Science and Technology, Seoul 136-791, South Korea, ^g Department of Chemical Engineering, School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang, Gyungbuk 790-784, South Korea, ^h National Creative Research Initiative Center for Oxide Nanocrystalline Materials, School of Chemical Engineering, Seoul National University, Seoul 151-744, South Korea, ⁱ Chemical Engineering Department, Washington State University, Pullman, WA 99164-2710, USA). **Nanoscale enzyme reactors in mesoporous carbon for improved performance and lifetime of biosensors and biofuel cells. *Biosensors and Bioelectronics*, Volume 26(2) (2010): 655-660**

Nanoscale enzyme reactors (NERs) of glucose oxidase in conductive mesoporous carbons were prepared in a two-step process of enzyme adsorption and follow-up enzyme crosslinking. MSU-F-C, a mesoporous carbon, has a bottleneck pore structure with mesocellular pores of 26 nm connected with window mesopores of 17 nm. This structure enables the ship-in-a-bottle mechanism of NERs, which effectively prevents the crosslinked enzymes in mesocellular pores from leaching through the smaller window mesopores. This NER approach not only stabilized the enzyme but also expedited electron transfer between the enzyme and the conductive MSU-F-C by maintaining a short distance between them. In a comparative study with GOx that was simply adsorbed without crosslinking, the NER approach was proven to be effective in improving the sensitivity of glucose biosensors and the power density of biofuel cells. The power density of biofuel cells could be further improved by manipulating several factors, such as by adding a mediator, changing the order of adsorption and crosslinking, and inserting a gold mesh as an electron collector.

Keywords: Biofuel cells; Biosensors; Enzyme crosslinking; Mesoporous carbon; Nanoscale enzyme reactors

Hua Kuang^{1, a}, Wei Chen^{1, a}, Dinghua Xu^a, Liguang Xu^a, Yingyue Zhu^a, Liqiang Liu^a, Huaqin Chu^a, Chifang Peng^a, Chuanlai Xu^a and Shuifang Zhu^a. (^a State Key Lab of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, JiangSu 214122, PR China). **Fabricated aptamer-based electrochemical “signal-off” sensor of ochratoxin A. *Biosensors and Bioelectronics*, Volume 26(2) (2010): 710-716**

An ultrasensitive and rapid electrochemical platform for the specific detection of ochratoxin A (OTA) was developed. In this method, three single-stranded DNA molecules, including the aptamer, were immobilized on the surface of an electrode. Binding of the OTA target analyte to the aptamer changed the redox current of methylene blue (MB), which was used as the electrochemical probe, in a manner that was dependent on OTA concentration. With signal

enhancement from gold nanoparticle-functionalized DNA, the sensitivity of this method for OTA was as low as 30 pg/mL, and the effective sensing range was from 0.1 to 20 ng/mL. To investigate the sensing process, the conformational switch of the aptamer was studied by circular dichroism (CD), which confirmed the recognition of the aptamer by the target OTA. Given its sensitivity and rapid detection, we believe this approach has the potential to be a main technology for the detection of toxins in the field of food safety, and in other areas.

Keywords: Ultrasensitive; Toxin detection; Electrochemical detection; Aptamer

Yan Li^a, Honglan Qi^a, Qiang Gao^a, Jia Yang^a and Chengxiao Zhang^a. (^a Key Laboratory of Applied Surface and Colloid Chemistry (Shaanxi Normal University), Ministry of Education, School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, PR China). **Nanomaterial-amplified “signal off/on” electrogenerated chemiluminescence aptasensors for the detection of thrombin. Biosensors and Bioelectronics, Volume 26(2) (2010): 754-759**

Two electrogenerated chemiluminescence (ECL) aptasensors for the detection of thrombin were developed using the thrombin binding aptamer (TBA) taken as a molecular recognition element and nanomaterial as a carrier of the ECL capture/signal probe. In the “signal off” aptasensor, the thiolated capture probe (ss-DNA, 12-mer) was self-assembled on the gold nanoparticles (GNPs) which were self-assembled on the surface of gold electrode, and hybridized with six-base segment of the ss-DNA sequence (Tgt-aptamer, 21-mer) containing TBA-I (ss-DNA, 15-mer) tagged with ruthenium complex, producing a high ECL intensity. Introduction of the analyte thrombin triggered the dissociation of the Tgt-aptamer tagged with ruthenium complex from the aptasensors, led to significantly decrease in ECL intensity. The decreased ECL intensity was in proportion to the concentration of thrombin in a range from 2.7×10^{-12} to 2.7×10^{-9} M with a detection limit of 8×10^{-13} M. In the “signal on” aptasensor, the thiolated TBA-I was self-assembled on the gold electrode for capturing thrombin onto the electrode and then the TBA-II (ss-DNA, 29-mer) labeled with single-walled carbon-nanotubes (SWNT)-ECL tag was bound with epitope of thrombin, producing a high ECL intensity. The increased ECL intensity was linearly with the concentration of thrombin from 1.0×10^{-14} M to 1.0×10^{-11} M with a detection limit of 3×10^{-15} M. The present work demonstrates that using nanomaterial as a carrier for capture probe and signal probe is a promising way to amplify the ECL signal and to improve the sensitivity of the aptasensors.

Keywords: Aptasensor; Electrogenerated chemiluminescence; Thrombin; Gold nanoparticles; Single-walled carbon-nanotubes

Application of oxygen-independent biosensor for testing yeast fermentation capacity

Bogumila Kurtinaitienė^a, Julija Razumienė^a, Vidutė Gurevičienė^a, Vytautas Melvydas^b, Liucija Marcinkevičienė^a, Irina Bachmatova^a, Rolandas Meškys^a and Valdas Laurinavičius^a. (^a Institute of Biochemistry, Mokslininkų 12, LT-08662 Vilnius, Lithuania, ^b Institute of Botany, Žaliojų Ežerų 49, LT-08406 Vilnius, Lithuania). **Biosensors and Bioelectronics, Volume 26(2) (2010): 766-771**

The pyrroloquinoline quinone (PQQ)-dependent soluble glucose dehydrogenase based carbon paste electrodes were investigated and applied for glucose monitoring in the oxygen deficient media. Reagentless biosensors possessing a wide linear range (up to 5 mM glucose with a detection limit of 0.12 mM) were designed. The oxygen-insensitive response of the biosensor creates the opportunity to use it as a flow-through device for continuous monitoring of glucose in media during the wine yeast fermentation process. The analysis of glucose assimilation rate by yeast strains using the developed biosensor correlated well ($R^2 = 0.9938$) with convenient yeast testing methods.

Keywords: PQQ-dependent glucose dehydrogenase; Direct electron transfer; Biosensor; Monitoring

Usa Sungkanak^a, Assawapong Sappat^b, Anurat Wisitsoraat^b, Chamras Promptmas^a and Adisorn Tuantranont^b. (^a Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, Nakhon Pathom 73170, Thailand, ^b Nanoelectronics and MEMS Laboratory, National Electronics and Computer Technology Center, 112 Paholyothin Rd., Klong Luang, Pathumthani 12120, Thailand). Ultrasensitive detection of *Vibrio cholerae* O1 using microcantilever-based biosensor with dynamic force microscopy. *Biosensors and Bioelectronics*, Volume 26(2) (2010): 784-789

This work presents the first demonstration of a cantilever based *cholerae* sensor. Dynamic force microscopy within atomic force microscope (AFM) is applied to measure the cantilever's resonance frequency shift due to mass of cell bound on microcantilever surface. The *Vibrio cholerae* O1, a food and waterborne pathogen that caused cholera disease in human, is a target bacterium cell of interest. Commercial gold-coated AFM microcantilevers are immobilized with monoclonal antibody (anti-*V. cholerae* O1) by self-assembled monolayer method. *V. cholerae* O1 detection experiment is then conducted in concentrations ranging from 1×10^3 to 1×10^7 CFU/ml. The microcantilever-based sensor has a detection limit of $\sim 1 \times 10^3$ CFU/ml and a mass sensitivity, $\Delta m/\Delta F$, of ~ 146.5 pg/Hz, which is at least two orders of magnitude lower than other reported techniques and sufficient for *V. cholerae* detection in food products without pre-enrichment steps. In addition, *V. cholerae* O1 antigen-antibody binding on microcantilever is confirmed by scanning electron microscopy. The results demonstrate that the new biosensor is promising for high sensitivity, uncomplicated and rapid detection of *V. cholerae* O1.

Keywords: Microcantilever; *Vibrio cholerae* O1; Biosensor; Dynamic force microscopy

Asta Kausaite-Minkstimiene^a, Viktor Mazeiko^a, Almira Ramanaviciene^{a, b} and Arunas Ramanavicius^{a, c}. (^a NanoTechnas – Center of Nanotechnology and Material Science, Faculty of Chemistry, Vilnius University, Naugarduko 24, 03225 Vilnius, Lithuania, ^b Laboratory of Immunotechnology, State Research Institute Center for Innovative Medicine, Zygimantu 9, 01102 Vilnius, Lithuania, ^c Institute of Chemistry, State Research Institute Center for Physical and Technological Sciences, A. Gostauto g. 11, LT-01108 Vilnius, Lithuania). Enzymatically synthesized polyaniline layer for extension of linear detection region of amperometric glucose biosensor. *Biosensors and Bioelectronics*, Volume 26(2) (2010): 790-797

In this article a new method for fabrication of enzymatic electrodes suitable for design of amperometric glucose biosensor and/or anode of biofuel cell powered by glucose is presented.

Glucose oxidase (GOx) E.C. 1.1.3.4. from *Penicillium vitale* was immobilized on the carbon rod electrode by cross-linking it with glutaraldehyde (GOx-electrode). Catalytic activity of immobilized GOx was exploited for polymerisation of aniline by taking a high concentration of hydrogen peroxide produced during the catalytic action of immobilized GOx and locally lowered pH due to the formation of gluconic acid; it created optimal conditions for the polymerisation of aniline. The GOx layer was self-encapsulated within formed polyaniline (PANI) matrix (GOx/PANI-electrode). Properties of the GOx/PANI-electrode have been studied and results were compared with GOx-electrode. The results show that the upper detection limit of glucose using GOx-electrode was dramatically changed by the formation of PANI layer. An increase in the upper detection limit, optimal pH region for operation and stability of GOx based electrode modified by PANI was detected when comparing that of an unmodified GOx-electrode.

Keywords: Amperometric glucose biosensor; Immobilized glucose oxidase; Biofuel cells; Conducting polymers; Polyaniline

Lidiane Raquel Verola Mataveli^{1, a}, Natália de Jesus Antunes^a, Maisa Ribeiro Pereira Lima Brigagão^a, Cristiana Schmidt de Magalhães^a, Célio Wisniewski^a and Pedro Orival Luccas^a. (^a Departamento de Ciências Exatas, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva, 700, CEP 37130-000, Alfenas, MG, Brazil). **Evaluation of a simple and low cost potentiometric biosensor for pharmaceutical and *in vivo* adrenaline determination. Biosensors and Bioelectronics, Volume 26(2) (2010): 798-802**

In this work the polyphenol oxidase (PPO) was the main component of a biosensor for adrenaline determination. The activity of this enzyme was measured in several vegetables. Banana (*Musa* sp.) extracts presented better results with 974 UA (units of activity). The biosensor was constructed with a polyethylene tube (0.8 mm i.d.) filled with: carbon paste containing 50 UA of the PPO in phosphate buffer (pH = 7.00) solution and vaseline as agglutinant. When the biosensor was applied in medicine samples it provided a linear range from 8.00×10^{-9} to 8.00×10^{-4} mol L⁻¹; the results obtained with the proposed method and the Brazilian Pharmacopoeia method were in agreement (*t*-test). When it was applied in blood samples, the matrix-matching calibration was used, and the linear range was from 8.00×10^{-7} to 8.00×10^{-3} mol L⁻¹. *In vivo* studies were also done. The obtained results for those electrodes, which were inserted in the jugular vein of Wistar rats, were very promising.

Keywords: Adrenaline; Biosensors; Polyphenol oxidase; *In vivo* measurements

Sunil A. Patil^{a, b}, Falk Harnisch^a, Balasaheb Kapadnis^b and Uwe Schröder^a. (^aInstitute of Environmental and Sustainable Chemistry, Sustainable Chemistry and Energy Research, TU Braunschweig, Hagenring 30, 38106 Braunschweig, Germany, ^bDepartment of Microbiology, University of Pune, 411 007 Pune, India). **Electroactive mixed culture biofilms in microbial bioelectrochemical systems: The role of temperature for biofilm formation and performance. Biosensors and Bioelectronics, Volume 26(2) (2010): 803-808**

In this paper we investigate the temperature dependence and temperature limits of waste water derived anodic microbial biofilms. We demonstrate that these biofilms are active in a temperature range between 5 °C and 45 °C. Elevated temperatures during initial biofilm growth not only accelerate the biofilm formation process, they also influence the bioelectrocatalytic

performance of these biofilms when measured at identical operation temperatures. For example, the time required for biofilm formation decreases from above 40 days at 15 °C to 3.5 days at 35 °C. Biofilms grown at elevated temperatures are more electrochemically active at these temperatures than those grown at lower incubation temperature. Thus, at 30 °C current densities of 520 $\mu\text{A cm}^{-2}$ and 881 $\mu\text{A cm}^{-2}$ are achieved by biofilms grown at 22 °C and 35 °C, respectively. Vice versa, and of great practical relevance for waste water treatment plants in areas of moderate climate, at low operation temperatures, biofilms grown at lower temperatures outperform those grown at higher temperatures. We further demonstrate that all biofilms possess similar lower (0 °C) and upper (50 °C) temperature limits – defining the operational limits of a respective microbial fuel cell or microbial biosensor – as well as similar electrochemical electron transfer characteristics.

Keywords: Microbial fuel cell; Microbial biosensor; Biosensor; Bioelectrochemical system; Temperature; Microbial biofilms; Electroactive biofilms; Waste water treatment

Gulsu Sener^{a, b}, Erdogan Ozgur^a, Erkut Yilmaz^a, Lokman Uzun^a, Ridvan Say^c and Adil Denizli^a. (^a Hacettepe University, Department of Chemistry, Ankara, Turkey, ^b Hacettepe University, Division of Nanotechnology and Nanomedicine, Ankara, Turkey, ^c Anadolu University, Department of Chemistry, Eskisehir, Turkey). **Quartz crystal microbalance based nanosensor for lysozyme detection with lysozyme imprinted nanoparticles. Biosensors and Bioelectronics, Volume 26(2) (2010): 815-821**

The aim of this study is to prepare quartz crystal microbalance (QCM) nanosensor for the real-time detection of lysozyme. In the first part, the lysozyme imprinted (MIP) nanoparticles were prepared by mini-emulsion polymerization. The MIP nanoparticles were characterized by TEM, zeta-sizer and FTIR–ATR measurements. Particle size was found around 50 nm. The MIP nanoparticles were attached by dropping of nanoparticle solution to gold surface and then, dried at 37 °C for 6 h. QCM nanosensor was characterized with AFM and ellipsometer. The observations indicated that the nanoparticle film was almost monolayer. The detection limit was found as 1.2 ng/mL. The specificity of the QCM nanosensor was shown by using albumin as a competitor molecule. The results show that the QCM nanosensor has high selectivity and sensitivity with a wide range of lysozyme concentrations in both aqueous solutions (0.2–1500 $\mu\text{g/mL}$) and natural sources (egg white) (460–1500 ng/mL).

Keywords: QCM; Nanoparticles; Lysozyme; Molecular imprinting; Nanosensors

Shiyang Pan^a, Jian Xu^a, Yongqian Shu^b, Fang Wang^a, Wenyong Xia^a, Qingqing Ding^b, Ting Xu^a, Chun Zhao^a, Meijuan Zhang^a, Peijun Huang^a and Shan Lu^c. (^aDepartment of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, China, ^b Department of Oncology, the First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, China, ^c Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01605, United States). **Double recognition of oligonucleotide and protein in the detection of DNA methylation with surface plasmon resonance biosensors. Biosensors and Bioelectronics, Volume 26(2) (2010): 850-853**

DNA methylation plays an essential role in maintenance of cellular function. A growing number of human diseases have been found to be associated with aberrant DNA methylation, especially cancer. However, current technologies used in DNA methylation detection are complicated and

time consuming. A promotor of the Adenomatous polyposis coli (APC) gene, a well-studied tumor suppressor gene, was used as the detection target DNA sequence. The double recognition mechanism was realized with oligonucleotide probe hybridization and specific protein binding. First, complementary target DNA was captured by the probe immobilized onto a surface plasmon resonance (SPR) sensor chip. Then, the recombinant methyl-CpG binding domain (MBD) protein was passed over the surface to recognize and bind to methylated CpG sites. Binding resulted in an increase in the refractive index, and a detectable optical signal was generated. Five picomoles of methylated APC promotor DNA could be easily detected with this method. The entire detection could be completed within 1 h. This work represents the first SPR based biosensor technology, which achieves simple and specific DNA methylation detection and avoids complicated bisulfite treatment and methylation-sensitive restriction digestion. It will improve our ability to detect DNA methylation specifically and rapidly, and promote our understanding of the role of DNA methylation in gene regulation and diseases.

Keywords: DNA methylation; Surface plasmon resonance; Biosensor; MBD; APC

Xiao-Mei Chen^a, Geng-Huang Wu^a, Jin-Mei Chen^a, Ya-Qi Jiang^a, Guo-Nan Chen^c, Munetaka Oyama^d, Xi Chen^{a, b} and Xiao-Ru Wang^a. (^a Department of Chemistry and Key Laboratory of Analytical Sciences of the Ministry of Education, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China, ^bState Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China, ^c Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety, Fuzhou University, Fuzhou 350002, China, ^d Division of Research Initiatives, International Innovation Center, Kyoto University, Nishikyo-ku, Kyoto 615-8520, Japan). A novel electrochemiluminescence sensor based on bis(2,2'-bipyridine)-5-amino-1,10-phenanthroline ruthenium(II) covalently combined with graphite oxide. *Biosensors and Bioelectronics*, Volume 26(2) (2010): 872-876

This communication reports a novel electrochemiluminescence (ECL) sensor based on covalently linking bis(2,2'-bipyridine)-5-amino-1,10-phenanthroline ruthenium(II) (Ru(II)-NH₂) with graphite oxide (GO) on a glassy carbon electrode. 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and N-hydroxy-succinimide were applied to activate the carboxyl groups on the GO surface and catalyze the formation of amido link between Ru(II)-NH₂ and carboxyl groups on GO. The composite film was characterized using atomic force microscopy, transmission electron microscopy and Fourier transform infrared absorption spectroscopy. Based on ECL experimental results, the composite film modified electrode displayed high electrochemical activity towards the oxidation of 2-(dibutylamino) ethanol (DBAE). Under optimized conditions, the linear response of ECL intensity to DBAE concentration was valid in the range 6.0×10^{-7} – 2.0×10^{-4} mol L⁻¹ ($r^2 = 0.9948$) with a detection limit (S/N = 3) of 5.0×10^{-8} mol L⁻¹. Furthermore, the ECL sensor presented good characteristics in terms of stability and reproducibility, promising the development of ECL sensors for biologically important compounds.

Keywords: Graphite Oxide; Bis(2,2'-bipyridine)-5-amino-1,10-phenanthroline ruthenium(II); Electrochemiluminescence; 2-(Dibutylamino)ethanol; Covalent linkage

Sofía Carbajosa^{a, b}, Moustafá Malki^{a, b}, Renaud Caillard^c, María F. Lopez^c, F. Javier Palomares^c, José A. Martín-Gago^{c, d}, Nuria Rodríguez^d, Ricardo Amils^{b, d}, Victor M. Fernández^a and Antonio L. De Lacey^a. (^a Instituto de Catálisis, CSIC, c/Marie Curie 2, 28049 Madrid, Spain, ^b Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, 28049 Madrid, Spain, ^c Instituto de Ciencia de Materiales de Madrid, CSIC, 28049 Madrid, Spain, ^d Centro de Astrobiología, INTA-CSIC, 28850 Torrejón de Ardoz, Spain). **Electrochemical growth of *Acidithiobacillus ferrooxidans* on a graphite electrode for obtaining a biocathode for direct electrocatalytic reduction of oxygen. *Biosensors and Bioelectronics*, Volume 26(2) (2010): 877-880**

An aspect in microbial fuel cell research that is currently of great interest is the development of bacterial cathodes. Bacterial cathodes that catalyze oxygen reduction to water at low pH have the advantage of overcoming the kinetic limitations due to the requirement of 4 protons per molecule reduced. In this work we have studied the performance of a biocathode using as electrocatalyst an acidophile microorganism: *Acidithiobacillus ferrooxidans*. Growth of the microorganism directly on the electrode took place using an applied voltage of 0 V vs. SCE as the only energy source and without adding redox mediators to the solution. Current densities of up to 5 A m⁻² were measured for O₂ reduction in the *At. ferrooxidans* cathode at pH 2.0 and the electrocatalytic wave was shifted 300 mV to higher potential compared to the control graphite electrodes without the bacterium.

Keywords: Microbial fuel cell; Biocathode; Acidophile; Oxygen reduction; Extracellular electron transfer

Recent progress in bio-sensing techniques with encapsulated enzymes

Byung-Wook Park^a, Do-Young Yoon^b and Dong-Shik Kim^a. (^a Department of Chemical & Environmental Engineering, University of Toledo, Toledo, OH 43606, United States, ^b Department of Chemical Engineering, Kwangwoon University, Seoul 139-701, Republic of Korea). ***Biosensors and Bioelectronics*, Volume 26(1) (2010): 1-10**

Biosensors with encapsulated enzymes have advantages of high substrate selectivity and sustained enzyme activity. Enzymes are encapsulated in various materials, such as liposome, polymer, and sol-gel or hydro-gel, depending on sensing conditions. By stabilizing the enzymes via encapsulation with new methods and materials the enzyme activity may be maintained for a longer time, and even the selectivity can possibly be enhanced. In general increased mass transfer limitation seems to be the major challenge to investigate more. Novel materials, encapsulation techniques, and sensing mechanisms have been studied intensively for encapsulated enzyme biosensors. In this review, we focus on the recent progress in encapsulated enzyme biosensors published in peer-reviewed journals over the last 10 years. The articles are categorized and reviewed in four groups based on encapsulation techniques incorporating with liposome, polymer, sol-gel or hydro-gel, and peptide. Research articles that are considered critical and noticeable are selected and compared according to these categorizations. Based on these article analyses, we suggested future direction in the encapsulated enzyme biosensor research.

Keywords: Biosensor; Enzyme encapsulation; Nanoparticle; Nanotube; Electrochemistic; Spectrophotometric

Feng Long^a, Miao He^a, Anna Zhu^a, Baodong Song^a, Jianwu Sheng^a and Hanchang Shi^a. (^aEnvironmental Simulation and Pollution Control State Key Joint Laboratory, Department of Environment Science and Engineering, Tsinghua University, Beijing, 100084, China). Compact quantitative optic fiber-based immunoarray biosensor for rapid detection of small analytes. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 16-22

Immunoarrays have been proven to be powerful tools for high-throughput analysis of multiple analytes. In this paper, a proof-of-concept development of a novel optic fiber-based immunoarray biosensor for the detection of multiple small analytes is presented. This was developed through immobilization of two kinds of hapten conjugates, MC-LR-OVA and NB-OVA, onto the same fiber optic probe. The technique is significantly different from conventional immunoarray sensors. Microcystin-LR (MC-LR) and trinitrotoluene (TNT) could be detected simultaneously and specifically within an analysis time of about 10 min for each assay cycle. The limits of detection for MC-LR and TNT were 0.04 µg/L and 0.09 mg/L, respectively. Good regeneration performance, binding properties, and robustness of the sensor surface of the proposed immunoarray biosensor ensure the cost-effective and accurate measurement of small analytes. The change in concentration of the hapten conjugates immobilized onto the sensor surface was also proven to have no significant effect on the performance of immunoarray sensor, which is essential to the application of the immunoarray in real samples detection. This compact and portable quantitative immunoarray provides an excellent multiple assay platform for clinical and environmental samples.

Keywords: Immunosensor; Array; Fluorescence; Small analyte; Quantitative detection

Yuri Antonio Diaz-Fernandez^a, Elisa Mottini^a, Luca Pasotti^a, Emanuela Fabiola Craparo^b, Gaetano Giammona^b, Gennara Cavallaro^b and Piersandro Pallavicini^a. (^aDipartimento di Chimica Generale, Università di Pavia, viale Taramelli 12, 27100 Pavia, Italy, ^b Dipartimento di Chimica e Tecnologie Farmaceutiche, via Archirafi 32, 90123 Palermo, Italy). Multicomponent polymeric micelles based on polyaspartamide as tunable fluorescent pH-window biosensors. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 29-35

PHEA-PEG₅₀₀₀-C₁₆ is a polyaspartamide polymer with appended hydrophilic PEG₅₀₀₀ functions and hydrophobic n-C₁₆ units forming biocompatible micelles with a CAC as low as 1.8×10^{-7} M. The protonation and acidity constants of the polymer's amino and carboxylic groups have been determined by potentiometric titrations at five different concentrations higher than CAC, finding concentration-independent values. Viscosity and polarity of the micellar core have been investigated by means of fluorescent probes, finding local values comparable to those of pure toluene and to the core of sodium dodecyl sulphate micelles, independently on the protonation degree of the polymer. The fluorophore pyrene, the lipophilic N,N'-dimethyl-N"-dodecylamine and 2-dodecylpyridine self-assemble in the hydrophobic core of PHEA-PEG₅₀₀₀-C₁₆ micelles originating a micellar device that behaves as a rare "off-on-off" fluorescence sensor for pH windows, with no interference by the amino and carboxylic functions of the polymer. The "on" state of the sensor includes the physiological 6–8 pH interval, and can be finely shifted in both directions of the pH axis by comicellization of charged cosurfactants. Dialysis experiments demonstrate that the micellar device exhibits an efficient retention ability of all molecular components, including cosurfactants, thus candidating for in vivo use.

Keywords: Polymeric micelles; Fluorescent biosensor; PH window; Self-assembling

Ramiz S.J. Alkasir^a, Mallikarjunarao Ganesana^a, Yu-Ho Won^b, Lia Stanciu^b and Silvana Andreescu^a. (^a Department of Chemistry and Biomolecular Science, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699-5810, United States, ^b School of Materials Engineering and Birck Nanotechnology Center, Purdue University, West Lafayette, IN 47907, United States). **Enzyme functionalized nanoparticles for electrochemical biosensors: A comparative study with applications for the detection of bisphenol A. Biosensors and Bioelectronics, Volume 26(1) (2010): 43-49**

We developed electrochemical biosensors based on enzyme functionalized nanoparticles of different compositions for the detection of bisphenol A. We utilized for the first time magnetic nickel nanoparticles as an enzyme immobilization platform and electrode material to construct screen-printing enzyme biosensors for bisphenol A. We compared the analytical performance of these sensors with those based on iron oxide (Fe₃O₄) and gold nanoparticles. The proposed biosensor format exhibited fast and sensitive amperometric responses to bisphenol A with a response time of less than 30 s. Among the three configurations, nickel provided comparable or better characteristics in terms of detection limit and sensitivity than Fe₃O₄ and gold nanoparticles. The biosensors were characterized by good reproducibility, stability of more than 100 assays (residual activity for nickel was 98%) and a wide linear range which spanned from 9.1×10^{-7} to 4.8×10^{-5} M for nickel, 2.2×10^{-8} to 4.0×10^{-5} M for Fe₃O₄ and 4.2×10^{-8} to 3.6×10^{-5} M for gold. The highest sensitivity was obtained with nickel. The detection limits for the three types of biosensors were: 7.1×10^{-9} , 8.3×10^{-9} and 1×10^{-8} M for nickel, Fe₃O₄ and gold nanoparticles in that order, respectively. These results demonstrate that nickel nanoparticles can be successfully used in the construction of electrochemical enzyme sensors for the detection of phenolic compounds.

Keywords: Tyrosinase; Bisphenol A; Magnetic; Iron oxide; Nickel; Gold; Nanoparticles; Screen-printed electrodes

Liu Deng^a, Li Shang^a, Dan Wen^a, Junfeng Zhai^a and Shaojun Dong^a. (^a State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, PR China). **A membraneless biofuel cell powered by ethanol and alcoholic beverage. Biosensors and Bioelectronics, Volume 26(1) (2010): 70-73**

In this study, we reported on the construction of a stable single-chamber ethanol/O₂ biofuel cell harvesting energy from the ethanol and alcoholic beverage. We prepared a composite film which consisted of partially sulfonated (3-mercaptopropyl)-trimethoxysilane sol-gel (PSSG) and chitosan (CHI). The combination of ion-exchange capacity sol-gel and biopolymer chitosan not only provided the attached sites for mediator MDB and AuNPs to facilitate the electron transfer along the substrate reaction, but also gave the suitable microenvironment to retain the enzyme activity in long term. The ethanol bioanode was constructed with the film coimmobilized dehydrogenase (ADH), Meldola's blue (MDB) and gold nanoparticles (AuNPs). The MDB/AuNPs/PSSG-CHI-ADH composite modified electrode showed prominent electrocatalytic activity towards the oxidation of ethanol. The oxygen biocathode consisted of laccase and AuNPs immobilized on the PSSG-CHI composite membrane. The AuNPs/PSSG-CHI-laccase modified electrode catalyzed four-electron reduction of O₂ to water, without any mediator. The assembled single-chamber biofuel cell exhibited good stability and power output towards

ethanol. The open-circuit voltage of this biofuel cell was 860 mV. The maximum power density of the biofuel cell was 1.56 mW cm^{-2} at 550 mV. Most interestingly, this biofuel cell showed the similar performance when the alcoholic beverage acted as the fuel. When this biofuel cell ran with wine as the fuel, the maximum power output density was 3.21 mA cm^{-2} and the maximum power density was 1.78 mW cm^{-2} at 680 mV of the cell voltage. Our system exhibited stable and high power output in the multi-component substrate condition. This cell has great potential for the development and practical application of bioethanol fuel cell.

Keywords: Alcohol dehydrogenase; Laccase; Biofuel cell; Alcoholic beverage; Bioethanol

Mariana S. Fernandes^a, Nuno S. Dias^a, Alexandre F. Silva^a, Jivago S. Nunes^b, Senentxu Lanceros-Méndez^b, José H. Correia^a and Paulo M. Mendes^a. (^aDepartment of Industrial Electronics, University of Minho, Campus Azurém, 4800-058 Guimarães, Portugal, ^b Department of Physics, University of Minho, 4710-057 Braga, Portugal). **Hydrogel-based photonic sensor for a biopotential wearable recording system. Biosensors and Bioelectronics, Volume 26(1) (2010): 80-86**

Wearable devices are used to record several physiological signals, providing unobtrusive and continuous monitoring. These systems are of particular interest for applications such as ambient-assisted living (AAL), which deals with the use of technologies, like brain-computer interface (BCI). The main challenge in these applications is to develop new wearable solutions for acquisition of electroencephalogram (EEG) signals. Conventional solutions based on brain caps, are difficult and uncomfortable to wear. This work presents a new optical fiber biosensor based on electro-active gel – polyacrylamide (PAAM) hydrogel – with the ability to measure the required EEG signals and whose technology principle leads to contactless electrodes. Experiments were performed in order to evaluate the electro-active properties of the hydrogel and its frequency response, using an electric and optical setup. A sinusoidal electric field was applied to the hydrogel while the light passes through the sample. An optical detector was used to collect the resultant modulated light. The results have shown an adequate sensitivity in the range of μV , as well as a good frequency response, pointing the PAAM hydrogel sensor as an eligible sensing component for wearable biopotential recording applications.

Keywords: Wearable braincap; Contactless sensor; Brain-computer interface; Ambient-assisted living

Ya Cao^a, Jing Wang^a, Yuanyuan Xu^a and Genxi Li^{a, b}. (^a Department of Biochemistry and National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, 210093 Nanjing, China, ^b Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, 200444 Shanghai, China). **Combination of enzyme catalysis and electrocatalysis for biosensor fabrication: Application to assay the activity of indoleamine 2,3-dioxygenase. Biosensors and Bioelectronics, Volume 26(1) (2010): 87-91**

A new strategy to fabricate electrochemical biosensor is reported in this paper based on the selective combination of enzyme catalysis and electrocatalysis, thus an electrochemical method to assay the activity of indoleamine 2,3-dioxygenase (IDO) is proposed. Tryptophan, the substrate of IDO, is firstly covalently immobilized on a gold electrode surface. Oxidation of the tryptophan residue catalyzed by IDO and the subsequent hydrolyzation of the product by acetic

acid may yield kynurenine, which may induce the immobilization of dithiobis [succinimidylpropionate] (DSP)-modified platinum nanoparticles (Pt NPs) onto the surface of the gold electrode. Since Pt NPs can electrochemically catalyze the reduction of H₂O₂ to produce electrochemical signals and the electrochemical wave can be correlated with the enzyme activity, electrochemical method to detect IDO activity is thus achieved. Under optimized conditions, IDO activity can be assayed in the range of 20–400 U/mL with a detection limit of 6.84 U/mL. The proposed biosensor shows high sensitivity, acceptable reliability, and can be used for the investigation of the enzymatic inhibition by inhibitors as well as the screen of the enzymatic activity in complex matrix such as serum samples.

Keywords: Indoleamine 2,3-dioxygenase; Platinum nanoparticle; Enzyme catalysis; Electrocatalysis

Pratik Banerjee^{a, b} and Arun K. Bhunia^b. (^a Laboratory of Food Microbiology and Immunochemistry, Alabama A&M University, Normal, AL 35762, USA, ^b Molecular Food Microbiology Laboratory, Department of Food Science, Purdue University, 745 Agriculture Mall Drive, West Lafayette, IN 47907, USA). Cell-based biosensor for rapid screening of pathogens and toxins. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 99-106

Development and validation of a mammalian cell-based biosensor for application in food defense and food safety was investigated. Three prototypes of the biosensor capable of handling different sample types were developed and tested with food and beverages. The sensing element is a B lymphocyte Ped-2E9 cell-line, encapsulated in collagen matrix in 3D scaffold. The uniqueness of this biosensor is that it detects analyte interaction with mammalian cells and is able to distinguish pathogenic from non-pathogenic and active from inactive toxins, rendering accurate estimation of the risk associated with the agents. This sensor gave positive signal for a broad range of bacterial pathogens; *Listeria monocytogenes*, enterotoxigenic *Bacillus*, *Vibrio*, *Micrococcus* and *Serratia*, and toxins; α -hemolysin from *Staphylococcus aureus*, phospholipase C from *Clostridium perfringens*, cytolyisin from sea anemone *Stoichactis helianthus*, listeriolysin O from *L. monocytogenes*, and enterotoxin from *Bacillus*. Detection limit for toxins was 10–40 ng in 2 h while for a model bacterial pathogen, *L. monocytogenes*, 10³–10⁴ CFU/ml in 4–6 h, even in the presence of a mixture of higher concentrations of non-pathogenic species of the same genera or common background microflora. With inoculated food and beverage, the sensor detected *L. monocytogenes* and *Bacillus cereus* at a low initial concentration of 10²–10⁴ CFU/g from ready-to-eat meat and rice, and only active toxins at nanogram quantities from rice, milk and water samples. Though all the three prototypes performed well with beverages, Devices II & III are most suitable for testing particulate foods. These data present promising evidence for possible application of this biosensor for rapid detection of multiple pathogens or toxins for food defense and food safety application.

Keywords: Cell-based biosensor; Pathogens; Toxins; Food; Water

Xinyan Bi^a and Kun-Lin Yang^a. (^a 4 Engineering Drive 4, Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore 117576, Singapore). Liquid crystals decorated with linear oligopeptide FLAG for applications in immunobiosensors. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 107-111

Immunobiosensors are emerging as powerful tools for diagnostic applications. In this paper, we exploit optical properties of liquid crystal (LC) and the specific epitope–antibody interaction to develop an optical immunobiosensor which can be used to detect monoclonal anti-FLAG M2, a model antibody. The sensitive layer of this immunobiosensor is a monolayer of immobilized oligopeptide CDYKDDDDK (FLAG) at an LC/aqueous interface. The linear oligopeptide can function as an epitope which can be recognized by anti-FLAG M2. When the LC is modified with 50 μM of FLAG and then exposed to an aqueous solution containing anti-FLAG, anti-FLAG binds to the immobilized FLAG epitope and triggers orientational transition of LC. Because of the optical birefringence of the LC, the orientational transition is accompanied by changes in the optical images of LC from dark to bright under crossed polarizers. The detection limit for the system is 27 ng/mL of anti-FLAG, and the response time is <1 h. The binding of anti-FLAG to its motif FLAG oligopeptide is also very specific. When a variant epitope CDAKDDDDK is used, no apparent optical response can be observed after the addition of anti-FLAG.

Keywords: Immunobiosensor; Oligopeptide; FLAG; Liquid crystals

Dipali Bagal-Kestwal^a, Rakesh Mohan Kestwal^a, Bo-Chuan Hsieh^b, Richie L.C. Chen^b, Tzong-Jih Cheng^{b, c} and Been-Huang Chiang^a. (^a Institute of Food Science and Technology, National Taiwan University, No. 1, Roosevelt Road, Section 4, Taipei, Taiwan, ROC, ^b Department of Bio-Industrial Mechatronics Engineering, College of Agriculture, National Taiwan University, No.1, Roosevelt Road, Section 4, Taipei, Taiwan, ROC, ^c Department of Biomedical Engineering, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan, ROC). **Electrochemical $\beta(1 \rightarrow 3)$ -d-glucan biosensors fabricated by immobilization of enzymes with gold nanoparticles on platinum electrode. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 118-125**

$\beta(1 \rightarrow 3)$ -d-Glucan sensors were fabricated using bi-enzyme and tri-enzyme immobilized systems with gold nanoparticles (GNPs) to increase sensitivity. The plant $\beta(1 \rightarrow 3)$ -d-glucanase (βG), glucose oxidase (GOD) or/and peroxidase (POD) in agarose–corn flour–gelatin (ACG) matrix were coated on platinum disc electrode to detect soluble $\beta(1 \rightarrow 3)$ -d-glucan. The atomic force microscopy (AFM) revealed that GNPs embedded in ACG formed tiny islands/clusters with enzymes. Both of bi-enzyme sensor (ACG– βG –GOD–GNPs/Pt) and tri-enzyme sensor (ACG– βG –GOD–POD–GNPs/Pt) had response time less than 20 s for $\beta(1 \rightarrow 3)$ -d-glucan. A linear calibration plot for bi-enzyme sensor was obtained for $\beta(1 \rightarrow 3)$ -d-glucan concentration ranged from 100 to 1000 ng mL⁻¹ ($R^2 = 0.983$). The lower detection limit was 30 ng mL⁻¹ using applied potential of 200 mV and scan rate of 50 mV s⁻¹; with signal to noise ratio (S/N) of 3. Fabricated tri-enzyme sensor was also operable under similar conditions with LOD of 50 ng mL⁻¹ ($R^2 = 0.989$) at –175 mV applied potential and scan rate of 50 mV s⁻¹. Both sensors were durable and could be repeatedly used for at least 14 times. When the tri-enzyme sensor was employed to analyze $\beta(1 \rightarrow 3)$ -d-glucan content in alcoholic beverages, the results were comparable to those obtained by standard method.

Keywords: $\beta(1 \rightarrow 3)$ -d-Glucan biosensor; $\beta(1 \rightarrow 3)$ -d-Glucanase; Glucose oxidase; Peroxidase; Gold nanoparticles; Agarose–corn flour–gelatin matrix

Marta Sanchez-Paniagua Lopez^a, Franck Charmantray^b, Virgil Helaine^b, Laurence Hecquet^b and Christine Mousty^c. (^a Sección Departamental Química Analítica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain, ^b Laboratoire de Synthèse et Etude de Systèmes à Intérêt Biologique, UMR 6504, Université Blaise Pascal, Clermont-Ferrand, France, ^c Laboratoire des Matériaux Inorganiques, UMR 6002, Université Blaise Pascal, Clermont-Ferrand, France). Electrochemical detection of transketolase activity using a tyrosinase biosensor. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 139-143

This paper proposes a new concept of transketolase (TK) activity profiling. A tyrosinase (PPO) biosensor, based on the immobilization of this enzyme in a Mg₂Al–Cl layered double hydroxide, was developed for the amperometric detection of N-acetyl-l-tyrosine ethyl ester monohydrate (N-Ac-Tyr-OEt) at –0.2 V. This compound was released during an enzymatic reaction catalyzed by TK with N-acetyl-O-(2R, 3S, 5-trihydroxy-4-oxopentyl)-l-tyrosine ethyl ester used as donor substrate. This tyrosinase biosensor was optimized for the detection of TK activity, including PPO optimum substrate concentration, electrolyte nature, pH, and influence of bovine serum albumin (BSA). It was found that N-Ac-Tyr-OEt release is dependent on TK concentration (U/mL) in the electrolyte medium. These results demonstrate the sensitivity and specificity of the tyrosinase biosensor designed for *in vitro* detection of TK activity, which is known to be involved in several diseases.

Keywords: PPO; Biosensor; Transketolase; Tyrosine; Layered double hydroxides; Amperometry

Jinghua Chen^{a, b}, Jing Zhang^c, Huanghao Yang^a, Fengfu Fu^a and Guonan Chen^a. (^aMinistry of Education Key Laboratory of Analysis and Detection Technology for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, Department of Chemistry, Fuzhou University, 2 Xueyuan Road, Fuzhou 350108, Fujian, China, ^b Department of Pharmaceutical Analysis, Faculty of Pharmacy, Fujian Medical University, Fuzhou 350004, China, ^c Pharmaceutical Department of Fujian College of Medical Occupation and Technology, Fuzhou 350101, China). A strategy for development of electrochemical DNA biosensor based on site-specific DNA cleavage of restriction endonuclease. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 144-148

A new strategy for development of electrochemical DNA biosensor based on site-specific DNA cleavage of restriction endonuclease and using quantum dots as reporter was reported in this paper. The biosensor was fabricated by immobilizing a capture hairpin probe, thiolated single strand DNA labeled with biotin group, on a gold electrode. BfuCI nuclease, which is able to specifically cleave only double strand DNA but not single strand DNA, was used to reduce background current and improve the sensitivity. We demonstrated that the capture hairpin probe can be cleaved by BfuCI nuclease in the absence of target DNA, but cannot be cleaved in the presence of target DNA. The difference before and after enzymatic cleavage was then monitored by electrochemical method after the quantum dots were dissolved from the hybrids. Our results suggested that the usage of BfuCI nuclease obviously improved the sensitivity and selectivity of the biosensor. We successfully applied this method to the sequence-selective discrimination between perfectly matched and mismatched target DNA including a single-base mismatched target DNA, and detected as low as 3.3×10^{-14} M of complementary target DNA. Furthermore, our above strategy was also verified with fluorescent method by designing a fluorescent molecular beacon (MB), which combined the capture hairpin probe and a pair of fluorophore

(TAMRA) and quencher (DABCYL). The fluorescent results are consistent with that of electroanalysis, further indicating that the proposed new strategy indeed works as we expected.

Keywords: Electrochemical DNA Biosensor; Site-specific DNA cleavage; Restriction endonuclease; Cymbidium mosaic virus

Youlin Zhang^{a, b}, Qinghui Zeng^a, Yajuan Sun^a, Xiaomin Liu^a, Langping Tu^a, Xianggui Kong^a, Wybren Jan Buma^b and Hong Zhang^b. (^a Key Laboratory of Excited State Processes, Changchun Institute of Optics, Fine Mechanics and Physics, Chinese Academy of Sciences, 3888 Eastern South Lake Road, Changchun 130033, China, ^b Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands). **Multi-targeting single fiber-optic biosensor based on evanescent wave and quantum dots. Biosensors and Bioelectronics, Volume 26(1) (2010): 149-154**

Highly sensitive, multi-analyte assay is a long-standing challenge for a single fiber-optic evanescent wave biosensor (FOB). In this paper, we report the first realization of such kind of FOB using CdSe/ZnS core/shell quantum dots (QDs) as labels. A direct binding assay model between antibody and antigen was employed to demonstrate the advantages of using QDs, instead of conventional fluorescein isothiocyanate (FITC), in lifting the sensitivity. Especially, multiplexed immunoassay was demonstrated in a single fiber FOB constructed with four differently sized QDs. Furthermore, the phenomenon that the affinity of the QD-labeled human IgG (QD-IgG) with goat anti-human IgG (anti-IgG) was lower than that of the FITC-labeled human IgG (FITC-IgG) was investigated and was ascribed to the differences in size and mass of the two. Our study indicates that the affinity could be improved by controlling the amount of IgG binding on QDs.

Keywords: Fiber-optic evanescent wave biosensor; Multiplexed immunoassay; Quantum dots; CdSe/ZnS; Affinity

F. Sommerhage^{a, b, d}, A. Baumann^c, G. Wrobel^{a, b}, S. Ingebrandt^{a, b, d} and A. Offenhäusser^{a, b}. (^a Institute of Bio- and Nanosystems (IBN2), Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany, ^b Jülich-Aachen Research Alliance, Fundamentals of Future Information Technologies (JARA-FIT), Jülich, Germany, ^c Institute of Structural Biology and Biophysics (ISB1), Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany, ^d Department of Informatics and Microsystems Technology (I/MST), University of Applied Sciences Kaiserslautern, Campus Zweibrücken, D-66482 Zweibrücken, Germany). **Extracellular recording of glycine receptor chloride channel activity as a prototype for biohybrid sensors. Biosensors and Bioelectronics, Volume 26(1) (2010): 155-161**

There is a continuously growing scientific and technological interest to develop and improve the application of artificial sensors. Biological components which are capable to transduce neutral signals into specific, robust and reproducible indicators frame an attractive alternative to construct biohybrid sensors. Since naturally "occurring" biosensors are only sparsely compatible with artificial devices, genetic engineering of eukaryotic cells provides an attractive approach, where cells can be tailored such to detect target compounds with exquisite specificity and

sensitivity. We have developed the prototype for a single-cell-based anion-selective biohybrid sensor. HEK293 cells were stably transfected with a gene encoding glycine receptor alpha₁ subunits. These cells were employed as transducers for glycine-evoked chloride currents in a concentration-dependent way. Cultured on substrate-integrated micro-devices, anionic membrane currents of cells were monitored extracellularly with field-effect transistors (FETs) and gold microelectrode arrays (MEAs). The results supported predictions of state-of-the-art models for cell-sensor coupling mechanisms and confirmed that extracellularly recorded anion currents cause similar signals, regardless whether obtained with field-effect transistors or microelectrodes. The whole-cell sensor successfully tracked glycine concentrations differing by three orders of magnitude. To our knowledge this contribution for the first time marks the functional characterization of an anion-selective biohybrid sensor.

Keywords: Glycine receptor; Field-effect transistor; Microelectrode array; (bio)Hybrid system; Chloride channel; Single-cell-based sensor

Dana Krinke^{a, 1}, Heinz-Georg Jahnke^{a, 1}, Till G.A. Mack^b, Anika Hirche^c, Frank Striggow^b and Andrea A. Robitzki^a. (^a Centre for Biotechnology and Biomedicine (BBZ), University of Leipzig, Division of Molecular Biological-Biochemical Processing Technology, Deutscher Platz 5, 04103 Leipzig, Germany, ^b KeyNeurotek Pharmaceuticals AG, Zenit Technologiepark, Leipziger Str. 44, 39120 Magdeburg, Germany, ^c Translational Centre for Regenerative Medicine, University of Leipzig, Semmelweisstr. 14, 04103 Leipzig, Germany). A novel organotypic tauopathy model on a new microcavity chip for bioelectronic label-free and real time monitoring. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 162-168

Herewith we developed a novel 3D *in vitro* Alzheimer's disease (AD) model, based on the human neuroblastoma cell line SH-SY5Y, which is well differentiated without the application of any agents. Furthermore AD-like pathological neurodegeneration can be induced by okadaic acid (OA) mediated hyperphosphorylation of the microtubule associated protein tau. Moreover, we established stable "rapid tauopathy cell lines" expressing additional EGFP-fused (enhanced green fluorescent protein) wildtype or a pathology-promoting mutant tau variant (P301L) by lentiviral transduction. For the sensitive and feasible quantitative detection of pathological effects on neuronal 3D-cultures by electrochemical impedance spectroscopy (EIS) we optimized and redesigned a microcavity array (MCA). The cellular contribution to impedance could be increased by the factor of 2.5 and the variance decreased by 40%. Using our optimized MCA and impedance measurement setup we were able to detect quantitatively an OA concentration- and time-dependent decrease of the impedance in 3D SH-SY5Y cultures. Moreover, we were able to detect and quantify distinct, AD-related effects triggered by tau-mutant (P301L) expression and hyperphosphorylation in our organotypic 3D-cultures with the help of impedance spectroscopy.

Keywords: Microcavity array; Impedance spectroscopy; SH-SY5Y cell line; Okadaic acid; Tau hyperphosphorylation; Alzheimer's disease model

Shinichi Oota^a, Yuta Hatae^b, Kei Amada^c, Hidekazu Koya^c and Mitsuyasu Kawakami^c (^aMaterials Science and Production Engineering, Graduate School of Engineering, Fukuoka Institute of Technology, Higashi-ku, Fukuoka 811-0295, Japan, ^bFunctional Materials Engineering, Graduate School of Engineering, Fukuoka Institute of Technology, Higashi-ku, Fukuoka 811-0295, Japan, ^c Department of Life, Environment and Materials

Science, Faculty of Engineering, Fukuoka Institute of Technology, Higashi-ku, Fukuoka 811-0295, Japan). Development of mediated BOD biosensor system of flow injection mode for *shochu* distillery wastewater. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 262-266

Although microbial biochemical oxygen demand (BOD) sensors utilizing redox mediators have attracted much attention as a rapid BOD measurement method, little attempts have been made to apply the mediated BOD biosensors to the flow injection analysis system. In this work, a mediated BOD sensor system of flow injection mode, constructed by combining an immobilized microbial reactor with an electrochemical flow cell of three electrodes configuration, has been developed to estimate BOD of *shochu* distillery wastewater (SDW). It was demonstrated consequently that the mediated sensing was realized by employing phosphate buffer containing potassium hexacyanoferrate as the carrier. The output current was found to yield a peak with a sample injection, and to result from reoxidation of reduced mediator at the electrode. By employing the peak area as the sensor response, the effects of flow rate and pH of the carrier on the sensitivity were investigated. The sensor system using a microorganism of high SDW-assimilation capacity showed good performance and proved to be available for estimation of BOD of SDW.

Keywords: Biochemical oxygen demand; Flow injection; Mediator; *Shochu* distillery wastewater

Yinyin Wei^a, Ying Li^a, Xiaoqian Liu^a, Yuezhong Xian^a, Guoyue Shi^a and Litong Jin^a. (^aDepartment of Chemistry, East China Normal University, 3663 Zhong Shan Road North, Shanghai 200062, China). ZnO nanorods/Au hybrid nanocomposites for glucose biosensor. *Biosensors and Bioelectronics*, Volume 26(1) (2010): 275-278

ZnO nanorods/Au hybrid nanocomposites (ZnO/Au) with Au nanocrystals growing on the surface of ZnO nanorods were synthesized via a simple and facile hydrothermal route. The prepared ZnO/Au nanocomposites were characterized by X-ray diffraction (XRD), scanning electron microscope (SEM) and transmission electron microscopy (TEM) for the morphology study. The composites had a good electron transferring and biocompatibility. The glucose biosensor was fabricated by entrapping glucose oxidase (GOx) in this composite matrix using cross-linking method with glutaraldehyde and Nafion solutions. The proposed biosensor responded to glucose linearly over concentration range of 0.1–33.0 μM ($R^2 = 0.9956$), and the detection limit was 10 nM (S/N = 3) at an operating potential of +0.55 V in pH 7.4 phosphate buffered solution (PBS). The biosensor exhibited a high and reproducible sensitivity, short response time (within 5 s), good storage stability and high affinity to GOx ($K_M^{\text{app}} = 0.41 \text{ mM}$). The effects of electroactive interferents at the testing conditions can be negligible which showed a good selectivity of the biosensor. It is estimated that this ZnO/Au is an attractive material for the fabrication of efficient amperometric biosensors.

Keywords: ZnO/Au; Hybrid nanocomposites; Glucose oxidase; Glucose biosensor

M. Holgado^a, C.A. Barrios^b, F.J. Ortega^c, F.J. Sanza^a, R. Casquel^a, M.F. Laguna^a, M.J. Bañuls^c, D. López-Romero^b, R. Puchades^c and A. Maquieira^c. (^a Centro Láser UPM, Universidad Politécnica de Madrid. Campus sur de la UPM, 28031 Madrid, Spain, ^b

Instituto de Sistemas Optoelectrónicos y Microtecnología, Universidad Politécnica de Madrid, ETSI de Telecomunicación. Ciudad Universitaria s/n. 28040 Madrid, Spain, ^c Instituto de Reconocimiento Molecular. Universidad Politécnica de Valencia. Camino de Vera s/n. 46022 Valencia, Spain). Label-free biosensing by means of periodic lattices of high aspect ratio SU-8 nano-pillars. *Biosensors and Bioelectronics*, Volume 25(12) (2010): 2553-2558

We developed biophotonic sensing arrays of $60 \times 60 \mu\text{m}^2$ made of periodic lattices of high aspect ratio SU-8 nano-pillars in order to demonstrate their capability for label-free molecule detection, as well as the sensitivity enhancement in comparison with a single layer of SU-8. The biophotonic sensing arrays, that we call BICELLS (Biophotonic sensing cells), are interrogated vertically by using micron spot size Fourier transform visible and IR spectrometry (FT-VIS-IR). We monitored the surface immobilization of bovine serum albumin (BSA) antigen and anti-BSA antibody (aBSA) recognition. The bioassay exhibits a limit of detection (LOD) in the order of 2 ng/ml limited by the wavenumber uncertainty during the interrogation process. We also estimated and compared the theoretical bilayer thickness with previous results.

Keywords: Label-free biosensing; Micro-nano fabrication; SU-8; Optical interrogation

Xia Sun^a and Xiangyou Wang^a. (^a School of Agriculture and Food Engineering, Shandong University of Technology, NO. 12, Zhangzhou Road, Zibo 255049, Shandong Province, PR China). Acetylcholinesterase biosensor based on prussian blue-modified electrode for detecting organophosphorous pesticides. *Biosensors and Bioelectronics*, Volume 25(12) (2010): 2611-2614

In this study, a novel acetylcholinesterase (AChE) biosensor was developed based on dual-layer membranes (chitosan membrane and prussian blue membrane) modifying glassy carbon electrode (GCE). A chitosan membrane was used for immobilizing AChE through glutaraldehyde cross-linking attachment to recognize pesticides selectively. A prussian blue (PB) membrane was electrodeposited on the surface of GCE to enhance electron transfer. Before the detection, the chitosan enzyme membrane was quickly fixed on the surface of PB/GCE with O-ring to prepare an amperometric AChE-PB/GCE sensor for organophosphorus (OP) pesticides. The electrochemical behaviour of AChE-PB/GCE was studied, and the results showed that the chitosan membrane as carrier can absorb a large amount of enzyme, and PB has a significant synergistic effect towards enzymatic catalysis. As a result of these two important enhancement factors, the proposed biosensor exhibited extreme sensitivity to OP pesticides compared to the other kinds of AChE biosensor. The influences of phosphate buffer pH, substrate concentration, incubation time of pesticide on the response of the fabricated biosensor were investigated. Under optimum conditions, the inhibition rates of these pesticides were proportional to their concentrations in the range of $0.01\text{--}10 \mu\text{g l}^{-1}$, $0.05\text{--}10 \mu\text{g l}^{-1}$, $0.03\text{--}5 \mu\text{g l}^{-1}$, and $0.05\text{--}10 \mu\text{g l}^{-1}$, respectively. The detection limits were found to be 2.5 ng l^{-1} for dichlorvos, 15 ng l^{-1} for omethoate, 5 ng l^{-1} for trichlorfon and 10 ng l^{-1} for phoxim. Moreover, the biosensor exhibited good reproducibility and stability, and it was suitable for trace detection of OP pesticide residue.

Keywords: Biosensor; Prussian blue; Acetylcholinesterase; Pesticide residue; Modified electrode

Limin Liu^a, Bo Shen^a, Jianjun Shi^a, Fang Liu^a, Guo-Yuan Lu^a and Jun-Jie Zhu^a. (^aKey Laboratory of Analytical Chemistry for Life Science, Ministry of Education of China,

School of Chemistry and Chemical Engineering, Nanjing University, Hankou Road 22, Nanjing 210093, PR China). A novel mediator-free biosensor based on co-intercalation of DNA and hemoglobin in the interlayer galleries of α -zirconium phosphate. *Biosensors and Bioelectronics*, Volume 25(12) (2010): 2627-2632

A novel mediator-free biosensor was constructed by the co-intercalation of negatively charged DNA and positively charged hemoglobin (Hb) in the interlayer galleries of layered α -zirconium phosphate (α -ZrP) with the delamination-assembly procedure at pH 5.5. X-ray diffraction and field-emission scanning electron microscopy results revealed the featured layered structure for the re-assembled DNA/Hb/ α -ZrP composite. Infrared spectroscopy and circular dichroism results confirmed the coexistence of Hb and DNA in the composite and the considerably retained protein conformation of intercalated Hb. The direct electron transfer of Hb was facilitated by the co-intercalation of DNA and Hb. Because of the synergistic effect of α -ZrP host and co-intercalated DNA guest, the DNA/Hb/ α -ZrP modified electrode exhibited good electrocatalytic response to H_2O_2 with higher sensitivity of $0.79 \text{ A M}^{-1} \text{ cm}^{-2}$ and lower detection of $4.28 \times 10^{-7} \text{ M}$ in the linear range of 7.28×10^{-7} to $9.71 \times 10^{-5} \text{ M}$. Furthermore, the electrocatalytic activity of Hb in the DNA/Hb/ α -ZrP composite retained at high temperature ($85 \text{ }^\circ\text{C}$) or in the presence of organic solvent (CH_3CN), which could be the protection of α -ZrP nanosheets.

Keywords: Hemoglobin (Hb); DNA; DNA/Hb/ α -ZrP composite; Direct electron transfer (DET)

Rakesh Singh Moirangthem^{a, b, c}, Yia-Chung Chang^b, Shih-Hsin Hsu^b and Pei-Kuen Wei^b. (^a Department of Engineering and System Science, National Tsing Hua University, Hsinchu 300, Taiwan, ^b Research Centre for Applied Sciences, Academia Sinica, Taipei 115, Taiwan, ^c Nano Science and Technology Program, Taiwan International Graduate Program, Academia Sinica, Taipei 115, Taiwan). Surface plasmon resonance ellipsometry based sensor for studying biomolecular interaction. *Biosensors and Bioelectronics*, Volume 25(12) (2010): 2633-2638

A simple surface plasmon resonance (SPR) ellipsometry equipped with a dove prism and micro-fluidic flow cell is adopted to investigate and study basic properties of biomolecular interaction. Using a dove prism greatly simplifies the optical alignment and the use of micro-fluidic cell helps reduce significantly the volume of the biological sample required in the experiment. By recording the ellipsometry data in terms of relative changes in the ellipsometric parameters, Ψ and Δ as sensor signals we can understand the biomolecular interaction. Spectroscopic measurements were performed to check the bulk sensitivity, which further helps determine the corresponding wavelength for maximum sensitivity. Furthermore, dynamic measurements at a fixed wavelength were also done and allow the observation of real-time response to the changes in surface properties on a metallic film. Such a simple technique gives an index resolution around 1.64×10^{-6} which is better than the conventional SPR method based on the resonance angular detection. This technique yields sensitivity sufficient enough to detect changes in the effective thickness of biomolecular layer. Biological processes such as adsorption of protein to metal and protein-protein interaction can be understood from the optical response of the sample surface. Such technique is a promising candidate in developing profitable and user-friendly biosensors. Furthermore, this kind of characterization technique is non-destructive, label free, and sensitive with a sub-nanometer resolution in thickness.

Keywords: Spectroscopic ellipsometry; Kretschmann configuration; Dove prism; Surface plasmon resonance; Biosensor

Xin Wang^a, Yujie Feng^a, Jia Liu^a, He Lee^a, Chao Li^a, Nan Li^a and Nanqi Ren^a. (^aState Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, No. 73 Huanghe Road, Nangang District, Harbin 150090, China). **Sequestration of CO₂ discharged from anode by algal cathode in microbial carbon capture cells (MCCs). Biosensors and Bioelectronics, Volume 25(12) (2010): 2639-2643**

Due to increased discharge of CO₂ is incurring problems, CO₂ sequestration technologies require substantial development. By introducing anodic off gas into an algae grown cathode (*Chlorella vulgaris*), new microbial carbon capture cells (MCCs) were constructed and demonstrated here to be an effective technology for CO₂ emission reduction with simultaneous voltage output without aeration (610 ± 50 mV, 1000 Ω). Maximum power densities increased from 4.1 to 5.6 W/m³ when the optical density (OD) of cathodic algae suspension increased from 0.21 to 0.85 (658 nm). Compared to a stable voltage of 706 ± 21 mV (1000 Ω) obtained with cathodic dissolved oxygen (DO) of 6.6 ± 1.0 mg/L in MCC, voltage outputs decreased from 654 to 189 mV over 70 h in the control reactor (no algae) accompanied with a decrease in DO from 7.6 to 0.9 mg/L, indicating that cathode electron acceptor was oxygen. Gas analysis showed that all the CO₂ generated from anode was completely eliminated by catholyte, and the soluble inorganic carbon was further converted into algal biomass. These results showed the possibility of a new method for simultaneous carbon fixing, power generation and biodiesel production during wastewater treatment without aeration.

Keywords: CO₂; Sequestration; Microbial fuel cells; *Chlorella vulgaris*

Shengnan Xie^a and S. Patrick Walton^{1a}. (^a Applied Biomolecular Engineering Laboratory, Cellular and Biomolecular Laboratory, Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI 48824, USA). **Development of a dual-aptamer-based multiplex protein biosensor. Biosensors and Bioelectronics, Volume 25(12) (2010): 2663-2668**

Parallel biosensors for proteins are becoming more essential for the thorough and systematic investigation of complex biological processes. These tools also enable improved clinical diagnoses relative to single-protein analyses due to their greater information content. If implemented correctly, affinity-based techniques can provide unique advantages in terms of sensitivity and flexibility. Aptamers are increasingly being used as the affinity reagents of choice for protein biosensing applications. Here, we describe the development and characterization of an aptamer-based method for parallel protein analyses that relies on recognition of the target protein by two unique aptamers targeting different epitopes on the protein. Our results show that the technique achieved simultaneous and quantitative detection of thrombin and platelet-derived growth factor-BB (PDGF-BB) with high specificity both in buffered solutions and in serum samples.

Keywords: Biosensor; Multiplex; Aptamer; Thrombin; PDGF-BB; Quantitative PCR

Xiaoying Liu^a, Xiandong Zeng^a, Nannan Mai^a, Yong Liu^a, Bo Kong^a, Yonghong Li^a, Wanzhi Wei^a and Shenglian Luo^a. (^a State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University,

Hunan, Changsha 410082, PR China). Amperometric glucose biosensor with remarkable acid stability based on glucose oxidase entrapped in colloidal gold-modified carbon ionic liquid electrode. *Biosensors and Bioelectronics*, Volume 25(12) (2010): 2675-2679

A colloidal gold-modified carbon ionic liquid electrode was constructed by mixing colloidal gold-modified graphite powder with a solid room temperature ionic liquid n-octyl-pyridinium hexafluorophosphate (OPPF₆). Glucose oxidase (GOD) was entrapped in this composite matrix and maintained its bioactivity well and displayed excellent stability. The effect conditions of pH, applied potential and GOD loading were examined. Especially, the glucose oxidase entrapped in this carbon ionic liquid electrode fully retained its activity upon stressing in strongly acidic conditions (pH 2.0) for over one hour. The proposed biosensor responds to glucose linearly over concentration range of 5.0×10^{-6} to 1.2×10^{-3} and 2.6×10^{-3} to 1.3×10^{-2} M, and the detection limit is 3.5×10^{-6} M. The response time of the biosensor is fast (within 10 s), and the life time is over two months. The effects of electroactive interferents, such as ascorbic acid, uric acid, can be significantly reduced by a Nafion film casting on the surface of resulting biosensor.

Keywords: Biosensor; Glucose oxidase; Carbon ionic liquid electrode; Colloidal gold; Acid stability

Shengbo Sang^a and Hartmut Witte^a. (^a Department of Biomechatronics, Faculty of Mechanical Engineering, Ilmenau University of Technology, 98693 Ilmenau, Germany). A novel PDMS micro membrane biosensor based on the analysis of surface stress. *Biosensors and Bioelectronics*, Volume 25(11) (2010): 2420-2424

The biological and medical application of biosensors is more and more important with the development of technology and society. Detection of cells and biological molecules utilizing biosensors based on the analysis of surface stress would facilitate inexpensive and high-throughput test and diagnosis. This paper presents a biocompatible surface stress-based polydimethylsiloxane (PDMS) micro membrane biosensor. Each biosensor chip consists of two available PDMS micro membranes, one acts as active membrane and the other as reference. Biosensors were functionalized using different functional materials respectively: MUA (11 Mercapto 1 undecanoic acid), MUO (11 Mercapto 1 undecanol) and DOT (Dodecane thiol). Two biosensor test systems were built based on a white light interferometer and a fiber optic interferometer respectively. Finally, testing experiments using *Escherichia coli* (*E. coli*) were performed based on the biosensor test systems we built. The results of the experiments showed that the MUA is a better functional material to functionalize the biosensor membranes than MUO and DOT for *E. coli* detection, some properties of *E. coli*, such as healthily living and dead status, can be analyzed based on the PDMS micro membrane biosensors.

Keywords: Biosensor; Surface stress; PDMS micro membrane; *Escherichia coli*; Interferometer

Guo-Jun Zhang^a, Zhan Hong Henry Luo^a, Min Joon Huang^a, Guang Kai Ignatius Tay^a and Eu-Jin Andy Lim^a. (^a Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), 11 Science Park Road, Singapore 117685, Singapore). Morpholino-functionalized silicon nanowire biosensor for sequence-specific label-free detection of DNA. *Biosensors and Bioelectronics*, Volume 25(11) (2010): 2447-2453

We investigated Morpholino-functionalized silicon nanowires (SiNWs) as a novel gene chip platform for the sequence-specific label-free detection of DNA. Morpholino attachment and subsequent Morpholino–DNA hybridization on silicon surface was characterized by X-ray photoelectron spectroscopy and fluorescence microscopy. The resultant Morpholino-modified surfaces showed high specificity of recognition for DNA. Subsequently, by using the same protocol, the surface of the SiNW biosensor was functionalized with Morpholino, and this was used for label-free Morpholino-DNA hybridization detection. Real-time measurements of the Morpholino-functionalized SiNW biosensor exhibited a decrease in a time-dependent conductance when complementary and mutant DNA samples were added. Furthermore, identification of fully complementary versus mismatched DNA samples was carried out by the Morpholino-functionalized SiNW biosensor. We demonstrated that DNA detection using the Morpholino-functionalized SiNW biosensor could be carried out to the hundreds of femtomolar range. The Morpholino-functionalized SiNWs show a novel biosensor for label-free and direct detection of DNA with good selectivity, and a promising application in gene expression.

Keywords: Silicon nanowire; Biosensor; Surface functionalization; Hybridization detection; Morpholino; DNA

A.K.M. Kafi^a, Asieh Ahmadalinezhad^a, Jingpeng Wang^{a, b}, Dan F. Thomas^b and Aicheng Chen^a. (^a Department of Chemistry, Lakehead University, Thunder Bay, Ontario P7B 5E1, Canada, ^b Department of Chemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada). **Direct growth of nanoporous Au and its application in electrochemical biosensing. *Biosensors and Bioelectronics*, Volume 25(11) (2010): 2458-2463**

In this work, we have directly grown three-dimensional nanoporous Au networks onto a Ti substrate using the hydrothermal technique. This newly designed material with a large surface area was used as a supporting matrix for immobilizing a redox protein, hemoglobin (Hb), to develop a high-performance hydrogen peroxide biosensor. Scanning electron microscope (SEM), X-ray photoelectron spectroscopy (XPS), and energy-dispersive X-ray (EDX) spectroscopy were employed to characterize the morphology and composition of the fabricated nanoporous Au network. Cyclic voltammetry (CV) and amperometry were used to study and to optimize the performance of the fabricated electrochemical biosensor. Our CV studies show the direct electron transfer of Hb immobilized on the nanoporous Au network. In addition, amperometric H₂O₂ sensing experiments revealed that the nanoporous Au-network based biosensor exhibits fast response, long linearity, a low detection limit, high stability and very good reproducibility. Under the optimized conditions, the linearity of the developed biosensor for the detection of H₂O₂ spans from 5×10^{-8} to 2×10^{-4} M with a detection limit of 2×10^{-8} M (based on S/N = 3).

Keywords: Nanoporous Au; Hemoglobin; Biosensors; Hydrogen peroxide

Michele Menotta^a, Rita Crinelli^a, Elisa Carloni^a, Marzia Bianchi^a, Elisa Giacomini^a, Ugo Valbusa^b and Mauro Magnani^a. (^a Department of Biomolecular Sciences, University of Urbino “Carlo Bo” Via Saffi 2 61029 Urbino (PU), Italy, ^b Nanomed Labs, Physics Department, University of Genova, Advanced Biotechnology Center, Largo R. Benzi, 10 Genova, 16132 Italy). **Label-free quantification of activated NF- κ B in biological samples by atomic force microscopy. *Biosensors and Bioelectronics*, Volume 25(11) (2010): 2490-2496**

Nuclear factor- κ B (NF- κ B) is a ubiquitous transcription factor involved in the pro-inflammatory response to several factor, and in auto-inflammatory diseases. The usual methods for detection of

NF- κ B DNA binding activity are the electrophoretic mobility shift assay (EMSA), and enzyme-linked immunosorbent assay (ELISA). Here we report a development of a quantitative atomic force microscopy (AFM) based technique, for the analysis of NF- κ B DNA binding activity. NF- κ B target sequence DNA has been employed to mica functionalization in order to set up a surface able to capture transcriptionally active NF- κ B protein complexes from cell lysates, with the aim to detect DNA binding capacity of NF- κ B from low amount of biological samples such as biopsy.

We were able to obtain images of the captured complex on the surface and furthermore we carried out an AFM images quantification. We were able to quantify relative and absolute quantities of NF- κ B at pico-Molar proteins concentration range from cultured cell samples and from biological fluid cells permitting us to estimate NF- κ B binding activity. The results obtained by AFM imaging have been compared and validated with EMSA. The present work represents the first quantification approach by AFM analysis. The results and the method may be used toward development of NF- κ B based bio-diagnostic nano-device.

Keywords: NF- κ B; AFM; Label-free quantification

Guo-Jun Lang, Jin-Yan Shang, Yan-Xia Chen, Ying-Jun Cui, Qiang Wang, Zhen-Hua Tang and Chuan-Xi Zhang. Expression of the housefly acetylcholinesterase in a bioreactor and its potential application in the detection of pesticide residues. World Journal of Microbiology and Biotechnology, Volume 26(10) (2010): 1795-1801

Acetylcholinesterase is a key enzyme of the animal nerve system. The enzyme is the primary target of organophosphorous (OP) and carbamate (CB) insecticides. The insect AChE is being extensively used in development of new insecticides or in vitro selection of the new designed insecticides, and in pharmacological and toxicological field. Rapid assays using AChE-based methods have been proposed as an efficient and rapid method for the detection of pesticides, especially in many Asian markets. In this study, the acetylcholinesterase gene was cloned from housefly (*Musca domestica*) susceptible to organophosphate (OP) and carbamate (CB) insecticides, and expressed in baculovirus-insect cells system using a bioreactor with oxygen supplementation. The recombinant housefly AChE was purified using ammonium sulfate precipitation and procainamide affinity chromatography, and approximately 0.42 mg of the purified AChE with high biological activity (118.9 U/mg) was obtained from 100 ml of culture solution. The purified AChE was highly sensitive to OP and CBs insecticides. In conclusion, an efficient expression and purification system has been developed for large-scale production of recombinant housefly AChE. The recombinant enzyme is potential to be used for the detection of pesticide residues.

Keywords: Acetylcholinesterase - *Musca domestica* - Expression - Bioreactor - Pesticide residue detection

Haiwei Chen, Ruo Yuan, Yaqin Chai, Jinfen Wang and Wenjuan Li. Glucose biosensor based on electrodeposited platinum nanoparticles and three-dimensional porous chitosan membranes. Biotechnology Letters, Volume 32(10) (2010): 1401-1404

The chitosan with three-dimensional porous structure greatly increased the effective electrode surface for loading of platinum nanoparticles and promoted efficient electron transfer. The resulting biosensor had a response time (within 5 s) and a linear response from 6 μM to 4.2 mM glucose with a detection limit of 2 μM (S/N = 3). Moreover, the methodology can be applied for the immobilization of other enzymes.

Keywords: Chitosan - Glucose biosensor - Glucose oxidase - Platinum nanoparticles - Porous membranes

Purpose of work A novel and sensitive glucose biosensor based on platinum nanoparticles and glucose oxidase held on a three-dimensional porous chitosan film has been developed.

L. Thöni^{aa}, V. Urumov^{bb}, L. Yurukova^{cc} and H.G. Zechmeister^{dd}. (^a Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Road, Bangor, Gwynedd LL57 2UW, UK, ^b Department of Chemistry, Norwegian University of Science and Technology, 7491 Trondheim, Norway, ^c Finnish Forest Research Institute, Kirkkosaaentie 7, 91500 Muhos, Finland, ^d Environmental Agency of Bolzano, 39055 Laives, Italy, ^e International Sakharov Environmental University, Minsk, Belarus, ^f National Botanical Garden, Academy of Science of Ukraine, Timiryazevs'ka St. 1, 01014 Kyiv, Ukraine, ^g Canakkale Onsekiz Mart University, 17100 Çanakkale, Turkey, ^h Environment Agency, Traðagøta 38, FO-165 Argir, Faroe Islands, ⁱ Veterinary and Agrochemical Research Centre, Leuvensesteenweg 17, 3080 Tervuren, Belgium, ^j University of Santiago de Compostela, Faculty of Biology, Department of Ecology, 15782 Santiago de Compostela, Spain, ^k Latvian Environment, Geology and Meteorology Agency, Maskavas Str. 165, Riga LV 1019, Latvia, ^l Joint Institute for Nuclear Research, Str. Joliot-Curie 6, 141980 Dubna, Russian Federation, ^m University of Navarra, Irunlarrea No 1, 31008 Pamplona, Spain, ⁿ Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31512 Krakow, Poland, ^o Jožef Stefan Institute, Department of Environmental Sciences, Jamova 39, 1000 Ljubljana, Slovenia, ^p University of Opole, Poland, ^q Faculty of Science, University of Novi Sad, Trg D. Obradovica 4, 21000 Novi Sad, Serbia, ^r Institute of Physics, Savanoriu Ave 231, 02300 Vilnius, Lithuania, ^s Muséum National d'Histoire Naturelle, 57 rue Cuvier, Case 39, 75005 Paris, France, ^t Tallinn Botanic Garden, Kloostrimetsa tee 52, 11913 Tallinn, Estonia, ^u Icelandic Institute of Natural History, Hlemmur 3, 125 Reykjavík, Iceland, ^v Institute of Landscape Ecology, Slovak Academy of Science, Štefánikova Str. 3, 814 99 Bratislava, Slovakia, ^w Chair of Landscape Ecology, University of Vechta, PO Box 1553, D-49356 Vechta, Germany, ^x Humlekärrshultsvägen 10, 572 41 Oskarshamn, Sweden, ^y Oikon Ltd., Institute for Applied Ecology, Avenija V. Holjevca 20, 10020 Zagreb, Croatia, ^z Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Kvetnove namesti 391, 252 43 Pruhonice, Czech Republic, ^{aa} FUB-Research Group for Environmental Monitoring, Alte Jonastrasse 83, 8640 Rapperswil, Switzerland, ^{bb} Saints Cyril and Methodius University, PO Box 162, 1000 Skopje, Former Yugoslav Republic of Macedonia, ^{cc} Institute of Botany, Bulgarian Academy of Sciences, Acad. G.Bonchev Str., Block 23, 1113 Sofia, Bulgaria, ^{dd} University of Vienna, Althanstraße 14, 1090 Vienna, Austria). Mosses as biomonitors of atmospheric heavy metal deposition: Spatial patterns and temporal trends in Europe. *Environmental Pollution*, Volume 158(10) (2010): 3144-3156

In recent decades, mosses have been used successfully as biomonitors of atmospheric deposition of heavy metals. Since 1990, the European moss survey has been repeated at five-yearly intervals. Although spatial patterns were metal-specific, in 2005 the lowest concentrations of

metals in mosses were generally found in Scandinavia, the Baltic States and northern parts of the UK; the highest concentrations were generally found in Belgium and south-eastern Europe. The recent decline in emission and subsequent deposition of heavy metals across Europe has resulted in a decrease in the heavy metal concentration in mosses for the majority of metals. Since 1990, the concentration in mosses has declined the most for arsenic, cadmium, iron, lead and vanadium (52–72%), followed by copper, nickel and zinc (20–30%), with no significant reduction being observed for mercury (12% since 1995) and chromium (2%). However, temporal trends were country-specific with sometimes increases being found.

Since 1990, heavy metal concentrations in mosses have declined in Europe for most metals.

Keywords: Biomonitoring; EMEP maps; Heavy metals; Metal deposition; Moss survey

Sally R. Gadsdon^a, Jeremy R. Dagley^b, Patricia A. Wolseley^c and Sally A. Power^a. (^aDivision of Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, UK, ^b City of London Corporation, Epping Forest, The Warren, Loughton, Essex IG10 4RW, UK, ^c Department of Botany, Natural History Museum, Exhibition Road, South Kensington, London SW7 5BD, UK). **Mosses as biomonitors of atmospheric heavy metal deposition: Spatial patterns and temporal trends in Europe. Environmental Pollution, Volume 158(10) (2010): 3144-3156**

The relationship between different features of lichen communities in *Quercus robur* canopies and environmental variables, including concentrations of NO₂ and NH₃ was investigated. NO₂ concentration was the most significant variable, it was positively correlated with the proportion of lichen cover comprising nitrophytes and negatively correlated with total lichen cover. None of the lichen community features were correlated with NH₃ concentrations, which were relatively low across the site. Since nitrophytes and nitrophobes are likely to react in opposite directions to nitrogenous compounds, total lichen cover is not a suitable indicator for these pollutants. It is, therefore, suggested that the proportion of lichen cover comprising nitrophytes may be a suitable simple indicator of air quality, particularly in locations where the pollution climate is dominated by oxides of nitrogen.

Response of lichen communities to nitrogenous pollutants.

Keywords: Ammonia; Canopy; Epiphytic lichens; Nitrogen dioxide; Nitrophyte

Bioengineering

Muhammad Ali^{1,3}, Muhammad Faisal Saddiqui^{2*}, Ihsan Ullah^{1,3}, and Jehan Bakht¹. (¹Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University, Pakistan, ²Faculty of Chemical and Natural Resource Engineering, University of Malaysia Pahang (UMP), Kuantan, Malaysia, ³Department of Biotechnology, Sarhad University of Science and Information Technology Pakistan, *Corresponding author E-mail: send2biotech@yahoo.com. Tel: +60179679520). **Analysis of HMWGS of historical set of**

Pakistani bread wheat varieties using SDS-PAGE. African Journal of Biotechnology Vol. 9 (27) (2010): 4286-4289

In the present study an attempt has been made to characterize thirty bread-wheat varieties of Pakistan for High Molecular Weight Glutenin Subunits (HMW- GS). Glutenin proteins form a continuous proteinaceous matrix in the cells and form a continuous viscoelastic network during the mixing process of dough development. Glutenin consists of High Molecular Weight (HMW) and Low Molecular Weight (LMW) subunits. The HMW Glutenin Subunits (HMW-GS) are chiefly vital for determining dough elasticity. The core objective of our research work was to inspect the glutenin subunits by sodium dodecyl-sulfate polyacrylamide gel-electrophoresis (SDS-PAGE) and compare the banding pattern with Chinese Spring High-Molecular-Weight Glutenin Subunits (HMW-GS). The bands were numbered according to Payne's numbering system and varieties were accordingly assigned theoretical quality scores. All the tested varieties indicated null allele for *gluA1* locus, 17 + 18 for *gluB1* locus and 2 + 12 for *gluD1* locus. This result indicating that all varieties have similar bread making quality alleles at HMWGS loci. The varieties containing 5 + 10 HMWGS allele at *gluD1* locus have better bread making quality. Better bread making wheat varieties may be produced by crossing the local varieties of *gluA1* locus, 17 + 18 for *gluB1* locus and 2 + 12 for *gluD1* locus with 5 + 10 HMWGS allele at *gluD1* locus.

Keywords: Wheat varieties, SDS-PAGE, HMWGS, Payne's numbering.

Shun-chang Pu, Ming-jun Chen, Zhong-you Ma, Ling Xie, Zeng-zhi Li and Bo Huang* . (Anhui Provincial Key Laboratory of Microbial Pest Control, Anhui Agricultural University, Hefei 230036, China. *Corresponding author. E-mail: Huangbochhy@hotmail.com. Tel: +865515786887. Fax: +865515786786). Genotyping isolates of the entomopathogenic fungus *Beauveria bassiana sensu lato* by multi-locus polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. African Journal of Biotechnology Vol. 9 (27) (2010): 4290-4294

Multi-locus denaturing gradient gel electrophoresis (DGGE) analysis was developed to investigate the genotypes of *Beauveria bassiana sensu lato*. Sensitive tests indicated all isolates with one or more nucleotide differences at EF-1 α and Bloc could be distinguished by DGGE except for one pair of strains that differed at four nucleotide positions. Ten, twelve and five genotypes were identified at the EF-1 α , Bloc and ITS locus, respectively, among seventeen isolates, which together differentiated 13 genotypes. These results demonstrated that multi-locus DGGE is a potentially useful molecular marker for genotyping, identifying and tracking the fates of experimentally released strains of *B. bassiana sensu lato*. Moreover, by multi-locus DGGE for scanning *B. bassiana sensu lato* isolates with different multi-locus sequences, genetic diversity of *B. bassiana sensu lato* was effectively investigated with substantially reduced time and cost in subsequent DNA sequencing.

Keywords: Denaturing gradient gel electrophoresis, multi-locus sequences, genotyping, sensitive tests.

Abbreviations: DGGE, Denaturing gradient gel electrophoresis; RAPD, random amplified polymorphic DNA; ISSR, inter simple sequence repeat; PCR-SSCP, polymerase chain reaction single-strand conformation polymorphism; SSR, simple sequence repeat.

Pollen Biotechnology

Mohammad T. Hedayati, Sabah Mayahi and David W. Denning. A study on *Aspergillus* species in houses of asthmatic patients from Sari City, Iran and a brief review of the health effects of exposure to indoor *Aspergillus*. Environmental Monitoring and Assessment, Volume 168(1-4) (2010):481-487

To study the distribution of *Aspergillus* spp. in outdoor and indoor air of asthmatic patients' houses, as well as a review on the health effects of exposure to indoor *Aspergillus*. Open plates containing malt extract agar media were used to isolate fungi from the indoor ($n=360$) and outdoor ($n=180$) air of 90 asthmatic patients' houses living in Sari City, Iran. Plates were incubated at room temperature for 7–14 days. Cultured *Aspergillus* spp. were identified by standard mycological techniques. All culture plates grew fungi, a testament to the ubiquitous nature of fungal exposure. *Cladosporium* spp. (29.2%), *Aspergillus* spp. (19.0%), and *Penicillium* spp. (18.3%) were most common inside the houses while *Cladosporium* spp. (44.5%), *Aspergillus* spp. (12.4%), and *Alternaria* spp. (11.1%) were most common outside the houses. *Aspergillus flavus* (30.1%) and *A. fumigatus* (23.1%) are the most commonly isolated species in indoor air. *Aspergillus flavus* (44.5%) and *A. fumigatus* (42.6%) were the most prevalent *Aspergillus* spp. outside. The most colony numbers of *Aspergillus* were isolated from kitchens (30.4%) and the least from bedrooms (21.1%). *Aspergillus flavus* was the most prevalent specie in all sampled rooms except in the kitchen where *A. fumigatus* was the most common. *Aspergillus flavus* is the most prevalent species among the *Aspergillus* spp. in the indoor and outdoor of a warm climate area. In these areas, *A. flavus* can be a major source of allergen in the air. Therefore, minimizing indoor fungal exposure could play an important role in reducing allergic symptoms in susceptible persons.

Keywords: Asthma - Indoor - Outdoor - Volatile - Allergen

Biotechnology Policy Issue

Åsa Svenfelt^a, Rebecka Engström^{1, a} and Mattias Höjer^a. (^a Division of Environmental Strategies Research – fms, Royal Institute of Technology (KTH), SE 100 44 Stockholm, Sweden). Use of explorative scenarios in environmental policy-making—Evaluation of policy instruments for management of land, water and the built environment. Futures, Volume 42(10) (2010): 1166-1175

In order to achieve the Swedish Environmental Quality Objectives (EQOs), three action strategies have been adopted by the Swedish parliament. The strategy addressed in this paper deals with the management of land, water and the built environment. The paper reports on a project involving authorities and researchers in which policy measures required for achieving relevant targets for the strategy were gathered, structured and analysed regarding their potential assuming alternative futures. Measures with proposed policy instruments were qualitatively evaluated against one business as usual scenario and four explorative scenarios varying along two dimensions; level of governance and level of embeddedness. The results show a heavy

predominance of administrative policy instruments. This policy strategy depends on a future development where such policy instruments are accepted. In order to achieve the EQOs regardless of future developments, more robust packages of measures including a larger variation in policy instruments need to be developed.

Keywords: Explorative scenarios; Environmental objectives; Policy measures

Agricultural Biotechnology

Alberto Zezza^a and Luca Tasciotti^a . (^a Agricultural Development Economics Division, Food and Agriculture Organization (FAO), Viale delle Terme di Caracalla, 00153 Rome, Italy). Urban agriculture, poverty, and food security: Empirical evidence from a sample of developing countries. *Food Policy*, Volume 35(4) (2010): 265-273

Urban agriculture may have a role to play in addressing urban food insecurity problems, which are bound to become increasingly important with the secular trend towards the urbanization of poverty and of population in developing regions. Our understanding of the importance, nature and food security implications of urban agriculture is however plagued by a lack of good quality, reliable data. While studies based on survey data do exist for several major cities, much of the evidence is still qualitative if not anecdotal. Using a recently created dataset bringing together comparable, nationally representative household survey data for 15 developing or transition countries, this paper analyzes in a comparative international perspective the importance of urban agriculture for the urban poor and food insecure. Some clear hints do come from our analysis. On the one hand, the potential for urban agriculture to play a substantial role in urban poverty and food insecurity reduction should not be overemphasised, as its share in income and overall agricultural production is often quite limited. On the other hand, though, its role should also not be too easily dismissed, particularly in much of Africa and in all those countries in which agriculture provides a substantial share of income for the urban poor, and for those groups of households to which it constitutes an important source of livelihoods. We also find fairly consistent evidence of a positive statistical association between engagement in urban agriculture and dietary adequacy indicators.

Keywords: Urban agriculture; Food security; Poverty; Nutrition; Household surveys

Bioenergy

Guangyuan Teng, Lijing Gao, Guomin Xiao, Hu Liu and Jianhua Lv. Biodiesel Preparation from *Jatropha curcas* Oil Catalyzed by Hydrotalcite Loaded With K₂CO₃. *Applied Biochemistry and Biotechnology*, Volume 162(6) (2010): 1725-1736

This paper discusses the synthesis of biodiesel catalyzed by solid base of K₂CO₃/HT using *Jatropha curcas* oil as feedstock. Mg–Al hydrotalcite was prepared using co-precipitation methods, in which the molar ratio of Mg to Al was 3:1. After calcined at 600 °C for 3 h, the Mg–Al hydrotalcite and K₂CO₃ were grinded and mixed according to certain mass ratios, in which some water was added. The mixture was dried at 65 °C, and after that it was calcined at 600 °C

for 3 h. Then, this Mg–Al hydrotalcite loaded with potassium carbonate was obtained and used as catalyst in the experiments. Analyses of XRD and SEM characterizations for catalyst showed the metal oxides formed in the process of calcination brought about excellent catalysis effect. In order to achieve the optimal technical reaction condition, five impact factors were also investigated in the experiments, which were mass ratio, molar ratio, reaction temperature, catalyst amount and reaction time. Under the best condition, the biodiesel yield could reach up to 96%.

Keywords: Biodiesel - Transesterification - Hydrotalcite - Solid base - *Jatropha curcas* oil

Hossein Amani, Mohammad Reza Mehrnia, Mohammad Hossein Sarrafzadeh, Manouchehr Haghighi and Mohammad Reza Soudi. Scale up and Application of Biosurfactant from *Bacillus subtilis* in Enhanced Oil Recovery. Applied Biochemistry and Biotechnology, Volume 162(2) (2010): 510-523

There is a lack of fundamental knowledge about the scale up of biosurfactant production. In order to develop suitable technology of commercialization, carrying out tests in shake flasks and bioreactors was essential. A reactor with integrated foam collector was designed for biosurfactant production using *Bacillus subtilis* isolated from agricultural soil. The yield of biosurfactant on biomass ($Y_{p/x}$), biosurfactant on sucrose ($Y_{p/s}$), and the volumetric production rate (Y) for shake flask were obtained about 0.45 g g^{-1} , 0.18 g g^{-1} , and $0.03 \text{ g l}^{-1} \text{ h}^{-1}$, respectively. The best condition for bioreactor was 300 rpm and 1.5 vvm, giving $Y_{x/s}$, $Y_{p/x}$, $Y_{p/s}$, and Y of 0.42 g g^{-1} , 0.595 g g^{-1} , 0.25 g g^{-1} , and $0.057 \text{ g l}^{-1} \text{ h}^{-1}$, respectively. The biosurfactant maximum production, 2.5 g l^{-1} , was reached in 44 h of growth, which was 28% better than the shake flask. The obtained volumetric oxygen transfer coefficient ($K_L a$) values at optimum conditions in the shake flask and the bioreactor were found to be around 0.01 and 0.0117 s^{-1} , respectively. Comparison of $K_L a$ values at optimum conditions shows that biosurfactant production scaling up from shake flask to bioreactor can be done with $K_L a$ as scale up criterion very accurately. Nearly 8% of original oil in place was recovered using this biosurfactant after water flooding in the sand pack.

Keywords: *Bacillus subtilis* - Bioreactor - Biosurfactant - Enhanced oil recovery - Foam - Scale up

Yi-Hung Chen^a, Jhih-Hong Chen^a, Ching-Yuan Chang^b and Chia-Chi Chang^b. (a) Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei 106, Taiwan, (b) Graduate Institute of Environmental Engineering, National Taiwan University, Taipei 106, Taiwan). Biodiesel production from tung (*Vernicia montana*) oil and its blending properties in different fatty acid compositions. Bioresource Technology, Volume 101(24) (2010): 9521-9526

The feasibility of biodiesel production from tung (*Vernicia montana*) oil was investigated with respect to the transesterification yield and biodiesel properties. Tung oil has poor oxidation stability due to the instability of the conjugated carbon–carbon double bonds in the α -elaeostearic acid. The methyl elaeostearate is the predominant component (82.2 wt.%) of the tung oil biodiesel. The tung oil biodiesel has the low cold filter plugging point of $-11 \text{ }^\circ\text{C}$, ester content of 94.9 wt.%, and oxidation stability ($110 \text{ }^\circ\text{C}$) of 0.3 h. Moreover, the tung oil biodiesel

exhibits the high density of 903 kg/m³ at 15 °C, kinematic viscosity of 7.84 mm²/s at 40 °C, and iodine value of 161.1 g I₂/100 g. The properties of the tung oil biodiesel can be improved by blending with canola and palm oil biodiesels to satisfy the biodiesel specifications. In addition, multiple linear correlations between biodiesel properties and its methyl ester composition were established.

Keywords: Tung oil; Blending; Fatty acid methyl esters; Non-edible oil; Linear regression

Zhenzhong Wen^a, Xinhai Yu^{a, b}, Shan-Tung Tu^a, Jinyue Yan^{c, d} and Erik Dahlquist^c. (^a Key Laboratory of Safety Science of Pressurized System (MOE), School of Mechanical Engineering, East China University of Science and Technology, Shanghai 200237, China, ^b State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China, ^c School of Sustainable Development of Society and Technology, Mälardalen University, SE-721 23 Västerås, Sweden, ^d School of Chemical Science and Engineering, Royal Institute of Technology Stockholm, SE-100 44 Stockholm, Sweden). **Biodiesel production from waste cooking oil catalyzed by TiO₂-MgO mixed oxides. *Bioresource Technology*, Volume 101(24) (2010): 9570-9576**

Mixed oxides of TiO₂-MgO obtained by the sol-gel method were used to convert waste cooking oil into biodiesel. Titanium improved the stability of the catalyst because of the defects induced by the substitution of Ti ions for Mg ions in the magnesia lattice. The best catalyst was determined to be MT-1-923, which is comprised of an Mg/Ti molar ratio of 1 and calcined at 923 K, based on an assessment of the activity and stability of the catalyst. The main reaction parameters, including methanol/oil molar ratio, catalyst amount, and temperature, were investigated. The catalytic activity of MT-1-923 decreased slowly in the reuse process. After regeneration, the activity of MT-1-923 slightly increased compared with that of the fresh catalyst due to an increase in the specific surface area and average pore diameter. The mixed oxides catalyst, TiO₂-MgO, showed good potential in large-scale biodiesel production from waste cooking oil.

Keywords: Biodiesel; Waste cooking oil; Solid catalyst; Stability; Heterogeneous

M.A. Martín^a, J.A. Siles^a, A.F. Chica^a and A. Martín^a. (^a Departamento de Química Inorgánica e Ingeniería Química, Facultad de Ciencias, Universidad de Córdoba, Campus Universitario de Rabanales, Edificio C-3, Ctra Madrid-Cádiz, km 396, 14071 Córdoba, Spain). **Biomethanization of orange peel waste. *Bioresource Technology*, Volume 101(23) (2010): 8993-8999**

Recent research has demonstrated that orange peel waste is a potentially valuable resource that can be developed into high value products such as methane. Following a pre-treatment to extract D-limonene, the anaerobic digestion of orange peel waste was evaluated at laboratory and pilot scale under mesophilic and thermophilic conditions. D-Limonene removals of 70% were reached with pre-treatment. The results showed the convenience of thermophilic conditions for treating this waste as the methane production rate and biodegradability were higher than at mesophilic temperature. At pilot scale, a thermophilic continuously stirred-tank reactor working in semi-continuous mode was employed. The OLR was found to be in the range of 1.20–3.67 kg COD/m³ d; the most appropriate range for working under stable conditions at SRT of 25 d. The methane yield coefficient was found to be 0.27–0.29 L_{STP} CH₄/g added COD and the

biodegradability 84–90% under these conditions. However, acidification occurred at the highest OLR.

Keywords: Orange peel waste; D-Limonene; Anaerobic digestion; Thermophilic and mesophilic conditions; Biodegradability

Germán Buitrón^a and Carolina Carvajal^a. (^a **Laboratory for Research on Advanced Processes for Water Treatment, Instituto de Ingeniería, Unidad Académica Juriquilla, Universidad Nacional Autónoma de México, Blvd. Juriquilla 3001, Querétaro 76230, Mexico**). **Biohydrogen production from Tequila vinasses in an anaerobic sequencing batch reactor: Effect of initial substrate concentration, temperature and hydraulic retention time. *Bioresource Technology*, Volume 101(23) (2010): 9071-9077**

The effect of the temperature (25 and 35 °C), the hydraulic retention time, HRT, (12 and 24 h) and initial substrate concentration on hydrogen production from Tequila vinasse was studied using a sequencing batch reactor. When 25 °C and 12-h HRT were applied, only insignificant biogas quantities were produced; however, using 24 h of HRT and temperatures of 25 and 35 °C, biogas containing hydrogen was produced. A maximum volumetric hydrogen production rate of 50.5 mL H₂ L⁻¹ h⁻¹ (48 mmol H₂ L_{reactor}⁻¹ d⁻¹) and an average hydrogen content in the biogas of 29.2 ± 8.8% were obtained when the reactor was fed with 3 g COD L⁻¹, at 35 °C and 12-h HRT. Methane formation was observed when the longer HRT was applied. Results demonstrated the feasibility to produce hydrogen from this waste without a previous pre-treatment.

Keywords: Hydrogen; Tequila; Vinasses; Sequencing batch reactor; Methane

Andrea K. Forrest^a, Rocio Sierra^b and Mark T. Holtzaple^a. (^a **Department of Chemical Engineering, Texas A&M University, College Station, TX 77843, United States**, ^b **Departamento de Ingeniería Química, Universidad de los Andes, Bogota, Colombia**). **Effect of biodiesel glycerol type and fermentor configuration on mixed-acid fermentations. *Bioresource Technology*, Volume 101(23) (2010): 9185-9189**

The MixAlco process biologically converts biomass to carboxylate salts that may be converted to a variety of chemicals and fuels. This study examines the viability of different types of glycerol as a potential feedstock. Batch fermentations of crude biodiesel glycerol were compared to distilled and refined glycerol. Continuous fermentations were performed in a CSTR and a packed-bed fermentor with refined glycerol. While crude and distilled glycerol are difficult to process industrially, all types of glycerol performed well during MixAlco fermentations, producing acid concentrations above 22 g/L and conversions of greater than 65%. The CSTR configuration produced excellent acid concentrations (16 g/L) while the packed-bed configuration produced high amounts of cell material for use in cell extract products or starter cultures.

Keywords: Fermentation; MixAlco; Carboxylic acids; Glycerol; Biodiesel

Anniina Kivistö^a, Ville Santala^a and Matti Karp^a. (^a **Tampere University of Technology, Department of Chemistry and Bioengineering, Tampere, Finland**). **Hydrogen production**

from glycerol using halophilic fermentative bacteria. Bioresource Technology, Volume 101(22) (2010): 8671-8677

Glycerol-based hydrogen production by the halophilic bacteria *Halanaerobium saccharolyticum* subspecies *saccharolyticum* and *senegalensis* was studied as batch experiments. The main metabolites of glycerol fermentation of both strains were hydrogen, carbon dioxide, and acetate. Subspecies *saccharolyticum* also produced 1,3-propanediol (1,3-PD), butyrate, and ethanol. The highest hydrogen yields were achieved with 2.5 g/l glycerol and 150 g/l salt at pH 7.4 (subsp. *saccharolyticum*, yield 0.6 mol/mol glycerol) and at pH 7.0 (subsp. *senegalensis*, yield 1.6 mol/mol glycerol). The hydrogen yield of subsp. *senegalensis* has potential for practical applications after scale-up and bioprocess optimizations and metabolic engineering after genome-wide sequencing could be applied to improve the yield of subsp. *saccharolyticum*.

Keywords: Glycerol fermentation; Biohydrogen production; 1,3-Propanediol production; *Halanaerobium saccharolyticum*

S. Tian^{a,c}, X.L. Luo^{b,c,d}, X.S. Yang^a and J.Y. Zhu^{c,d}. (^a College of Life Science, Capital Normal University, Beijing, China, ^b Key Laboratory Pulp and Paper Engineering, South China University of Technology, Guangzhou, China, ^c USDA Forest Service, Forest Products Laboratory, Madison, WI, USA, ^d Dept. of Biological Systems Engineering, University of Wisconsin-Madison, Madison, WI, USA). **Robust cellulosic ethanol production from SPORL-pretreated lodgepole pine using an adapted strain *Saccharomyces cerevisiae* without detoxification[†]. Bioresource Technology, Volume 101(22) (2010): 8678-8685**

This study reports an ethanol yield of 270 L/ton wood from lodgepole pine pretreated with sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) using an adapted strain, *Saccharomyces cerevisiae* Y5, without detoxification. The enzymatic hydrolysate produced from pretreated cellulosic solids substrate was combined with pretreatment hydrolysate before fermentation. Detoxification of the pretreatment hydrolysate using overliming or XAD-4 resin before being combined with enzymatic hydrolysate improved ethanol productivity in the first 4 h of fermentation and overall fermentation efficiency. However, detoxification did not improve final ethanol yield because of sugar losses. The Y5 strain showed excellent ethanol productivities of 2.0 and 0.8 g/L/h averaged over a period of 4 and 24 h, respectively, in the undetoxified run. The furan metabolization rates of the Y5 strain were significantly higher for the undetoxified run than those for the detoxified runs, suggesting it can tolerate even higher furan concentrations than those studied. Preliminary mass and energy balances were conducted. SPORL produced an excellent monomeric sugar recovery value of about 85% theoretical and a net energy output of 4.05 GJ/ton wood with an ethanol energy production efficiency of 178% before distillation.

Keywords: Cellulosic ethanol; Woody/forest biomass; Fermentation; Detoxification; Pretreatment

Sung Jin Yoo^a, Hong-shik Lee^a, Bambang Veriansyah^b, Jaehoon Kim^b, Jae-Duck Kim^b and Youn-Woo Lee^a. (^a School of Chemical and Biological Engineering and Institute for Chemical Processes, Seoul National University, 599 Gwanangno, Gwanak-gu, Seoul 151-744, Republic of Korea, ^b Supercritical Fluid Research Laboratory, Energy Research Division, Korea Institute of Science and Technology (KIST), 39-1 Hawolgok-dong,

Seongbuk-gu, Seoul 136-791, Republic of Korea). Synthesis of biodiesel from rapeseed oil using supercritical methanol with metal oxide catalysts. Bioresource Technology, Volume 101(22) (2010): 8686-8689

This study examined the synthesis of biodiesel using supercritical or subcritical methanol with metal oxide catalysts. The transesterification of rapeseed oil was carried out with the metal oxide catalysts (SrO, CaO, ZnO, TiO₂ and ZrO₂) to determine the most effective heterogeneous catalyst having the highest catalytic activity with minimum weight loss caused by dissolution. SrO and CaO dissolved in the biodiesel during the reaction because they were transformed to strontium methoxide and calcium methoxide, respectively. ZnO was the optimum catalyst for the transesterification of rapeseed oil owing to its high activity and minimum weight loss in supercritical methanol. The optimal reaction conditions included a molar ratio of methanol to oil of 40 in the presence of 1.0 wt.% ZnO and a reaction time of 10 min. The supercritical process with ZnO as a catalyst appears economically viable.

Keywords: Biodiesel; Transesterification; Metal oxide; Supercritical methanol; Heterogeneous catalyst

Chris J. Hulatt^a and David N. Thomas^a. (^a School of Ocean Sciences, College of Natural Sciences, Bangor University, Menai Bridge, Anglesey, LL59 5AB, UK). Dissolved organic matter (DOM) in microalgal photobioreactors: A potential loss in solar energy conversion?. Bioresource Technology, Volume 101(22) (2010): 8690-8697

Microalgae are considered to be a potential alternative to terrestrial crops for bio-energy production due to their relatively high productivity per unit area of land. In this work we examined the amount of dissolved organic matter exuded by algal cells cultured in photobioreactors, to examine whether a significant fraction of the photoassimilated biomass could potentially be lost from the harvestable biomass. We found that the mean maximum amount of dissolved organic carbon (DOC) released measured 6.4% and 17.3% of the total organic carbon in cultures of *Chlorella vulgaris* and *Dunaliella tertiolecta*, respectively. This DOM in turn supported a significant growth of bacterial biomass, representing a further loss of the algal assimilated carbon. The release of these levels of DOC indicates that a significant fraction of the photosynthetically fixed organic matter could be lost into the surrounding water, suggesting that the actual biomass yield per hectare for industrial purposes could be somewhat less than expected. A simple and inexpensive optical technique, based on chromophoric dissolved organic matter (CDOM) measurements, to monitor such losses in commercial PBRs is discussed.

Keywords: Microalgae; Dissolved organic matter; Biofuel; Bio-energy; Photobioreactor

Prachand Shrestha^{a, 1}, Samir Kumar Khanal^b, Anthony L. Pometto III^c and J. (Hans) van Leeuwen^{a, d, e}. (^a Department of Civil, Construction, and Environmental Engineering, Iowa State University, Ames, IA 50011, USA, ^b Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI 96822, USA, ^c Department of Food Science and Human Nutrition, Clemson University, Clemson, SC 29634, USA, ^d Department of Food Sciences and Human Nutrition, Iowa State University, Ames, IA 50011, USA, ^e Department of Agricultural and Biosystems Engineering, Iowa State

University, Ames, IA 50011, USA). Ethanol production via *in situ* fungal saccharification and fermentation of mild alkali and steam pretreated corn fiber. *Bioresource Technology*, Volume 101(22) (2010): 8698-8705

The effect of mild alkali and steam pretreatments on fungal saccharification and sequential simultaneous-saccharification and fermentation (SSF) of corn fiber to ethanol was studied. The corn fiber was pretreated with: (i) 2% NaOH (w/w) at 30 °C for 2 h and (ii) steaming at 100 °C for 2 h. Ethanol yields were 2.6 g, 2.9 g and 5.5 g ethanol/100 g of corn fiber, respectively, for *Phanerochaete chrysosporium*, *Gloeophyllum trabeum* and *Trichoderma reesei* saccharification and sequential SSFs. SSF with commercial cellulase enzyme – Spezyme-CP had 7.7 g ethanol/100 g corn fiber. Mild alkali pretreatment resulted in higher glucose yields following fungal saccharification of corn fiber. However, the ethanol yields were comparatively similar for untreated and mild alkali pretreated corn fiber. Solid-substrate fermentation of corn fiber with fungi can be improved to either eliminate or reduce the dosage of commercial cellulase enzymes during SSF.

Keywords: Pretreatment; Lignocellulose; Wood-rot fungi; SSF; Ethanol

Aloia Roman^{a,b}, Gil Garrote^{a,b}, José Luis Alonso^{a,b} and Juan Carlos Parajó^{a,b}. (^a Department of Chemical Engineering, Faculty of Science, University of Vigo (Campus Ourense), As Lagoas, 32004 Ourense, Spain, ^b CITI (Centro de Investigación, Transferencia e Innovación) – University of Vigo, Tecnopole, San Cibrao das Viñas, Ourense, Spain). Bioethanol production from hydrothermally pretreated *Eucalyptus globulus* wood. *Bioresource Technology*, Volume 101(22) (2010): 8706-8712

Eucalyptus globulus wood samples were pretreated in aqueous media under non-isothermal conditions to reach maximal temperatures (T_{MAX}) in the range 195–250 °C, in order to assess the effects of the pre-treatment severity on the fractionation of wood and on the susceptibility of processed samples toward enzymatic hydrolysis. Both the fraction of cellulose susceptible to hydrolysis and the hydrolysis rate increased with the severity of the pre-treatments, but the overall glucose yield decreased for substrates pretreated at T_{MAX} above 220 °C owing to cellulose losses. Using substrates pretreated at $T_{MAX} = 220$ °C, up to 94% of polysaccharides were recovered in the hydrolysis media as mono- or oligo-saccharides. High glucose to ethanol conversions were obtained operating at low enzyme charges in Simultaneous Saccharification and Fermentation mode.

Keywords: Autohydrolysis; Enzymatic hydrolysis; SSF; *Eucalyptus globulus*; Bioethanol

Shih-Hsin Ho^a, Wen-Ming Chen^b and Jo-Shu Chang^{a,c,d}. (^a Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan, ^b Department of Seafood Science, National Kaohsiung Marine University, Kaohsiung 811, Taiwan, ^c Sustainable Environment Research Center, National Cheng Kung University, Tainan, Taiwan, ^d Center for Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan). *Scenedesmus obliquus* CNW-N as a potential candidate for CO₂ mitigation and biodiesel production. *Bioresource Technology*, Volume 101(22) (2010): 8725-8730

This study aimed to achieve higher CO₂ consumption ability and lipid productivity of an indigenous microalgal isolate *Scenedesmus obliquus* CNW-N by a two-stage cultivation strategy.

The microalga strain was first cultivated with 10% CO₂ using a nutrient-rich medium to promote cell growth, which was followed by a nutrient-deficient condition to trigger lipid accumulation. The optimal biomass productivity, lipid productivity, and CO₂ consumption rate were 292.50 mg L⁻¹ d⁻¹, 78.73 mg L⁻¹ d⁻¹ (38.9% lipid content per dry weight of biomass), and 549.90 mg L⁻¹ d⁻¹, respectively. This performance is superior to the results from most of the related studies. Under the nutrient-deficient condition, the microalgal lipid was mainly composed of C16/C18 fatty acids (accounting for 89% of total fatty acids), which is suitable for biodiesel synthesis.

Keywords: Microalgae; *Scenedesmus obliquus*; CO₂ fixation; Lipid production

Hui Wang^{a,b}, Ming Fang^a, Zhong Fang^a and Huaiyu Bu^c. (^a Key Laboratory of Synthetic and Natural Functional Molecule Chemistry (Ministry of Education), College of Chemistry & Materials Science, Northwest University, Xi'an 710069, PR China, ^b National Key Laboratory of Photoelectric Technology and Functional Materials (Culture Base), National Photoelectric Technology and Functional Materials & Application of Science and Technology International Cooperation Base, Institute of Photonics & Photon-Technology, Northwest University, Xi'an 710069, PR China, ^c College of Life Science, Northwest University, Xi'an, Shaanxi 710069, PR China). **Effects of sludge pretreatments and organic acids on hydrogen production by anaerobic fermentation. *Bioresource Technology*, Volume 101(22) (2010): 8731-8735**

Using apple pomace (AP) as substrate, a series of batch experiments were conducted to investigate the effects of activated sludge pretreated by ultraviolet and ultrasonic on the anaerobic fermentative bio-H₂ production. The results show that a maximum cumulative H₂ production (CHP_m) of 107.0 ml g⁻¹-TS and an average H₂ production rate (AHPR) of 15.0 ml g⁻¹-TS h⁻¹ were obtained by using the pretreated sludge that was irradiated by ultraviolet lamp (25 w) for 15 min as inoculant. Based on the optimal sludge pretreatment method, the experiments of bio-H₂ production under the optimal substrate pretreatment condition (soaked in the ammonia liquor of 6% for 24 h) were further carried out, and the CHP_m of 138.8 ml g⁻¹-TS was achieved, increased by 80.6% compared to the un-pretreated group (76.8 ml g⁻¹-TS). The effects of the various organic acids on the fermentative H₂ production were also investigated under the above optimal sludge pretreatment condition.

Keywords: Bio-hydrogen production; Sludge pretreatment; Ultraviolet; Ultrasonic; Organic acids

Konrad Koch^a, Manfred Lübken^b, Tito Gehring^b, Marc Wichern^b and Harald Horn^a. (^a Institute of Water Quality Control, Technische Universität München, Am Coulombwall, 85748 Garching, Germany, ^b Institute of Environmental Engineering, Ruhr-Universität Bochum, Universitätsstraße 150, 44780 Bochum, Germany). **Biogas from grass silage – Measurements and modeling with ADM1. *Bioresource Technology*, Volume 101(21) (2010): 8158-8165**

Mono fermentation of grass silage without the addition of manure was performed over a period of 345 days under mesophilic conditions (38 °C). A simulation study based on the IWA Anaerobic Digestion Model No. 1 (ADM1) was done in order to show its applicability to

lignocellulosic biomass. Therefore, the influent was fractioned by established fodder analysis (Weender analysis and van Soest method). ADM1 was modified with a separate compound of inert decay products similar to the approach of Activated Sludge Model No. 1 (ASM1). Furthermore, a function, which described the influence of solids on the process of hydrolysis, has been integrated to reproduce reliable ammonium concentrations. The model was calibrated by using the modified Nash–Sutcliffe coefficient to evaluate simulation quality. It was possible to fit observed data by changing only hydrogen inhibition constants and the maximum acetate uptake rate. The extended ADM1 model showed good agreement with measurements and was suitable for modeling anaerobic digestion of grass silage.

Keywords: Grass silage; Mesophilic anaerobic digestion; Biogas technology; Mathematical modeling; ADM1

T. Zhang^a, Z. Chi^a, C.H. Zhao^a, Z.M. Chi^a and F. Gong^a. (^a Unesco Chinese Center of Marine Biotechnology and Institute of Marine Biodiversity and Evolution, Ocean University of China, Yushan Road, No. 5. Qingdao, China). **Bioethanol production from hydrolysates of inulin and the tuber meal of Jerusalem artichoke by *Saccharomyces* sp. W0. Bioresource Technology, Volume 101(21) (2010): 8166-8170**

It has been confirmed that *Saccharomyces* sp. W0 can produce high concentration of ethanol. However, this yeast strain cannot secrete inulinase. Therefore, in this study, inulin was hydrolyzed into reducing sugar by the recombinant inulinase produced by *Pichia pastoris* X-33/pPICZaA-*INU1*. It was found that 38.2 U of the recombinant inulinase per gram of inulin was suitable for the inulin hydrolysis and ethanol production by *Saccharomyces* sp. W0 and the fermentation period was 120 h. At the end of the fermentation, over 14.6 ml of ethanol per 100 ml of the fermented medium was produced, the ethanol productivity was over 0.384 g of ethanol/g of inulin and over 98.8% of total sugar was utilized. When the *Saccharomyces* sp. W0 was grown in the mixture of 4.0% hydrolysate of soybean meal and 20.0% of the hydrolysate of inulin for 120 h, over 14.9 ml of ethanol per 100 ml of the fermented medium was yielded, the ethanol productivity was over 0.393 g of ethanol/g of inulin and 98.9% of total sugar was used by the yeast strain. When *Saccharomyces* sp. W0 carrying the same inulinase gene was grown in the medium containing 50 g of the tuber meal of Jerusalem artichoke per 100 ml for 144 h, over 12.1 ± 0.35% ml of ethanol per 100 ml of the fermented medium was yielded, the ethanol productivity was 0.319 ± 0.9 g of ethanol/g of sugar and 3.7% (w/v) of total sugar and 0.5% (w/v) of reducing sugar were left in the fermented media.

Keywords: Bioethanol; Inulin; The tuber meal of Jerusalem artichoke; *Saccharomyces* sp.; Fermentation

S. Ferreira^a, N. Gil^a, J.A. Queiroz^{a, b}, A.P. Duarte^{a, b} and F.C. Domingues^{a, b}. (^a Research Unit of Textile and Paper Materials, University of Beira Interior, Av. Marquês D' Ávila e Bolama, 6201-001 Covilhã, Portugal, ^b CICS – Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6201-556 Covilhã, Portugal). **Bioethanol from the Portuguese forest residue *Pterospartum tridentatum* – An evaluation of pretreatment strategy for enzymatic saccharification and sugars fermentation. Bioresource Technology, Volume 101(20) (2010): 7797-7803**

Under the current energy scenario, the development of alternatives to fossil fuels, like bioethanol from lignocellulosic materials, is highly relevant. Therefore it is important to search and study

new raw materials and to optimize the different steps that lead to bioethanol production. In this work, acid diluted pretreatment was optimized considering the release of sugars. Under the optimal conditions, the reducing sugars yield was of 293.4 mg/g of dry biomass in liquid fraction. The tested pretreated samples of *Pterospartum tridentatum* that presented a higher glucose yield in enzymatic saccharification where those that were subject to a pretreatment at 180 °C for 75 min with 2.75% (w/w) of sulfuric acid when using a biomass/liquid ratio of 2.25 g/10 mL leading to a maximum yield of glucose that was 92% of the theoretical maximum. From the fermentation of filtrates it was possible to obtain a maximum ethanol yield of 0.26 g ethanol/g total sugars, without previous detoxification.

Keywords: *Pterospartum tridentatum*; Acid diluted pretreatment; Enzymatic hydrolysis; Fermentability; Lignocellulosic ethanol

Jin-Suk Lee^a and Shiro Saka^b. (^a Bioenergy Research Center, Korea Institute of Energy Research, Yuseong-gu, Daejeon 305-343, Republic of Korea, ^b Department of Socio-Environmental Energy Science, Graduate School of Energy Science, Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto 606-8501, Japan). **Biodiesel production by heterogeneous catalysts and supercritical technologies. Bioresource Technology, Volume 101(19) (2010): 7191-7200**

Intensive studies are underway to develop more efficient biodiesel conversion processes. Among the various new technologies, both solid catalyst and non-catalytic supercritical processes are recognized as those that can be turned to practical use in the near future. The current status and challenging issues for these two technologies are, therefore, reviewed in this work as innovative biodiesel production technologies.

Keywords: Biodiesel; Solid catalyst; Non-catalytic supercritical process; Stability; Commercialization

Aninidita Karmakar^a, Subrata Karmakar^b and Souti Mukherjee^a. (^a Department of Post Harvest Engineering, Faculty of Agricultural Engineering, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741 252, India, ^b Department of Farm Machinery and Power, Faculty of Agricultural Engineering, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741 252, India). **Properties of various plants and animals feedstocks for biodiesel production. Bioresource Technology, Volume 101(19) (2010): 7201-7210**

As an alternative fuel biodiesel is becoming increasingly important due to diminishing petroleum reserves and adverse environmental consequences of exhaust gases from petroleum-fuelled engines. Biodiesel, the non-toxic fuel, is mono alkyl esters of long chain fatty acids derived from renewable feedstock like vegetable oils, animal fats and residual oils. Choice of feedstocks depends on process chemistry, physical and chemical characteristics of virgin or used oils and economy of the process.

Extensive research information is available on transesterification, the production technology and process optimization for various biomaterials. Consistent supply of feedstocks is being faced as a major challenge by the biodiesel production industry. This paper reviews physico-chemical

properties of the plant and animal resources that are being used as feedstocks for biodiesel production. Efforts have also been made to review the potential resources that can be transformed into biodiesel successfully for meeting the ever increasing demand of biodiesel production.

Keywords: Renewable oil; Biodiesel; Feedstocks; Transesterification; FFA

Shinji Sakai^a, Yuping Liu^b, Tetsu Yamaguchi^c, Rie Watanabe^d, Masaaki Kawabe^d and Koei Kawakami^b. (^a Division of Chemical Engineering, Department of Materials Engineering, Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama-cho, Toyonaka, Osaka 560-8531, Japan, ^b Department of Chemical Engineering, Faculty of Engineering, Kyushu University, 744 Motoooka, Nishi-ku, Fukuoka 819-0395, Japan, ^c Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center, 1465-5 Aikawa, Kurume, Fukuoka 839-0861, Japan, ^d Japan Vilene Company, Ltd., 7 Kita-Tone, Koga, Ibaraki, 306-0213, Japan). **Production of butyl-biodiesel using lipase physically-adsorbed onto electrospun polyacrylonitrile fibers. Bioresource Technology, Volume 101(19) (2010): 7344-7349**

Butyl-biodiesel production using electrospun polyacrylonitrile fibers with *Pseudomonas cepacia* lipase immobilized through physical adsorption was studied. About 80% conversion to butyl-biodiesel was achieved after 24 h by suspending the catalyst at 2.4 mg/mL in a mixture of rapeseed oil and *n*-butanol at a molar ratio of 1:3, containing water at 8000 ppm at 40 °C. A further 24 h of operation resulted in 94% conversion. The initial reaction rate detected for this process was 65-fold faster than those detected for Novozym 435 on a total catalyst mass basis. The immobilized lipase continued to work as a catalyst for 27 d, within a 15% reduction in conversion yield at the outlet of the reactor compared with the average value detected during the first 3 d of operation in a continuous butyl-biodiesel production system.

Keywords: Biodiesel; Butanolysis; *Pseudomonas cepacia*; Transesterification; Lipase

Susanjib Sarkar^a and Amit Kumar^a. (^a Department of Mechanical Engineering, 4-9 Mechanical Engineering Building, University of Alberta, Edmonton, Alberta, Canada T6G 2G8). **Large-scale biohydrogen production from bio-oil. Bioresource Technology, Volume 101(19) (2010): 7350-7361**

Large amount of hydrogen is consumed during the upgrading of bitumen into synthetic crude oil (SCO), and this hydrogen is exclusively produced from natural gas in Western Canada. Because of large amount of emission from natural gas, alternative sources for hydrogen fuel especially renewable feedstocks could significantly reduce CO₂ emissions. In this study, biomass is converted to bio-oil by fast pyrolysis. This bio-oil is steam reformed near bitumen upgrading plant for producing hydrogen fuel. A techno-economic model is developed to estimate the cost of hydrogen from biomass through the pathway of fast pyrolysis. Three different feedstocks including whole-tree biomass, forest residues (i.e. limbs, branches, and tops of tree produced during logging operations), and straw (mostly from wheat and barley crops) are considered for biohydrogen production. Delivered cost of biohydrogen from whole-tree-based biomass (\$2.40/kg of H₂) is lower than that of forest residues (\$3.00/kg of H₂) and agricultural residues (\$4.55/kg of H₂) at a plant capacity of 2000 dry tonnes/day. In this study, bio-oil is produced in the field/forest and transported to a distance of 500 km from the centralized remote bio-oil production plant to bitumen upgrading plant. Feedstock delivery cost and capital cost are

the largest cost contributors to the bio-oil production cost, while more than 50% of the cost of biohydrogen production is contributed by bio-oil production and transportation. Carbon credits of \$133, \$214, and \$356/tonne of CO₂ equivalent could make whole-tree, forest residues, and straw-based biohydrogen production competitive with natural gas-based H₂ for a natural gas price of \$5/GJ, respectively.

Keywords: Biohydrogen; Bio-oil; Fast pyrolysis; Techno-economic assessment; Carbon credits

Jianchu Ye^a, Song Tu^a and Yong Sha^a. (^a College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, Fujian, China). Investigation to biodiesel production by the two-step homogeneous base-catalyzed transesterification. *Bioresource Technology*, Volume 101(19) (2010): 7368-7374

For the two-step transesterification biodiesel production made from the sunflower oil, based on the kinetics model of the homogeneous base-catalyzed transesterification and the liquid-liquid phase equilibrium of the transesterification product, the total methanol/oil mole ratio, the total reaction time, and the split ratios of methanol and reaction time between the two reactors in the stage of the two-step reaction are determined quantitatively. In consideration of the transesterification intermediate product, both the traditional distillation separation process and the improved separation process of the two-step reaction product are investigated in detail by means of the rigorous process simulation. In comparison with the traditional distillation process, the improved separation process of the two-step reaction product has distinct advantage in the energy duty and equipment requirement due to replacement of the costly methanol-biodiesel distillation column.

Keywords: Biodiesel; Simulation; Two-step production; Reaction kinetics; Separation

Ángel Pérez^a, Abraham Casas^a, Carmen María Fernández^a, María Jesús Ramos^a and Lourdes Rodríguez^b. (^a Chemical Engineering Department, Institute of Chemical and Environmental Technologies, University of Castilla-La Mancha, Avd. Camilo José Cela s/n, 13071 Ciudad Real, Spain, ^b Research and Development Center for Environmental Recovery, Alquimia Environmental Solutions, S.L., Polígono Industrial Daimiel Sur., C/Calidad, 3, 13250 Daimiel (Ciudad Real), Spain). Winterization of peanut biodiesel to improve the cold flow properties. *Bioresource Technology*, Volume 101(19) (2010): 7375-7381

Biodiesel is susceptible to start-up and performance problems, consistent with its chemical composition, when vehicles and fuel systems are subjected to cold temperatures. In this work, a comprehensive evaluation of the crystallization behavior of different biodiesels was performed by measuring the cold filter plugging point (CFPP), cloud point (CP) and pour point (PP). Results were related to differential scanning calorimetry (DSC) thermograms. Peanut methyl esters in particular led to the most unfavorable properties due to the presence of long-chain saturated compounds (arachidic or C20:0, behenic or C22:0, and lignoceric or C24:0 acid methyl esters) approaching 6 wt.%. The cold flow properties may be improved with different winterization techniques to eliminate some of these compounds. In this work, various techniques are tested, and the best technique is found to be crystallization filtration using methanol, which reduces the CFPP from 17 °C to -8 °C with a biodiesel loss of 8.93 wt.%. Moreover, the cake

from filtration, enriched with long-chain saturated methyl esters, can be used as phase change material (PCM) for thermo-regulated materials.

Keywords: Cold flow properties; Biodiesel; Peanut; Winterization; Phase change material

Ayla Sant'Ana da Silva^a, Hiroyuki Inoue^b, Takashi Endo^b, Shinichi Yano^b and Elba P.S. Bon^a. (^a Enzyme Technology Laboratory, Chemistry Institute, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil, ^b Biomass Technology Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 3-11-32 Kagami-Yama, Higashi Hiroshima, Hiroshima 739-0046, Japan). **Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. *Bioresource Technology*, Volume 101(19) (2010): 7402-7409**

The effectiveness of ball milling (BM) and wet disk milling (WDM) on treating sugarcane bagasse and straw were compared. Pretreated materials were characterized by wide angle X-ray diffraction analysis, particle-size distribution and scanning electron microscopy and the effectiveness of pretreatments was evaluated by enzymatic hydrolysis and fermentation. Glucose and xylose hydrolysis yields at optimum conditions for BM-treated bagasse and straw were 78.7% and 72.1% and 77.6% and 56.8%, respectively. Maximum glucose and xylose yields for bagasse and straw using WDM were 49.3% and 36.7% and 68.0% and 44.9%, respectively. BM improved the enzymatic hydrolysis by decreasing the crystallinity, while the defibrillation effect observed for WDM samples seems to have favored enzymatic conversion. Bagasse and straw BM hydrolysates were fermented by *Saccharomyces cerevisiae* strains. Ethanol yields from total fermentable sugars using a C6-fermenting strain reached 89.8% and 91.8% for bagasse and straw hydrolysates, respectively, and 82% and 78% when using a C6/C5 fermenting strain.

Keywords: Sugarcane bagasse; Sugarcane straw; Ball milling pretreatment; Wet disk milling pretreatment; Enzymatic hydrolysis

Nancy N. Nichols^a, Bruce S. Dien^a and Michael A. Cotta^a. (^a Fermentation Biotechnology Research Unit, NCAUR, USDA-ARS, Peoria, IL 61604, USA). **Fermentation of bioenergy crops into ethanol using biological abatement for removal of inhibitors[†]. *Bioresource Technology*, Volume 101(19) (2010): 7545-7550**

Biological abatement was used to condition dilute acid-pretreated hydrolysates of three perennial herbaceous crops that are potential bioenergy feedstocks: switchgrass, reed canarygrass, and alfalfastems. Fungal isolate *Coniochaeta ligniaria* was inoculated into the hydrolysates to metabolize and remove inhibitory compounds prior to yeast fermentation of glucose. Switchgrass, reed canarygrass, and alfalfa stem samples were pretreated with dilute acid at 10% w/w biomass loading and subjected to bioabatement with strain NRRL30616, to prepare the material for simultaneous saccharification of cellulose and fermentation by *Saccharomyces cerevisiae*. Bioabatement eliminated the extended fermentation lag times associated with inhibitory compounds and observed for the unconditioned biomass hydrolysates controls. Bioabatement was as effective as lime conditioning at reducing fermentation lag times. Prolonged incubations with the bioabatement microbe resulted in consumption of some glucose and reduced production of ethanol.

Keywords: Inhibitor abatement; Bioabatement; Biomass; Biofuels; Ethanol

Carmen María Fernández^a, María Jesús Ramos^a, Ángel Pérez^a and Juan Francisco Rodríguez^a. (^a Departamento de Ingeniería Química, Instituto de Tecnologías Química y Medioambiental, Universidad de Castilla-La Mancha, Avd. Camilo José Cela s/n, 13071 Ciudad Real, Spain). Production of biodiesel from winery waste: Extraction, refining and transesterification of grape seed oil. *Bioresource Technology*, Volume 101(18) (2010): 7019-7024

In regions with a large wine production the usage of their natural waste to make biodiesel can result an interesting alternative. In this work, different methods of extraction, refining and transesterification of grape seed oil were assayed. Two techniques of oil extraction were compared: solvent extraction and pressing. Two conventional transesterifications of the refined oil were carried out using methanol and bioethanol, being the methyl and ethyl ester contents higher than 97 wt.%. Finally, several in situ transesterifications were done. In situ transesterification did not reach either the oil yield extraction or the alkyl ester contents but the obtained biodiesel had better oxidation stability in comparison with the conventional process.

Keywords: Grape seed; Oil extraction; Refining; Transesterification; Biodiesel

Junfeng Qian^{a,b}, Haixian Shi^b and Zhi Yun^b. (^a Jiangsu Provincial Key Laboratory of Fine Petrochemical Engineering, Jiangsu Polytechnic University, Changzhou 213164, PR China, ^b College of Chemistry and Chemical Engineering, Nanjing University of Technology, Nanjing 210009, PR China). Preparation of biodiesel from *Jatropha curcas* L. oil produced by two-phase solvent extraction. *Bioresource Technology*, Volume 101(18) (2010): 7025-7031

In the present work the preparation of biodiesel from *Jatropha curcas* L. oil produced by two-phase solvent extraction (TSE) was studied. The experimental results of TSE process showed that the optimal extraction conditions were 30 g samples, 240 mL of extraction solvent mixture and methanol/*n*-hexane volume ratio 60:40, extraction temperature 35 °C, extraction time 30 min. Further, the effect of methanol recycling on the TSE process was also investigated. After TSE process, the investigations were carried out on transesterification of methanol with oil-*n*-hexane solution coming from TSE process in the presence of sodium hydroxide as the catalyst. The conversion could achieve 98% with 3:1 *n*-hexane/oil weight ratio, 60 °C reaction temperature, 7:1 methanol/oil mole ratio, 1.1% alkali catalyst concentration and 120 min reaction time. The properties of fatty acid methyl ester (FAME) product prepared from *Jatropha curcas* L. oil produced by two-phase solvent extraction met the ASTM specifications for biodiesel.

Keywords: Biodiesel; Fatty acid methyl ester; Two-phase solvent extraction; *Jatropha curcas* L. oil; Free fatty acid

Rafael Sánchez^a, Alejandro Rodríguez^a, Ana Requejo^a, Ana Ferrer^a and Enrique Navarro^a. (^a Chemical Engineering Department, Faculty of Science, University of Córdoba, Spain). Soda pulp and fuel gases synthesis from *Hesperaloe funifera*. *Bioresource Technology*, Volume 101(18) (2010): 7032-7040

The main objective of this work is to evaluate the suitability of *Hesperaloe funifera* which is an alternative raw material, for pulping with soda–anthraquinone to produce pulp and paper. It was studied the influence of operational variables (temperature (155–185 °C), cooking time (20–60 min) and sodaconcentration (5–15%), with a constant addition of 1% of anthraquinone and a liquid/solid ratio of 8, insoda–anthraquinone cooking of *H. funifera* on pulps and paper sheets properties obtained. Finally, the cooking liquors were acidified to separate solid fractions that were subjected to pyrolysis and gasification in order to obtain synthesis and fuel gases.

H. funifera contains little lignin and abundant α -cellulose; this, together with the morphological characteristics of its fibers, makes it a potentially highly useful papermaking raw material.

Keywords: *Hesperaloe funifera*; Pulp; Fuzzy model; Fuel; Lignin

Mitchell Lever^a, Goen Ho^a and Ralf Cord-Ruwisch^b. (^a Environmental Technology Centre and School of Environmental Science, Murdoch University, Perth, Western Australia, Australia, ^b School of Biological Sciences and Biotechnology, Murdoch University, Perth, Western Australia, Australia). Ethanol from lignocellulose using crude unprocessed cellulase from solid-state fermentation. *Bioresource Technology*, Volume 101(18) (2010): 7083-7087

It was desired to study a simplified method of cellulase production using solid-state fermentation for its potential to be used on-site as part of a cellulose to ethanol conversion process, in lieu of expensive and energy intensive commercial enzyme preparations. Crude unprocessed cellulase extracts were produced by solid-state fermentation of *Trichoderma reesei* on ground wheat straw. While cellulase yields were not high they were sufficient to produce ethanol from wheat straw in simultaneous saccharification and fermentation with *Saccharomyces cerevisiae*. As little as an additional 5% of the material converted to ethanol may be employed for cellulase production suggesting an inordinate quantity of additional substrate would not be required. These findings suggest a simplified crude cellulase process at the site of ethanol production using a common lignocellulosic substrate may be employed in lieu of commercial enzyme preparations.

Keywords: Cellulase; Ethanol; Fermentation; Straw; Solid-state

Zhenle Yuan^a, Junhua Wang^a, Lina Wang^b, Weihui Xie^b, Ping Chen^a, Zhaoyin Hou^a, and Xiaoming Zheng^a. (^a Institute of Catalysis, Department of Chemistry, Zhejiang University, Hangzhou 310028, China, ^b Key Laboratory of Advanced Textile Materials and Manufacturing Technology (Zhejiang Sci-tech University), Hangzhou 310012, China). Biodiesel derived glycerol hydrogenolysis to 1,2-propanediol on Cu/MgO catalysts. *Bioresource Technology*, Volume 101(18) (2010): 7088-7092

Hydrogenolysis of biodiesel derived glycerol to 1,2-propanediol (1,2-PDO) has attracted much attention in recent years. In this work, glycerol hydrogenolysis to 1,2-PDO was performed over CuO/MgO catalysts prepared by impregnation and coprecipitation at 180 °C and 3.0 MPa H₂. It was found that the Cu(15)/MgO catalyst prepared by coprecipitation had the best activity. The conversion of glycerol and the selectivity of 1,2-PDO over Cu(15)/MgO reached 72.0% and 97.6%, respectively. And the conversion of glycerol was further increased to 82.0% when small amount of NaOH was added in the reaction mixture. Those highly active catalysts were characterized by X-ray diffraction, transmission electron microscopy, N₂-adsorption and

temperature-programmed reduction with H₂. Characterization results revealed that the activity of the prepared catalysts depended strongly on the particle sizes of both Cu and MgO. Catalysts that have smaller sized Cu and MgO particles are more active for glycerol hydrogenolysis.

Keywords: Biodiesel; Glycerol; Hydrogenolysis; 1,2-Propanediol; Catalysts

Xiaoran Zhang^a, Yu Shen^a, Wenlong Shi^a and Xiaoming Bao^a. (^a State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, PR China). **Ethanol cofermentation with glucose and xylose by the recombinant industrial strain *Saccharomyces cerevisiae* NAN-127 and the effect of furfural on xylitol production. *Bioresource Technology*, Volume 101(18) (2010): 7093-7099**

Saccharomyces cerevisiae strain NAN-127 (2n, prototroph), which contains the xylose reductase-xylitol dehydrogenase (XR-XDH) xylose metabolic pathway was used for the cofermentation of glucose and xylose. Oxygen supply was the most important factor for xylose fermentation and pH 4.5 and a ventilation rate of 0.04 vvm were optimal. The xylose utilization ratio reached 0.655 at an initial xylose concentration of 50 g L⁻¹ and was 0.9 at an initial concentration of 20 g L⁻¹. Addition of furfural at late logarithmic phase as electron acceptor to a final concentration of 3.0 g L⁻¹ decreased the xylitol yield by 17% under micro-aeration conditions without inhibiting cell growth, but also without an increase in ethanol yield. The results are important to the application of strain NAN-127 in the lignocellulosic ethanol process.

Keywords: Bioethanol; Xylose cofermentation; Furfural; XR-XDH xylose metabolic pathway; Recombinant industrial yeast

Jeung-yil Park^a, Riki Shiroma^a, Muhammad Imran Al-Haq^a, Ying Zhang^a, Masakazu Ike^a, Yumiko Arai-Sanoh^b, Atsuhiko Ida^b, Motohiko Kondo^b and Ken Tokuyasu^a. (^a National Food Research Institute, National Agriculture and Food Research Organization (NARO), 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan, ^b National Institute of Crop Science, NARO, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan). **A novel lime pretreatment for subsequent bioethanol production from rice straw – Calcium capturing by carbonation (CaCCO) process. *Bioresource Technology*, Volume 101(17) (2010): 6805-6811**

In order to establish an efficient bioethanol production system for rice straw, we developed a novel lime-pretreatment process (CaCCO process) that did not require a solid-liquid-separation step. This process adopted a step in which after pretreatment lime was neutralized by carbonation, resulting in a final pH of about 6. CaCO₃ produced by the process was kept in the reaction vessel, and no significant inhibitory effects on enzymatic saccharification and fermentation were observed. In the CaCCO process, solubilized carbohydrates, such as xylan, starch, and sucrose were also kept in the vessel, enabling high recoveries of monomeric sugars. Simultaneous saccharification and fermentation (SSF) of pretreated rice straw, 10% (g-rice straw/g-water), using *Saccharomyces cerevisiae* and *Pichia stipitis* yielded 19.1 g L⁻¹ ethanol that was 74% of the theoretical yield from glucose and xylose. Thus, this process represents a novel pretreatment method to utilize not only cellulose but also xylan, starch, and sucrose from biomass.

Keywords: Bioethanol; Carbonation; Enzymatic hydrolysis; Lime pretreatment; Rice straw

Abbreviations: CaCCO, calcium capturing by carbonation; SCs, soft carbohydrates; SSF, simultaneous saccharification and fermentation; HNW, HCl neutralization/washing

J.A. Siles^a, M.A. Martín^a, A.F. Chica^a and A. Martín^a. (^a Departamento de Química Inorgánica e Ingeniería Química, Facultad de Ciencias, Universidad de Córdoba, Campus Universitario de Rabanales, Edificio C-3, Ctra. Madrid-Cádiz, km 396, 14071 Córdoba, Spain). *anaerobic co-digestion of glycerol and wastewater derived from biodiesel manufacturing. Bioresource Technology, Volume 101(16) (2010): 6315-6321*

The anaerobic co-digestion of glycerol and wastewater derived from biodiesel manufacturing, in which COD was found to be 1054 and 428 g/L, respectively, was studied in batch laboratory-scale reactors at mesophilic temperature (35 °C). Glycerol was acidified with H₃PO₄ in order to recover the alkaline catalyst employed in the transesterification reaction (KOH) as agricultural fertiliser (potassium phosphates). Wastewater was subjected to an electrocoagulation process in order to reduce its oil content. After mixing, the anaerobic revalorisation of the wastewater was studied employing inoculum–substrate ratios ranging from 5.02 to 1.48 g VSS/g COD and organic loading rates of 0.27–0.36 g COD/g VSS d. Biodegradability was found to be around 100%, while the methane yield coefficient was 310 mL CH₄/g COD removed (1 atm, 25 °C). At the equilibrium time, the proportionality between \bar{r} and the load was constant, showing 1.55 mL CH₄/(g VSS g COD h). The results showed that anaerobic co-digestion reduces the clean water and nutrient requirement, with the consequent economical and environmental benefit.

Keywords: Biodiesel manufacturing; Glycerol-containing waste; Wastewater; Anaerobic co-digestion; Mesophilic temperature

Baoning Zhu^{a,b}, Ruihong Zhang^a, Petros Gikas^c, Joshua Rapport^a, Bryan Jenkins^a and Xiujin Li^b. (^a Department of Biological and Agricultural Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States, ^b Department of Environmental Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China, ^c Department of Environmental Engineering, Technical University of Crete, Chania 73100, Greece). *Biogas production from municipal solid wastes using an integrated rotary drum and anaerobic-phased solids digester system. Bioresource Technology, Volume 101(16) (2010): 6374-6380*

This research was conducted to develop an integrated rotary drum reactor (RDR)-anaerobic-phased solids (APS) digester system for the treatment of municipal solid waste (MSW) to produce biogas energy and achieve waste reduction. A commercial RDR facility was used to provide a 3-d pretreatment and sufficient separation of the organics from MSW and then the organics were digested in a laboratory APS-digester system for biogas production. The organics generated from the RDR contained 50% total solids (TS) and 36% volatile solids (VS) on wet basis. The APS-digester was started at an organic loading rate (OLR) of 3.1 gVS L⁻¹ d⁻¹ and operated at three higher OLRs of 4.6, 7.7 and 9.2 gVS L⁻¹ d⁻¹. At the OLR of 9.2 gVS L⁻¹ d⁻¹ the system biogas production rate was 3.5 L L⁻¹ d⁻¹ and the biogas and methane yields were 0.38 and 0.19 L gVS⁻¹, respectively. Anaerobic digestion resulted in 38% TS reduction and 53% VS reduction in the organic solids. It was found that the total VFA concentration reached a peak value of 15,000 mg L⁻¹ as acetic acid in the first 3 d of batch

digestion and later decreased to about 500 mg L⁻¹. The APS-digester system remained stable at each OLRs for over 100 d with the pH in the hydrolysis reactors in the range of 7.3–7.8 and the pH in the biogasification reactor in 7.9–8.1. The residual solids after the digestion had a high heating value of 14.7 kJ gTS⁻¹.

Keywords: Anaerobic digestion; Municipal solid wastes; Rotary drum reactor; Biogas

Xuan Li^a, Tae Hyun Kim^a and Nhuan P. Nghiem^b. (^a Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA 50011, United States, ^b Eastern Regional Research Center, Agricultural Research Service, US Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, PA 19038, United States). **Bioethanol production from corn stover using aqueous ammonia pretreatment and two-phase simultaneous saccharification and fermentation (TPSSF). Bioresource Technology, Volume 101(15) (2010): 5910-5916**

An integrated bioconversion process was developed to convert corn stover derived pentose and hexose to ethanol effectively. In this study, corn stover was pretreated by soaking in aqueous ammonia (SAA), which retained glucan (~100%) and xylan (>80%) in the solids. The pretreated carbohydrates-rich corn stover was converted to ethanol via two-phase simultaneous saccharification and fermentation (TPSSF). This single-reactor process employed sequential simultaneous saccharification and fermentation (SSF), i.e. pentose conversion using recombinant *Escherichia coli* KO11 in the first phase, followed by hexose conversion with *Saccharomyces cerevisiae* D5A in the second phase. In the first phase, 88% of xylan digestibility was achieved through the synergistic action of xylanase and endo-glucanase with minimal glucan hydrolysis (10.5%). Overall, the TPSSF using 12-h SAA-treated corn stover resulted in the highest ethanol concentration (22.3 g/L), which was equivalent to 84% of the theoretical ethanol yield based on the total carbohydrates (glucan + xylan) in the untreated corn stover.

Keywords: Integrated bioconversion process; Bioethanol; Soaking in aqueous ammonia (SAA); KO11; D5A yeast

Shi-wei Liu^a, Cong-xia Xie^b, Rui Jiang^b, Shi-tao Yu^a and Fu-sheng Liu^a. (^a College of Chemical Engineering, Qingdao University of Science and Technology, No. 53 Zhengzhou Road, Qingdao 266042, China, ^b College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, No. 53 Zhengzhou Road, Qingdao 266042, China). **Hydrogenation of biodiesel using thermoregulated phase-transfer catalyst for production of fatty alcohols. Bioresource Technology, Volume 101(15) (2010): 6278-6280**

The hydrogenation of biodiesel was investigated in presence of thermoregulated phase-transfer catalysts to produce fatty alcohols. The thermoregulated catalytic system Pd/IV (IV: P-ligand, tri-(methoxyl polyethylene glycol)-phosphite) exhibited an efficient catalytic performance for the hydrogenation. It was also found that the steric resistance of the P-ligand, to a large extent, affected the performance of catalytic system. Using Pd/IV as catalyst, the product could be easily separated from the catalytic system and the catalyst was of good reusability. Thus, a clean and environmentally friendly strategy for the production of fatty alcohol is provided.

Keywords: Thermoregulated phase-transfer catalyst; Biodiesel; Fatty alcohol; Hydrogenation

Bing-Feng Liu^a, Nan-Qi Ren^a, Guo-Jun Xie^a, Jie Ding^a, Wan-Qian Guo^a and De-Feng Xing^a. (^a State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, No. 73 Huanghe Road, 2nd Campus of HIT Box 2614, Harbin 150090, China). **Enhanced bio-hydrogen production by the combination of dark- and photo-fermentation in batch culture. Bioresource Technology, Volume 101(14) (2010): 5325-5329**

In this study, some key factors, for example, diluted ratio of effluents, the ratio of dark-photo bacteria, light intensity and light/dark cycle influencing hydrogen production by combining *Clostridium butyricum* and immobilized *Rhodospseudomonas faecalis* RLD-53 in batch culture, were investigated. Experimental results showed the photo-hydrogen yield decreased when increasing diluted ratio from 1:0.5 to 1:3, and it reached the maximum value of 4368 ml-H₂/l-effluents at the ratio of 1:0.5. When the ratio of dark-photobacteria was at 1:2, the hydrogen yield reached highest value of 4.946 mol-H₂/mol-glucose and cumulative hydrogen volume was 5357 ml-H₂/l-culture during the combination process. When the light intensity was at 10.25 W/m², the hydrogen volume of photo-fermentation and the combination process reached maximum value of 4260 ml-H₂/l-effluents and 5892 ml-H₂/l-culture, respectively. During the combination process, maximum total hydrogen yield was 5.374 mol-H₂/mol-glucose. Meanwhile, hydrogen production under light/dark cycle was evaluated.

Keywords: Hydrogen production; Dark-fermentation; Photo-fermentation; Diluted ratio; Light/dark cycle

Vishnu Menon^a, Gyan Prakash^a, Asmita Prabhune^a and Mala Rao^a. (^a Division of Biochemical Sciences, National Chemical Laboratory, Pune 411 008, India). **Biocatalytic approach for the utilization of hemicellulose for ethanol production from agricultural residue using thermostable xylanase and thermotolerant yeast. Bioresource Technology, Volume 101(14) (2010): 5366-5373**

A hydrolysis of 62% and 50% for OSX (Oat spelt xylan) and WBH (Wheat bran hemicellulose) were obtained in 36 h and 48 h using Accellerase™ 1000 at 50 °C wherein thermostable xylanase from alkalothermophilic *Thermomonospora* sp. yielded 67% (OSX) in 3 h and 58% (WBH) in 24 h at 60 °C, favouring a reduction in process time and enzyme dosage. The rate of hydrolysis with thermostable xylanase was increased by 20% with the addition of nonionic surfactant tween 80 or biosurfactant sophorolipid. The simultaneous saccharification and fermentation (SSF) of OSX and WBH using thermostable xylanase and *D. hansenii* in batch cultures produced 9.1 g/L and 9.5 g/L of ethanol, respectively and had a shorter overall process time than the separate hydrolysis and fermentation (SHF). The immobilized yeast cells in Ca-alginate matrix produced ethanol with a yield of 0.46 g/g from hemicellulosic hydrolysates and were reused six times with 100% fermentation efficiency.

Keywords: Hemicellulose, Thermostable xylanase, Thermotolerant yeast, Biosurfactant, Ethanol

Qing Shu^a, Zeeshan Nawaz^a, Jixian Gao^a, Yuhui Liao^a, Qiang Zhang^a, Dezheng Wang^a and Jinfu Wang^a. (^a Beijing Key Laboratory of Green Chemical Reaction Engineering and

Technology, Department of Chemical Engineering, Tsinghua University, Beijing 100084, China). Synthesis of biodiesel from a model waste oil feedstock using a carbon-based solid acid catalyst: Reaction and separation. *Bioresource Technology*, Volume 101, Issue 14, July 2010, Pages 5374-538

A solid acid catalyst that can keep high activity and stability is necessary when low cost feedstocks are utilized for biodiesel synthesis because the reaction medium contains a large amount of water. Three solid acid catalysts were prepared by the sulfonation of carbonized vegetable oil asphalt and petroleum asphalt. The structure of these catalysts was characterized by a variety of techniques. A new process that used the coupling of the reaction and separation was employed, which greatly improved the conversion of cottonseed oil (triglyceride) and free fatty acids (FFA) when a model waste oil feedstock was used. The vegetable oil asphalt-based catalyst showed the highest catalytic activity. This was due to the high density and stability of its acid sites, its loose irregular network, its hydrophobicity that prevented the hydration of –OH species, and large pores that provided more acid sites for the reactants.

Keywords: Biodiesel; Solid acid catalyst; Sequential catalysis reaction process; Reaction and separation

Junming Xu^a Jianchun Jiang^a, Jie Chen^a and Yunjuan Sun^a. (^a Institute of Chemical Industry of Forest Products, CAF, Nanjing 210042, China). Biofuel production from catalytic cracking of woody oils. *Bioresource Technology*, Volume 101(14) (2010): 5586-5591

The catalytic cracking reactions of several kinds of woody oils have been studied. The products were analyzed by GC–MS and FTIR and show the formation of olefins, paraffins and carboxylic acids. Several kinds of catalysts were compared. It was found that the fraction distribution of product was modified by using base catalysts such as CaO. The products from woody oils showed good cold flow properties compared with diesel used in China. The results presented in this work have shown that the catalytic cracking of woody oils generates fuels that have physical and chemical properties comparable to those specified for petroleum based fuels.

Keywords: Biofuel; Woody oils; Catalytic cracking

R.S. Prakasham^a, T. Sathish^a and P. Brahmaiah^a. (^a Bioengineering and Environmental Centre, Indian Institute of Chemical Technology, Hyderabad 500 607, India). Biohydrogen production process optimization using anaerobic mixed consortia: A prelude study for use of agro-industrial material hydrolysate as substrate. *Bioresource Technology*, Volume 101(14) (2010): 5708-5711

Efficient biohydrogen production from lignocellulosic hydrolysate assumes considerable practical and academic importance. The impact of glucose to xylose ratio, medium pH, inoculum size and age on biohydrogen production indicated that glucose to xylose ratio is the critical parameter for effective H₂ production compared to either pure glucose or xylose as carbon source. Inoculum size and its age contributed more than 70% to overall H₂ production and revealed significance at individual as well as interactive level. Maximum interaction of 39% and 32% was noticed with inoculum size and its age vs. glucose to xylose ratio (2:3), respectively.

The H₂ production yield enhanced from 140 to 357 ml/g substrate upon statistical optimization revealing >240% improvement.

Keywords: Biohydrogen; Anaerobic fermentation; Glucose:xylose ratio; Process optimization; Taguchi methodology

Hui Teng Tan^a, Keat Teong Lee^a and Abdul Rahman Mohamed^a. (^a School of Chemical Engineering, Universiti Sains Malaysia, Engineering Campus, Seri Ampangan, 14300 Nibong Tebal, Pulau Pinang, Malaysia). Second-generation bio-ethanol (SGB) from Malaysian palm empty fruit bunch: Energy and exergy analyses. *Bioresource Technology*, Volume 101(14) (2010): 5719-5727

Recently, second-generation bio-ethanol (SGB), which utilizes readily available lignocellulosic biomass has received much interest as another potential source of liquid biofuel comparable to biodiesel. Thus the aim of this paper is to determine the exergy efficiency and to compare the effectiveness of SGB and palm methyl ester (PME) processes. It was found that the production of bio-ethanol is more thermodynamically sustainable than that of biodiesel as the net exergy value (NE_{xV}) of SGB is 10% higher than that of PME. Contrarily, the former has a net energy value (NE_V) which is 9% lower than the latter. Despite this, SGB is still strongly recommended as a potential biofuel because SGB production can help mitigate several detrimental impacts on the environment.

Keywords: Second-generation bio-ethanol; Lignocellulosic biomass; Exergy; Biofuel; Palm methyl ester

Aijie Wang^a, Dan Sun^a, Nanqi Ren^a, Chong Liu^a, Wenzong Liu^a, Bruce E. Logan^b and Wei-Min Wu^c. (^a State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (SKLUWRE, HIT), Harbin 150090, PR China, ^b Department of Civil and Environmental Engineering, The Pennsylvania State University, University Park, PA 16802, United States, ^c Department of Civil and Environmental Engineering, Stanford University, Stanford, CA 94305-4020, United States). A rapid selection strategy for an anodophilic consortium for microbial fuel cells. *Bioresource Technology*, Volume 101(14) (2010): 5733-5735

A rapid selection method was developed to enrich for a stable and efficient anodophilic consortium (AC) for microbial fuel cells (MFCs). A biofilm sample from a microbial electrolysis cell was serially diluted up to 10⁻⁹ in anaerobic phosphate buffer solution and incubated in an Fe(III)-acetate medium, and an Fe(III)-reducing AC was obtained for dilutions up to 10⁻⁶. The activity of MFC inoculated with the enrichment AC was compared with those inoculated with original biofilm or activated sludge. The power densities and Coulombic efficiencies of the AC (226 mW/m², 34%) were higher than those of the original biofilm (209 mW/m², 23%) and activated sludge (192 mW/m², 19%). The start-up period of the AC (60 h) was also shorter than those obtained with the other inocula (biofilm, 95 h; activated sludge, 300 h). This indicated that such a strategy is highly efficient for obtaining an anodophilic consortium for improving the performance of an MFC.

Keywords: Anodophilic consortium (AC); Serial dilution; Dissimilatory Fe(III) reduction; Microbial fuel cell(MFC)

Farid Talebnia^a, Dimitar Karakashev^a and Irini Angelidaki^a. (^a Department of Environmental Engineering, Building 113, Technical University of Denmark, Lyngby 2800, Denmark). Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. *Bioresource Technology*, Volume 101(13) (2010): 744-4753

Wheat straw is an abundant agricultural residue with low commercial value. An attractive alternative is utilization of wheat straw for bioethanol production. However, production costs based on the current technology are still too high, preventing commercialization of the process. In recent years, progress has been made in developing more effective pretreatment and hydrolysis processes leading to higher yield of sugars. The focus of this paper is to review the most recent advances in pretreatment, hydrolysis and fermentation of wheat straw. Based on the type of pretreatment method applied, a sugar yield of 74–99.6% of maximum theoretical was achieved after enzymatic hydrolysis of wheat straw. Various bacteria, yeasts and fungi have been investigated with the ethanol yield ranging from 65% to 99% of theoretical value. So far, the best results with respect to ethanol yield, final ethanol concentration and productivity were obtained with the native non-adapted *Saccharomyces cerevisiae*. Some recombinant bacteria and yeasts have shown promising results and are being considered for commercial scale-up. Wheat straw biorefinery could be the near-term solution for clean, efficient and economically-feasible production of bioethanol as well as high value-added products.

Keywords: Wheat straw; Ethanol; Pretreatment; Enzymatic hydrolysis; Fermentation

C.A. Cardona^a, J.A. Quintero^a and I.C. Paz^a. (^a Departamento de Ingeniería Química, Universidad Nacional de Colombia Sede Manizales, Cra. 27 No. 64-60, Manizales, Colombia). Production of bioethanol from sugarcane bagasse: Status and perspectives. *Bioresource Technology*, Volume 101(13) (2010): 4754-4766

Lignocellulosic biomass is considered as the future feedstock for ethanol production because of its low cost and its huge availability. One of the major lignocellulosic materials found in great quantities to be considered, especially in tropical countries, is sugarcane bagasse (SCB). This work deals with its current and potential transformation to sugars and ethanol, considering pretreatment technologies, detoxification methods and biological transformation. Some modeling aspects are exposed briefly. Finally stability is discussed for considering the high nonlinear phenomena such as multiplicity and oscillations, which make more complex the control as a result of the inhibition problems during fermentation when furfural and formic acid from SCB hydrolysis are not absent.

Keywords: Sugarcane bagasse; Ethanol; Pretreatment; Lignocellulosic; Stability

Parameswaran Binod^a, Raveendran Sindhu^a, Reeta Rani Singhanian^a, Surender Vikram^a, Lalitha Devi^a, Satya Nagalakshmi^a, Noble Kurien^a, Rajeev K. Sukumaran^a and Ashok Pandey^a. (^a Centre for Biofuels, National Institute for Interdisciplinary Science and Technology, CSIR, Trivandrum 695 019, India). Bioethanol production from rice straw: An overview. *Bioresource Technology*, Volume 101(13) (2010): 4767-4774

Rice straw is an attractive lignocellulosic material for bioethanol production since it is one of the most abundant renewable resources. It has several characteristics, such as high cellulose and

hemicelluloses content that can be readily hydrolyzed into fermentable sugars. But there occur several challenges and limitations in the process of converting rice straw to ethanol. The presence of high ash and silica content in rice straw makes it an inferior feedstock for ethanol production. One of the major challenges in developing technology for bioethanol production from rice straw is selection of an appropriate pretreatment technique. The choice of pretreatment methods plays an important role to increase the efficiency of enzymatic saccharification thereby making the whole process economically viable. The present review discusses the available technologies for bioethanol production using rice straw.

Keywords: Rice straw; Pretreatment; Bioethanol; Lignocellulosic biomass

F.M. Gírio^a, C. Fonseca^a, F. Carvalheiro^a, L.C. Duarte^a, S. Marques^a and R. Bogel-Lukasik^a. (^a **Laboratório Nacional de Energia e Geologia, I.P., Unidade de Bioenergia, Estrada do Paço do Lumiar 22, 1649-038, Lisboa, Portugal**). **Hemicelluloses for fuel ethanol: A review. *Bioresource Technology*, Volume 101(13) (2010): 4775-4800**

Hemicelluloses currently represent the largest polysaccharide fraction wasted in most cellulosic ethanol pilot and demonstration plants around the world. The reasons are based on the hemicelluloses heterogeneous polymeric nature and their low fermentability by the most common industrial microbial strains. This paper will review, in a “from field to fuel” approach the various hemicelluloses structures present in lignocellulose, the range of pre-treatment and hydrolysis options including the enzymatic ones, and the role of different microbial strains on process integration aiming to reach a meaningful consolidated bioprocessing. The recent trends, technical barriers and perspectives of future development are highlighted.

Keywords: Fuel ethanol; Hemicelluloses; Consolidated bioprocessing; Biomass pre-treatments; Cell factories

Jun-Seok Kim^a, Soon-Chul Park^b, Jin-Woo Kim^c, Jae Chan Park^c, Sung-Min Park^c and Jin-Suk Lee^b. (^a **Department of Chemical Engineering, Kyonggi University, Suwon 443-760, Republic of Korea**, ^b **Bioenergy Research Center, Korea Institute of Energy Research, Daejeon 305-343, Republic of Korea**, ^c **Samsung Advanced Institute of Technology, Yongin 446-712, Republic of Korea**). **Production of bioethanol from lignocellulose: Status and perspectives in Korea. *Bioresource Technology*, Volume 101(13) (2010): 4801-4805**

The current status and challenges associated with the production and utilization of cellulosic ethanol in Korea are reviewed in this paper. Cellulosic ethanol has emerged as a promising option for mitigating Korea's CO₂ emissions and enhancing its energy security. Korea's limited biomass resources is the most critical barrier to achieving its implementation targets for cellulosic ethanol. Efforts to identify new suitable biomass resources for cellulosic ethanol production are ongoing and intensive. Aquatic biomasses including macroalgae and plantation wastes collected in the Southeast Asia region have been found to have great potential as feedstocks for the production of cellulosic ethanol. R&D explorations into the development of technologies that can convert biomass materials to ethanol more efficiently also are underway. It is expected that cellulosic ethanol will be in supply from 2020 and that, by 2030, its use will have effectively reduced Korea's total gasoline consumption by 10%.

Keywords: Cellulosic ethanol; New resources; Pretreatment; Continuous fermentation; Fuel distribution infrastructure

W.E. Mabee^a and J.N. Saddler^b. (^a School of Policy Studies and Department of Geography, Queen's University, Canada, ^b Forest Products Biotechnology, Faculty of Forestry, University of British Columbia, Canada). Bioethanol from lignocellulosics: Status and perspectives in Canada. *Bioresource Technology*, Volume 101(13) (2010): 4806-4813

Canada has invested significantly in the development of a domestic bioethanol industry, and it is expected that bioethanol from lignocellulosics will become more desirable to the industry as it expands. Development of the Canadian industry to date is described in this paper, as are examples of domestic research programs focused on both bioconversion and thermochemical conversion to generate biofuels from lignocellulosic biomass. The availability of lignocellulosic residues from agricultural and forestry operations, and the potential biofuel production associated with these residues, is described. The policy tools used to develop the domestic bioethanol industry are explored. A residue-based process could greatly extend the potential of the bioethanol industry in Canada. It is estimated that bioethanol production from residual lignocellulosic feedstocks could provide up to 50% of Canada's 2006 transportation fuel demand, given ideal conversion and full access to these feedstocks. Utilizing lignocellulosic biomass will extend the geographic range of the bioethanol industry, and increase the stability and security of this sector by reducing the impact of localized disruptions in supply. Use of disturbance crops could add 9% to this figure, but not in a sustainable fashion. If pursued aggressively, energy crops ultimately could contribute bioethanol at a volume double that of Canada's gasoline consumption in 2006. This would move Canada towards greater transportation fuel independence and a larger role in the export of bioethanol to the global market.

Keywords: Bioethanol; Lignocellulosic feedstock availability; Biofuel policy; Biofuel implementation; Canada

Xu Fang^a, Yu Shen^a, Jian Zhao^a, Xiaoming Bao^a and Yinbo Qu^a. (^a State Key Laboratory of Microbial Technology, and National Glycoengineering Research Center, Shandong University, 27 Shanda-nan Road, Jinan, Shandong 250100, China). Status and prospect of lignocellulosic bioethanol production in China. *Bioresource Technology*, Volume 101(13) (2010): 4814-4819

As a developing country with the largest population, China faces a serious challenge in satisfying its continuously increasing energy demand, especially for liquid fuel. Bioethanol production from lignocellulosic material is a potential and feasible method to solve the many problems in China, and it was supported by the Chinese government. Many research projects in China on lignocellulosics ethanol production have been carried out. After more than 30 years of research, several pilot scale facilities have been constructed. This review focuses on the recent research activities and developments in lignocellulosic ethanol production during the past decade in China. As case study, a corncob biorefinery process is introduced.

Keywords: Bioethanol production; Lignocellulosic biomass; Pretreatment; Cellulase production; Hydrolysis and fermentation

Carlos Ricardo Soccol^a, Luciana Porto de Souza Vandenberghe^a, Adriane Bianchi Pedroni Medeiros^a, Susan Grace Karp^{a, b}, Marcos Buckeridge^c, Luiz Pereira Ramos^d, Ana Paula

Pitarelo^d, Viridiana Ferreira-Leitão^e, Leda Maria Fortes Gottschalk^f, Maria Antonieta Ferrara^g, Elba Pinto da Silva Bon^f, Lidia Maria Pepe de Moraes^h, Juliana de Amorim Araújo^h and Fernando Araripe Gonçalves Torres^h. (^a Bioprocess Engineering and Biotechnology Department, Federal University of Paraná (UFPR), Curitiba, PR, Brazil, ^b SENAI-PR, R. Sen. Accioly Filho, 250, CIC-Curitiba, PR, Brazil, ^c Department of Botany, Institute of Biosciences, University of São Paulo, Rua do Matão 277, São Paulo, SP, Brazil, ^d Department of Chemistry, Federal University of Paraná (UFPR), CEP 81531-970, Curitiba, PR, Brazil, ^e National Institute of Technology, Ministry of Science and Technology, Av. Venezuela, 82 Sala 302, CEP 20081-312, Rio de Janeiro, RJ, Brazil, ^f Chemistry Institute, Federal University of Rio de Janeiro, Av. Athos da Silveira Ramos, 149, Centro de Tecnologia, Bloco A, 5^o andar, Sala 539, CEP 21941-909, Rio de Janeiro, RJ, Brazil, ^g Far-Manguinhos/FIOCRUZ. Rua Sizenando Nabuco, 100 Manguinhos, CEP 21041-250, Rio de Janeiro, RJ, Brazil, ^h Centro de Biotecnologia Molecular, Universidade de Brasília, CEP 70910-900, Brasília, DF, Brazil). **Bioethanol from lignocelluloses: Status and perspectives in Brazil. *Bioresource Technology*, Volume 101(13) (2010): 4820-4825**

The National Alcohol Program – PróAlcool, created by the government of Brazil in 1975 resulted less dependency on fossil fuels. The addition of 25% ethanol to gasoline reduced the import of 550 million barrels oil and also reduced the emission CO₂ by 110 million tons. Today, 44% of the Brazilian energy matrix is renewable and 13.5% is derived from sugarcane. Brazil has a land area of 851 million hectares, of which 54% are preserved, including the Amazon forest (350 million hectares). From the land available for agriculture (340 million hectares), only 0.9% is occupied by sugarcane as energy crop, showing a great expansion potential. Studies have shown that in the coming years, ethanol yield per hectare of sugarcane, which presently is 6000 L/ha, could reach 10,000 L/ha, if 50% of the produced bagasse would be converted to ethanol. This article describes the efforts of different Brazilian institutions and research groups on second generation bioethanol production, especially from sugarcane bagasse.

Keywords: Second generation bioethanol; Brazilian bioethanol program; Sugarcane; Bagasse; Hydrolysis

Rajeev K. Sukumaran^a, Vikram Joshua Surender^a, Raveendran Sindhu^a, Parameshwaran Binod^a, Kanakambaran Usha Janu^a, Kuttavan Valappil Sajna^a, Kuni Parambil Rajasree^a and Ashok Pandey^a. (^a Centre for Biofuels, Biotechnology Division, National Institute for Interdisciplinary Science and Technology (CSIR), Trivandrum 695019, India). **Lignocellulosic ethanol in India: Prospects, challenges and feedstock availability. *Bioresource Technology*, Volume 101(13) (2010): 4826-4833**

India has a pressing need for renewable transportation fuels and bio-ethanol is considered as one of the most important options. Currently the country mandates use of 5% ethanol blending in motor gasoline in several states. The ethanol for this is mainly sourced from molasses feedstock, but this is barely sufficient to meet the current demand. Lignocellulosic biomass is the alternative but the availability of this resource is poorly documented. Also the technologies for ethanol production from lignocellulosic biomass are under preliminary stages of development which warrants extensive R&D in this field. The review discusses the current status of molasses based ethanol production in India and its limitations, the state of technologies for second generation ethanol production and the availability of feedstock for bio-ethanol production.

Keywords: Bio-ethanol; Biomass; Lignocellulosic feedstock; Agro-residues; Cellulase

Chun Sheng Goh^a, Kok Tat Tan^a, Keat Teong Lee^a and Subhash Bhatia^a. (^a School of Chemical Engineering, Universiti Sains Malaysia, Engineering Campus, Seri Ampangan, 14300 Nibong Tebal, Pulau Pinang, Malaysia). Bio-ethanol from lignocellulose: Status, perspectives and challenges in Malaysia. *Bioresource Technology*, Volume 101(13) (2010): 4834-484

The present study reveals the perspective and challenges of bio-ethanol production from lignocellulosic materials in Malaysia. Malaysia has a large quantity of lignocellulosic biomass from agriculture waste, forest residues and municipal solid waste. In this work, the current status in Malaysia was laconically elucidated, including an estimation of biomass availability with a total amount of 47,402 dry kton/year. Total capacity and domestic demand of second-generation bio-ethanol production in Malaysia were computed to be 26,161 ton/day and 6677 ton/day, respectively. Hence, it was proven that the country's energy demand can be fulfilled with bio-ethanol if lignocellulosic biomass were fully converted into bio-ethanol and 19% of the total CO₂ emissions in Malaysia could be avoided. Apart from that, an integrated national supply network was proposed together with the collection, storage and transportation of raw materials and products. Finally, challenges and obstacles in legal context and policies implementation were elaborated, as well as infrastructures shortage and technology availabilities.

Keywords: Second-generation bio-ethanol; Lignocellulose; Biomass

Edgard Gnansounou^a. (^a Swiss Federal Institute of Technology, Bioenergy and Energy Planning Research Group, EPFL ENAC INTER GR-GN, 1015 Lausanne, Switzerland). Production and use of lignocellulosic bioethanol in Europe: Current situation and perspectives. *Bioresource Technology*, Volume 101(13) (2010): 4842-4850

Contrary to the case of the United States where a systematic management of the RD&D on lignocellulosic ethanol prevails, in Europe the research works remain fragmented despite the efforts made by the European Union and in few member states. In most of the European countries, sustainable lignocellulosic resources may not be widely available in the future for bioethanol production due to the possible competition between several potential usages. Thus the actual deployment of the lignocellulosic bioethanol in Europe will depend on the opportunity costs of biomass on one side and on the prices of ethanol and gasoline on the other side. While the papers on lignocellulosic ethanol often emphasize technology progress, this review paper also addresses policy measures. It is found that, especially in Europe where security of oil supply will be lower in long term, the policy instruments should explicitly reward the higher value of lignocellulosic ethanol compared to first the generation ethanol and gasoline.

Keywords: Second generation bioethanol; Sustainability; Energy substitution; Renewable energy

P. Alvira^a, E. Tomás-Pejó^a, M. Ballesteros^a and M.J. Negro^a. (^a CIEMAT, Renewable Energy Division, Biomass Unit, Avda. Complutense 22, Madrid 28040, Spain). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology*, Volume 101(13) (2010): 4851-4861

Biofuel produced from lignocellulosic materials, so-called second generation bioethanol shows energetic, economic and environmental advantages in comparison to bioethanol from starch or sugar. However, physical and chemical barriers caused by the close association of the main components of lignocellulosic biomass, hinder the hydrolysis of cellulose and hemicellulose to fermentable sugars. The main goal of pretreatment is to increase the enzyme accessibility improving digestibility of cellulose. Each pretreatment has a specific effect on the cellulose, hemicellulose and lignin fraction thus, different pretreatment methods and conditions should be chosen according to the process configuration selected for the subsequent hydrolysis and fermentation steps. This paper reviews the most interesting technologies for ethanol production from lignocellulose and it points out several key properties that should be targeted for low-cost and advanced pretreatment processes.

Keywords: Pretreatment; Bioethanol; Enzymatic hydrolysis

Matjaz Oslaj^a, Bogomir Mursec^a and Peter Vindis^a. (^a University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, Maribor, Slovenia). *Biogas production from maize hybrids. Biomass and Bioenergy, Volume 34(11): 2010: 1538-1545*

There is an increasing world wide demand for energy crops and animal manures for biogas production. This research project was aimed at optimising anaerobic digestion of maize, using a laboratory digester, and finding out which maturity class of corn and which hybrid of a particular maturity class produces the highest rate of biogas and biomethane. Also the chemical composition of gases was studied. The cornhybrids of FAO 300 – FAO 400, FAO 400 – FAO 500 and FAO 500 – FAO 600 maturity class were tested. Experiments took place in the lab for 35 days within four series of experiments with four repetitions according to the method DIN 38 414. Results show that the highest maturity class of corn (FAO 400, FAO 500) increases the amount of biomethane. The greatest gain of biomethane per hectare according to maturity class is found with hybrids of FAO 400 (7768.4 Nm³ ha⁻¹) and FAO 500 (7050.1 Nm³ ha⁻¹) maturity class. Among the corn hybrids of maturity class FAO 300 – FAO 400, the hybrid PR38F70 gives the greatest production of biomethane per hectare (7646.2 Nm³ ha⁻¹). Among the hybrids of maturity class FAO 400 – FAO 500, the greatest amount of biomethane was achieved by the hybrid PIXXIA (9440.6 Nm³ ha⁻¹). Among the hybrids of maturity class FAO 500 – FAO 600 the hybrid CODISTAR (FAO 500) gives the highest production of biomethane (8562.7 Nm³ ha⁻¹). Production of biomethane varied with corn hybrids from 50 to 60% of produced biogas.

Keywords: Biogas; Biomethane; *Hybrid*; Maize

Daniel G. De La Torre Ugarte^a, Lixia He^{1, a}, Kimberly L. Jensen^{2, a} and Burton C. English^{3, a}. (^a Department of Agricultural & Resource Economics, University of Tennessee, 302 Morgan Hall, Knoxville, TN 37996-4518, USA). *Expanded ethanol production: Implications for agriculture, water demand, and water quality. Biomass and Bioenergy, Volume 34(11) (2010): 1586-1596*

Feedstock production for large scale development of the U.S. ethanol industry and introduction of cellulose-to-ethanol technology will require extensive changes in land use and field management. Hence, this production will likely have significant impact on water demand and quality. This study compares two ‘what if’ scenarios for attaining a 227.1 hm³ of ethanol by 2030 and 3.8 hm³ of biodiesel by 2012. In the first scenario cellulose-to-ethanol technology is

introduced in 2012, while in the second scenario the technology is delayed until 2015. Results show that the timing of introduction of cellulose-to-ethanol technology will affect the water use and water quality related input use in primarily in the eastern part of the nation. Results also suggest policy emphasis on reduced and no-till practices needs to be complementary to increased crop residue use.

Keywords: Cellulose-to-Ethanol; Water resources; *Panicum virgatum*; Reduced tillage; Crop residue use

Nathan Parker^a, Peter Tittmann^b, Quinn Hart^c, Richard Nelson^d, Ken Skog^e, Anneliese Schmidt^f, Edward Gray^f and Bryan Jenkins^g. (^a Institute of Transportation Studies, University of California at Davis, One Shields Avenue, Davis, CA 95616, USA, ^b Department of Geography, University of California at Davis, One Shields Avenue, Davis, CA 95616, USA, ^c Department of Land, Air and Water Resources, University of California at Davis, One Shields Avenue, Davis, CA 95616, USA, ^d Engineering Extension, KSU College of Engineering, 133 Ward Hall, Kansas State University, Manhattan, KS 66506-2508, USA, ^e Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53726, USA, ^f The Antares Group Inc., 4351 Garden City Drive, Suite 301, Landover, MD 20785, USA, ^g Department of Biological and Agricultural Engineering, University of California at Davis, One Shields Avenue, Davis, CA 95616, USA). **Development of a biorefinery optimized biofuel supply curve for the Western United States. Biomass and Bioenergy, Volume 34(11) (2010): 1597-1607**

A resource assessment and biorefinery siting optimization model was developed and implemented to assess potential biofuel supply across the Western United States from agricultural, forest, urban, and energy crop biomass. Spatial information including feedstock resources, existing and potential refinery locations and a transportation network model is provided to a mixed integer-linear optimization model that determines the optimal locations, technology types and sizes of biorefineries to satisfy a maximum profit objective function applied across the biofuel supply and demand chain from site of feedstock production to the product fuel terminal. The resource basis includes preliminary considerations of crop and residue sustainability. Sensitivity analyses explore possible effects of policy and technology changes. At a target market price of 19.6 \$ GJ⁻¹, the model predicts a feasible production level of 610–1098 PJ, enough to supply up to 15% of current regional liquid transportation fuel demand.

Keywords: Biofuel; Supply assessment; Resource; Supply chain; GIS; Optimization

H. Yamada^{a,b}, R. Tanaka^b, O. Sulaiman^c, R. Hashim^c, Z.A.A. Hamid^c, M.K.A. Yahya^c, A. Kosugi^d, T. Arai^d, Y. Murata^d, S. Nirasawa^d, K. Yamamoto^b, S. Ohara^{a,b}, Mohd Nor Mohd Yusof^e, Wan Asma Ibrahim^e and Y. Mori^{a,d}. (^a Department of Global Agricultural Sciences, University of Tokyo, 1-1-1, Yayoi, Bunkyo 113-8657, Japan, ^b Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan, ^c School of Industrial Technology, Universiti Sains Malaysia, 11800, Penang, Malaysia, ^d Japan International Research Center for Agricultural Sciences, 1-1, Owashi, Tsukuba, Ibaraki 305-8686, Japan, ^e Forest Research Institute Malaysia (FRIM), Kepong, 52109 Selangor, Malaysia). **Old oil palm trunk: A promising source of sugars for bioethanol production. Biomass and Bioenergy, Volume 34(11) (2010): 1608-1613**

Oil palm trees are replanted at an interval of approximately 25 years because of decreased oil productivity of old trees. Consequently the felled trunks are the enormous amount of biomass resources in the palm oil producing countries such as Malaysia and Indonesia. In this report, we found that the felled oil palm trunk contains large quantity of sap, which accounts for approximately 70% of the whole trunk weight, and that sugars existing in the sap increased remarkably during storage after logging. Total sugar in the sap increased from 83 mg ml⁻¹ to 153 mg ml⁻¹, the concentration comparable to that of sugar cane juice, after 30 days of storage, followed by the gradual decrease. The sugars contained in the sap were glucose, sucrose, fructose and galactose, all of which are fermentable by ordinary industrial yeast strains. The results indicate that old oil palm trunk becomes a promising source of sugars by proper aging after logging and, thus, its sap can be a good feedstock for bioethanol.

Keywords: *Elaeis guineensis*; Trunk; Sap; Sugar; Ethanol

John Ruane^a, Andrea Sonnino^b and Astrid Agostini^c. (^a FAO Working Group on Biotechnology, UN Food and Agriculture Organization (FAO), Viale delle Terme di Caracalla, 00153 Rome, Italy, ^b FAO Office of Knowledge Exchange, Research and Extension, UN Food and Agriculture Organization (FAO), Viale delle Terme di Caracalla, 00153 Rome, Italy, ^c FAO Investment Centre, UN Food and Agriculture Organization (FAO), Viale delle Terme di Caracalla, 00153 Rome, Italy). **Bioenergy and the potential contribution of agricultural biotechnologies in developing countries. Biomass and Bioenergy, Volume 34(10) (2010): 1427-1439**

We provide an overview of the current status of bioenergy development, focusing on first- and second-generation liquid biofuels, considering drivers of growth and risks that have raised concerns over recent years. We also describe the main areas where biotechnologies are being, or can be, applied for production of first- and second-generation biofuels as well as microalgal biodiesel and biogas. Greatest attention is paid to second-generation biofuels in the review because of the large expectations they have created and because of the significant role that biotechnology applications are likely to play in their development. We close with some specific considerations regarding applying biotechnologies for bioenergy development in developing countries.

Keywords: Bioenergy; Biotechnology; Developing countries

Abbreviations: CBP, Consolidated bioprocessing; EU, European Union; FTL, Fischer-Tropsch liquid; GHGs, Greenhouse gases; GM, Genetically modified; GMOs, Genetically modified organisms; IPR, Intellectual property rights; LC, lignocellulosic; OECD, Organisation for Economic Cooperation and Development; R&D, Research and development; TAGs, Triacylglycerols

Svetlana Nikolić^a, Ljiljana Mojović^a, Dušanka Pejin^b, Marica Rakin^a and Maja Vukašinić^a. (^a Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia, ^b Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia). **Production of bioethanol from corn meal hydrolyzates by free and immobilized cells of *Saccharomyces cerevisiae* var. *Ellipsoideus*. Biomass and Bioenergy, Volume 34(10) (2010): 1449-1456**

The ethanol fermentation of enzymatically obtained corn meal hydrolyzates by free and immobilized *Saccharomyces cerevisiae* var. *ellipsoideus* yeast in a batch system was studied. The initial glucose and inoculum concentration and the time required for the efficient ethanol production were optimized taking into account parameters such as ethanol concentration, ethanol yield and volumetric productivity. The yeast cells were immobilized in Ca-alginate by electrostatic droplet generation method. An optimal initial inoculum concentration of 2% (v v⁻¹) and optimal fermentation time of 38 h for both immobilized and free yeasts were determined. Optimal initial glucose concentrations of 150 and 176 g l⁻¹ for free and immobilized system were achieved, respectively. The immobilized cell system was superior to the free cell system since higher ethanol tolerance and productivity and lower substrate inhibition were detected.

Keywords: Corn ethanol; *Zea mays*; *Saccharomyces cerevisiae* var. *ellipsoideus*; Immobilization; Ca-alginate

Jhosianna P.V. da Silva^a, Tatiana M. Serra^a, Marcelo Gossmann^b, Carlos R. Wolf^b, Mario R. Meneghetti^b and Simoni M.P. Meneghetti^a. (^a Universidade Federal de Alagoas, Instituto de Química e Biotecnologia, Laboratório de Oleoquímica, Maceió, Alagoas, CEP 57072-970, Brazil, ^b Universidade Luterana do Brasil, Instituto de Química, Canoas, Rio Grande do Sul, CEP 92420-280, Brazil). *Moringa oleifera* oil: Studies of characterization and biodiesel production. *Biomass and Bioenergy*, Volume 34(10) (2010): 1527-1530

This work describes studies with the seeds of *Moringa oleifera* (MO), obtained in the northeast of Brazil, evaluating some properties and chemical composition of the oil, as well any potential application in biodiesel production. The studied physicochemical properties of the MO biodiesel, suggest that this material may be used as fuel in diesel engines, mainly as a mixture to petrodiesel.

Keywords: Biodiesel; Transesterification; *Moringa oleifera*; Methanolysis

Shanta Satyanarayan^a, Ramakant^b and Shivayogi^b. (^a Formerly Deputy Director, WWT Division, NEERI, Nagpur 400 020, India, ^b Formerly Project Fellow, WWT Division, NEERI, Nagpur 400 020, India). Biogas production enhancement by soya sludge amendment in cattle dung digesters. *Biomass and Bioenergy*, Volume 34(9) (2010): 1278-1282

Biogas energy production from cattle dung is an economically feasible and eco-friendly in nature. But dependence only on cattle dung is a limiting factor. Rich nitrogen containing substrate addition to extra carbohydrate digester like cattle dung could improve the biogas production. Detailed performance of the digesters at different ratios of cattle dung and soya sludge has been discussed in this paper considering the cold countries climate. Soya sludge substrate not only has high nitrogen content of 4.0–4.8% but it also has high percentage of volatile solids content in the range of 97.8–98.8%. Soya sludge addition also improved the manurial value of the digested slurry and also improved the dewater-ability of the sludge. Results indicated an increment of 27.0% gas production at 25.0% amendment of soya sludge in non-homogenized cattle dung (NCD) digester. The amount of gas production increased to 46.4% in case of homogenized cattle dung (HCD) with respect to NCD feed at the same amendment.

Keywords: Cattle dung; Amendment; Soya sludge; Anaerobic; Biogas

Lijing Gao^a, Guangyuan Teng^a, Guomin Xiao^a and Ruiping Wei^a. (^a School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 211189, P.R. China). **Biodiesel from palm oil via loading KF/Ca–Al hydrotalcite catalyst. Biomass and Bioenergy, Volume 34(9) (2010): 1283-1288**

The solid base catalyst KF/Ca–Al hydrotalcite was obtained from Ca–Al layered double hydroxides and successfully used in the transesterification of methanol with palm oil to produce biodiesel. With the load of KF, the activity of Ca–Al mixed-oxides had been improved much. For the mass ratio 80 wt.%(KF·6H₂O to Ca–Al mixed-oxides) catalyst, under the optimal condition: 338 K, catalyst amount 5%(wt./wt. oil) and methanol/oil molar ratio 12:1, after 5 h reaction, the fatty acid methyl esters yield could reach 97.98%; for the mass ratio 100 wt.%(KF·6H₂O to Ca–Al mixed-oxides) ones, under the same reaction condition, only needed 3 h to get the FAME yield of 99.74%, and even only reacted 1 h, the FAME yield could obtain 97.14%.

Keywords: Biodiesel; Transesterification; Solid base; *Elaeis guineensis*

Suresh Chauhan^a. (^a The Energy and Resources Institute, Darbari Seth Block, India Habitat Centre, Lodhi Road, New Delhi-110003, India). **Biomass resources assessment for power generation: A case study from Haryana state, India. Biomass and Bioenergy, Volume 34(9) (2010): 1300-1308**

India generates over 370 million tonnes of biomass every year. In addition to the direct harvesting from plants, biomass is also produced as a by product in many agro based industries such as rice husk from rice mill, saw dust from saw mill, bagasse from sugar mills etc. It has been estimated that about 17 GW of power can be generated through cogeneration, combustion and gasification routes from the available biomass. However, for this potential to be realized, data on production, present usage patterns, prices and seasonal fluctuation on biomass is essentially required. The present article is based on the resource assessment of non-plantation surplus biomass with a view to using it for energy production and its utilization in the state of Haryana, India.

Being an agricultural state, Haryana has a huge potential of biomass availability in the form of crop residue and saw dust. In the agricultural sector, a total 24.697 Mt y⁻¹ of residue is generated, of which 71% is consumed in various domestic and commercial activities within the state. While in agro based industrial sector, a total of 646 kt y⁻¹ of sawdust is generated, of which only 6.65% is consumed in the state. Of the total generated biomass in the state, 45.51% is calculated as basic surplus, 37.48% as productive surplus and 34.10% as net surplus. The power generation potential from all these three categories of surplus biomass is 1.499 GW, 1.227 GW and 1.120 GW respectively.

Keywords: Biomass; Surplus; MNERS; HAREDA; Taluka

Berna Kavacik^a and Bahattin Topaloglu^a. (^a Department of Environmental Engineering, Ondokuz Mayıs University, Kurupelit, 55139 Samsun, Turkey). **Biogas production from co-digestion of a mixture of cheese whey and dairy manure. Biomass and Bioenergy, Volume 34(9) (2010): 1321-1329**

In this study, daily amount of biogas of different mixtures of cheese whey and dairy manure, rates of production of methane, removal efficiencies of chemical oxygen demand (COD), total solid (TS) matter and volatile solid (VS) matter from the mixtures were investigated at 25 and 34 °C. In the experimental studies, two different solid matter rates (8% and 10%) were studied. The hydraulic retention times (HRTs) were 5, 10 and 20 days. Removal efficiencies and amount of biogas produced in each HRT were determined. Maximum daily biogas production was obtained as $1.510 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ at HRT of 5 days in the mixture containing 8% total solid matters at 34 °C and the methane production rate was around $60 \pm 1\%$ in all experiments. Maximum removal efficiencies for TS, VS and COD were found as 49.5%, 49.4% and 54%, respectively at HRT of 10 days in the mixture containing 8% total solid matters at 34 °C.

Keywords: Cheese whey; Co-digestion; Dairy manure; Biogas; Acidifying

S. Robra^a, R. Serpa da Cruz^b, A.M. de Oliveira^b, J. A. Almeida Neto^a and J.V. Santos^b. (^a Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, Rod. Ilhéus/Itabuna km 16 s/n, CEP 45662-000 Ilhéus, Bahia, Brazil, ^b Departamento de Ciências Exatas e Tecnológicas, Universidade Estadual de Santa Cruz, Rod. Ilhéus/Itabuna km 16 s/n, CEP 45662-000 Ilhéus, Bahia, Brazil). **Generation of biogas using crude glycerin from biodiesel production as a supplement to cattle slurry. Biomass and Bioenergy, Volume 34(9) (2010): 1330-1335**

The influence of crude glycerin on biogas production and methane content of the produced biogas was studied, when added to cattle slurry. The experimental design consisted of 5% wt (Gli 5), 10% wt (Gli 10), and 15% wt (Gli 15) of crude glycerin added to cattle slurry, and one control digester without addition of crude glycerin. Anaerobic digestion was carried out in 4 laboratory size CSTR-type biogas digesters with a working volume of 3 L, in semi-continuous regime at mesophilic conditions, over a period of 10 weeks. The highest biogas yields (825.3 mL g^{-1} and 825.7 mL g^{-1} , respectively) relative to mass of volatile compounds added, were produced by the treatments Gli 5 and Gli 10. The control treatment produced 268.6 mL g^{-1} , whereas the treatment Gli 15 produced 387.9 mL g^{-1} . This low value was due to the breakdown of the process. Compared to the control, methane contents was increased by 9.5%, 14.3%, and 14.6%, respectively, for the treatments Gli 5, Gli 10, and Gli 15.

Keywords: Co-digestion; Biodigestion; Methane; Biofuel

Ingeborg Callesen^a, Poul Erik Grohnheit^b and Hanne Østergård^a. (^a Biosystems Division, Risø National Laboratory for Sustainable Energy, Technical University of Denmark – DTU, Building 301, P.O. Box 49, Frederiksborgvej 399, DK-4000 Roskilde, Denmark, ^b Systems Analysis Division, Risø National Laboratory for Sustainable Energy, Technical University of Denmark – DTU, Building 110, P.O. Box 49, Frederiksborgvej 399, DK-4000 Roskilde, Denmark). **Optimization of bioenergy yield from cultivated land in Denmark. Biomass and Bioenergy, Volume 34(9) (2010): 1348-1362**

A cost minimization model for supply of starch, oil, sugar, grassy and woody biomass for bioenergy in Denmark was developed using linear programming. The model includes biomass supply from annual crops on arable land, short rotation forestry (willow) and plantation forestry. Crop area distributions were simulated using cost data for year 2005. Five scenarios with

different constraints, e.g. on food and feed supply and on nitrogen balance were considered focusing on: a) constraints as the year 2005, b) landscape aesthetics and biodiversity c) groundwater protection, d) maintaining current food and feed production, or e) on site carbon sequestration. In addition, two oil price levels were considered. The crop area distributions differed between scenarios and were affected by changing fossil oil prices up to index 300 (using 55\$ per barrel in 2005 as index = 100). The bioenergy supply (district heating, electric power, biogas, RME or bioethanol) varied between 56 PJ in the “2005” scenario at oil index 100 and 158 PJ at oil index 300 in the groundwater scenario. Our simple model demonstrates the effect of prioritizing multiple uses of land resources for food, feed or bioenergy, while maintaining a low nitrogen load to the environment. In conclusion, even after drastic landuse changes the bioenergy supply as final energy will not exceed 184 PJ annually (including 26 PJ processed biowaste sources) by far lower than the annual domestic total energy consumption ranging between 800 and 850 PJ yr⁻¹.

Keywords: Bioenergy supply; Biomass feedstock; Cost minimization model; Landuse; Linear programming; Nitrogen load

Malle Mandre^a, Henn Pärn^a, Jaan Klõšeiko^a, Morten Ingerslev^b, Inge Stupak^b, Margus Kõrt^c and Keddy Paasrand^c. (^a Department of Ecophysiology, Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Viljandi mnt. 18B, 11216 Tallinn, Estonia, ^b Forest & Landscape Denmark, University of Copenhagen, Hoersholm, Kongevej 11, DK-2970 Hoersholm, Denmark, ^c Estonian Environmental Research Centre, Marja 4D, 10617 Tallinn, Estonia). Use of biofuel ashes for fertilisation of *Betula pendula* seedlings on nutrient-poor peat soil. *Biomass and Bioenergy*, Volume 34(9) (2010):1384-1392

Short-term effects of different doses (0.25; 0.5 and 1.0 kg m⁻²) of wood ash (WA), peat ash (PA) and their mixture (MA) applied to peat substrate on the mineral composition and growth of seedlings of *Betula pendula* were investigated. The experiments were conducted with 1-year-old seedlings planted in vegetation pots. The pH of the substrate was increased by 0.4–0.9 units during the vegetation period compared to the control. The peat substrate was poor in nutrients, except N. The substrate treated with WA had higher concentrations of K, Mg, Mn, Fe, P, Zn, Cr and Pb, but a lower N concentration compared to the control. The substrate treated with PA had higher concentrations of Ca, Mg, N and P. The concentrations in the MA treatment were intermediate between WA and PA. The ashes increased K and lowered the concentration of Ca. A decrease in N in seedlings was found under the influence of WA and MA. An increase in K and P was found in all compartments of seedlings, while the concentrations of Ca, Mg, Cu, Zn, Cd and Cr in seedlings were affected irregularly depending on types and doses of ashes used. The uptake of Cd, Cr and Pb did not reach phytotoxic levels; however, increased concentrations of Cd and Pb were found in roots. A positive influence of ash application on growth was found. The heights and root collar diameters of all ash-fertilised treatments exceeded those of the control seedlings in most cases.

Keywords: Biofuel ashes; Peat soil; *Betula pendula*; Mineral element; Height growth

Mirosław Mleczek^a, Paweł Rutkowski^b, Iwona Rissmann^a, Zygmunt Kaczmarek^c, Piotr Golinski^a, Kinga Szentner^a, Katarzyna Strażyńska^b and Agnieszka Stachowiak^b. (^a University of Life Sciences in Poznan, Department of Chemistry, Wojska Polskiego 75, 60-625 Poznan, Poland, ^b University of Life Sciences in Poznan, Department of Silviculture, Wojska Polskiego 69, 60-625 Poznan, Poland, ^c Institute of Plant Genetics, Polish Academy

of Sciences, Strzeszynska 34, 60-479 Poznan, Poland). **Biomass productivity and phytoremediation potential of *Salix alba* and *Salix viminalis*. Biomass and Bioenergy, Volume 34(9) (2010): 1410-1418**

The aim of this work was to determine selected *Salix* clones' capacities for biomass production and accumulation of heavy metal ions. Determination of the relationship between sorption of metals and biomass productivity was a further purpose of this study. Eight *Salix viminalis* cultivars and one *Salix alba* cultivar were analyzed. The taxa characterized by greatest biomass production were *S. alba* var. Chermesina and *S. viminalis* '1056' (respectively 6.8 and 4.3 kg of fresh mass per shrub per year).

The results have revealed significant differences among clones. The clones most effective in accumulating all five metals were *S. viminalis* '1154' and '1054'. The studied *Salix* population was significantly diverse as regards accumulation efficiency. The differences between the highest and lowest heavy metal content in extreme clones were for: Cd 84%, Cu 90%, Hg 167%, Pb 190% and Zn 36%. At the same time, significant differences were observed in *Salix* structure. The greatest cellulose content was observed in *S. viminalis* 'Sprint' (49.69%) and the lowest in *S. viminalis* '1059' (42.09%).

Keywords: Accumulation; Biomass; Heavy metals; *Salix* clone; Soil

Abbreviations: BAF, bioaccumulation factor; Hyperaccumulator plant, metallophyte that accumulates an exceptionally high level of a metal to a specified concentration or to a specified multiple of the concentration found in non-accumulators; PAH, polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyl; Phytoremediation, use of plants to accumulate, remove or render harmless toxic compounds contaminating the environment; TPH, total petroleum hydrocarbon

Dave Timmons^a and César Viteri Mejía^a. (^a Department of Resource Economics, University of Massachusetts, 80 Campus Center Way, Amherst, MA 01003-9246, USA). Biomass energy from wood chips: Diesel fuel dependence?. Biomass and Bioenergy, Volume 34(9) (2010): 1419-1425

Most renewable energy sources depend to some extent on use of other, non-renewable sources. In this study we explore use of diesel fuel in producing and transporting woody biomass in the state of New Hampshire, USA. We use two methods to estimate the diesel fuel used in woody biomass production: 1) a calculation based on case studies of diesel consumption in different parts of the wood chip supply chain, and 2) to support extrapolating those results to a regional system, an econometric study of the variation of wood-chip prices with respect to diesel fuel prices. The econometric study relies on an assumption of fixed demand, then assesses variables impacting supply, with a focus on how the price of diesel fuel affects price of biomass supplied. The two methods yield similar results. The econometric study, representing overall regional practices, suggests that a \$1.00 per liter increase in diesel fuel price is associated with a \$5.59 per Mg increase in the price of wood chips. On an energy basis, the diesel fuel used directly in wood chip production and transportation appears to account for less than 2% of the potential energy in the wood chips. Thus, the dependence of woody biomass energy production on diesel fuel does not appear to be extreme.

Keywords: Woody biomass; Wood chips; Biomass transportation; Net energy ratio; Biomass electricity

Sukumar Puhan^a, N. Saravanan^b, G. Nagarajan^c and N. Vedaraman^d. (^a Department of Mechanical Engineering, Veltech Engineering college, Avadi, Chennai, India, ^b ERC Engines, Tata Motors, Pimpri, Pune, India, ^c Department of Mechanical Engineering, Anna University, Chennai, India, ^d Chemical Engineering Division, Central Leather Research Institute, Adyar, Chennai, India, Received 20 January 2008). **Effect of biodiesel unsaturated fatty acid on combustion characteristics of a DI compression ignition engine. Biomass and Bioenergy, Volume 34(8) (2010): 1079-1088**

Several research works have been carried out on biodiesel combustion, performance and emissions till today. But very few studies have been made about the chemistry of biodiesel that affects the diesel engine operation. Biodiesel is derived from vegetable oil or animal fats, which comprises of several fatty acids with different chain length and bonding. The present work focuses on the effect of biodiesel molecular weight, structure (Cis & Trans), and the number of double bonds on the diesel engine operation characteristics. Three types of biodiesel with different molecular weight and number of double bond were selected for the experimental studies. The biodiesels were prepared and analyzed for fuel properties according to the standards. A constant speed diesel engine, which develops 4.4 kW of power, was run with biodiesels and its performance was compared with diesel fuel. The results show that Linseed oil methyl ester with high linolenic (unsaturated fatty acid ester) does not suit best for diesel engine due to high oxides of nitrogen emission and low thermal efficiency.

Keywords: *Linum usitatissimum*; *Cocos nucifera*; *Jatropha integerrima* Compacta; Combustion; Performance; Emission

Prakash C. Jena^a, Hifjur Raheman^a, G.V. Prasanna Kumar^a and Rajendra Machavaram^a. (^a Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur 721302, India). **Biodiesel production from mixture of mahua and simarouba oils with high free fatty acids. Biomass and Bioenergy, Volume 34(8) (2010): 1108-1116**

A suitable process comprising acid pretreatment followed by main base transesterification reaction was developed to produce biodiesel from mixture of Mahua (M) and Simarouba (S) oils with high free fatty acids (FFA). The acid pretreatment reduced the high FFAs of the mixture of oils to around 1% which were then transesterified with methanol and KOH as catalyst at a reaction temperature of 60 °C. A genetic algorithm coupled with artificial neural network (ANN-GA) model to obtain the best pretreatment process parameters for bringing down the FFA level of individual vegetable oils to around 1% was modified to include the wide range of oils and validated for mixtures of M and S oils. The quality of biodiesel produced was analyzed by gas chromatography (GC), which indicated above 90% ester conversion. The fuel properties of biodiesel were found to be comparable to diesel and were conforming to the latest biodiesel standards.

Keywords: Mixture of oils; *Madhuca indica*; *Simarouba glauca*; ANN-GA; Biodiesel; Production; Fuel properties

Gi-Wook Choi^{a,1}, Hyun-Ju Um^{b,1}, Hyun-Woo Kang^a, Yule Kim^a, Mina Kim^b and Yang-Hoon Kim^b. (^a Changhae Institute of Cassava and Ethanol Research,

Changhae Ethanol Co., LTD, Palbok-Dong 829, Dukjin-Gu, Jeonju 561-203, South Korea, ^bDepartment of Microbiology, Chungbuk National University, 410 Sungbong-Ro, Heungduk-Gu, Cheongju 361-763, South Korea). Bioethanol production by a flocculent hybrid, CHFY0321 obtained by protoplast fusion between *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. Biomass and Bioenergy, Volume 34(8) (2010): 1232-1242

Fusion hybrid yeast, CHFY0321, was obtained by protoplast fusion between non-flocculent-high ethanolfermentative *Saccharomyces cerevisiae* CHY1011 and flocculent-low ethanol fermentative *Saccharomyces bayanus* KCCM12633. The hybrid yeast was used together with the parental strains to examine ethanol production in batch fermentation. Under the conditions tested, the fusion hybrid CHFY0321 flocculated to the highest degree and had the capacity to ferment well at pH 4.5 and 32 °C. Simultaneous saccharification and fermentation for ethanol production was carried out using a cassava (*Manihot esculenta*) powder hydrolysate medium containing 19.5% (w v⁻¹) total sugar in a 5 l lab scale jar fermenter at 32 °C for 65 h with an agitation speed of 2 Hz. Under these conditions, CHFY0321 showed the highest flocculating ability and the best fermentation efficiency for ethanol production compared with those of the wild-type parent strains. CHFY0321 gave a final ethanol concentration of $89.8 \pm 0.13 \text{ g l}^{-1}$, a volumetric ethanol productivity of $1.38 \pm 0.13 \text{ g l}^{-1} \text{ h}^{-1}$, and a theoretical yield of $94.2 \pm 1.58\%$. These results suggest that CHFY0321 exhibited the fermentation characteristics of *S. cerevisiae* CHY1011 and the flocculent ability of *S. bayanus* KCCM12633. Therefore, the strong highly flocculent ethanolfermentative CHFY0321 has potential for improving biotechnological ethanol fermentation processes.

Keywords: Ethanol production; *Saccharomyces* hybrids; Protoplast fusion; Flocculation; Cassava (*Manihot esculenta*)

A. Dalla Marta^a, M. Mancini^a, R. Ferrise^a, M. Bindi^a and S. Orlandini^a. (^a Department of Plant, Soil and Environmental Science, University of Florence, Piazzale delle Cascine 18 - 50144 Firenze, Italy). Energy crops for biofuel production: Analysis of the potential in Tuscany. Biomass and Bioenergy, Volume 34(7) (2010): 1041-1052

The possibility of using biomass as a source of energy in reducing green-house gas emissions is a matter of great interest. In particular, biomass from agriculture represent one of the largest and most diversified sources to be exploited and more specifically, ethanol and diesel deriving from biomass have the potential to be a sustainable means of replacing fossil fuels for transportation. Nevertheless, the cultivation of dedicated energy crops does meet with some criticism (competitiveness with food crop cultivation, water requirements, use of fertilizers, etc.) and the economical and environmental advantages of this activity depend on accurate evaluations of the total efficiency of the production system. This paper illustrates the production potential of two energy crops, sunflower (*Helianthus annuus*) and maize (*Zeamais*), cultivated with different water and fertilization supplies in the region of Tuscany, in central Italy. A 50-year climatic series of 19 weather stations scattered around Tuscany was used to run the crop model CropSyst for obtaining crop biomass predictions. The effect of climate change and variability was analyzed and the potential production of bioenergy was investigated in terms of pure vegetable oil (sunflower) and bioethanol (maize). The results demonstrated that despite a

reduction in crop yields and an increase of their variability due to climate change, the cultivation of maize in the regional set-aside areas would be capable of supplying approximately 50% of the energy requirements in terms of biofuel for transportation obtained, while the cultivation of a sunflower crops would supply less than 10%.

Keywords: Bioenergy; Agroclimatology; Modelling; Pure vegetable oil; Bioethanol

Min Sun^a, Zhong-Hua Tong^a, Guo-Ping Sheng^a, Yong-Zhen Chen^a, Feng Zhang^a, Zhe-Xuan Mu^b, Hua-Lin Wang^b, Raymond J. Zeng^a, Xian-Wei Liu^a, Han-Qing Yu^a, Li Wei^c and Fang Ma^c. (^a Department of Chemistry, University of Science & Technology of China, 96 Jinzhai Road, Hefei 230026, China, ^b School of Chemical Engineering, Hefei University of Technology, Hefei 230092, China, ^c State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China). **Microbial communities involved in electricity generation from sulfide oxidation in a microbial fuel cell. Biosensors and Bioelectronics, Volume 26(2) (2010): 470-476**

Simultaneous electricity generation and sulfide removal can be achieved in a microbial fuel cell (MFC). In electricity harvesting from sulfide oxidation in such an MFC, various microbial communities are involved. It is essential to elucidate the microbial communities and their roles in the sulfide conversion and electricity generation. In this work, an MFC was constructed to enrich a microbial consortium, which could harvest electricity from sulfide oxidation. Electrochemical analysis demonstrated that microbial catalysis was involved in electricity output in the sulfide-fed MFC. The anode-attached and planktonic communities could perform catalysis independently, and synergistic interactions occurred when the two communities worked together. A 16S rRNA clone library analysis was employed to characterize the microbial communities in the MFC. The anode-attached and planktonic communities shared similar richness and diversity, while the LIBSHUFF analysis revealed that the two community structures were significantly different. The exoelectrogenic, sulfur-oxidizing and sulfate-reducing bacteria were found in the MFC anodic chamber. The discovery of these bacteria was consistent with the community characteristics for electricity generation from sulfide oxidation. The exoelectrogenic bacteria were found both on the anode and in the solution. The sulfur-oxidizing bacteria were present in greater abundance on the anode than in the solution, while the sulfate-reducing bacteria preferably lived in the solution.

Keywords: Biocatalysis; Microbial community diversity; Microbial fuel cells; Sulfate-reducing bacteria; Sulfide oxidation; Sulfur-oxidizing bacteria

Hossain A. B. M. S., Nasrulhaq Boyce A., Salleh A. and Chandran S. (Programme of Biotechnology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia. *Corresponding author. E-mail: sharif@um.edu.my. Tel: 006-3-7967-4356. Fax: 006-3-7967-4356). Biodiesel production from waste soybean oil biomass as renewable energy and environmental recycled process. African Journal of Biotechnology Vol. 9 (27) (2010): 4233-4240

Alternative fuel is currently an important issue all over the world due to the efforts on reducing global warming which is contributed by the combustion of petroleum or petrol diesel. Biodiesel is non-toxic, biodegradable, produced from renewable sources and contributes a minimal amount of net green house gases, such as CO₂, SO₂ and NO emissions to the atmosphere. The study was carried out to produce biodiesel from waste/recycled oils to reduce the cost of biodiesel, waste

and pollution. Some important variables such as volumetric ratio, types of reactants and catalytic activities were selected to obtain a high quality biodiesel fuel within the specifications of the American Standard for Biodiesel Testing Method (ASTM D 6751) and European Norm (EN 14214). The highest biodiesel yield was obtained (71.2%) under the conditions of 1:1 volumetric oil-to-methanol weight ratio, 0.5% NaOH catalyst at 50°C reaction temperature and 320 rpm stirring speed. The results showed that biodiesel produced from different oil to methanol ratios, alcohol types and shaking time exhibited considerable differences. There was also a considerable difference of biodiesel yield produced by using methanol, ethanol and 1-butanol. The biodiesel yield increased in the order of 1-butanol < ethanol < methanol. There was a little difference in viscosity, acid value and chemical elements (Fe, Mg, Ca, Na, P etc.) at different parameters. The research showed that biodiesel obtained under optimum conditions from completely waste oil was of good quality and could be used as a diesel fuel which is considered as potential use of waste cooking oil. In addition, bioenergy could be renewed and environmental recycling process could be maintained potentially using waste soybean cooking oil.

Key words: Waste soybean oil, biodiesel, viscosity, acid value, element content.

Abbreviations: TAN, Total acid number; FFAs, free fatty acids; FAME, fatty acid methyl esters.

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
16. Applied Microbiology & Biotechnology
17. Aquaculture
18. Allergy
19. Australian Journal of Plant Physiology
20. Biocatalysis and Transformation
21. Biocontrol
22. Biocontrol Potential and its exploitation in sustainable Agriculture
23. Biodegradation
24. Biodeterioration & Biodegradation
25. Biodiversity and Conservation
26. Biological Agriculture and Horticulture
27. Biomass and Bioenergy
28. Biomedical and Environmental Sciences
29. Biomedical Engineering
30. Bioresource Technology
31. Bioscience, Biotechnology and Biochemistry
32. Biosensors-and –Bioelectronics
33. Bioseparation
34. Biotechnolgy Letters
35. Biotechnology Advances
36. Biotechnology and Applied Biochemistry
37. Biotechnology and Bioengineering
38. Botanical Review

39. Canadian Journal of Microbiology
40. Cell & Tissue Banking
41. Clinical Microbiology Reviews
42. Critical Reviews in Biotechnology
43. Crop Research Hisar
44. Current Microbiology
45. Current Opinion in Biotechnology
46. Current Science
47. Cytotechnology
48. Ecology and Environmental Corner
49. Ecological Engineering
50. Ecotoxicology
51. Environmental Conservation
52. Environmental Research
53. Environmental Pollution
54. Enzyme and Microbial Technology
55. Every Man's Science
56. Fems Microbiology & Ecology
57. Food & Agricultural Immunology
58. Global Environmental Change
59. Hydrometallurgy
60. Immunological Research
61. Indian Agriculturist
62. Indian Biologist
63. Indian Farming
64. Indian Journal of Agricultural Science
65. Indian Journal of Biotechnology
66. Indian Journal of Ecology
67. Indian Journal of Experimental Biology
68. Indian Journal of Environmental Toxicology
69. Indian Journal of Environmental Health
70. Indian Journal of Plant Physiology
71. International Biodeterioration & Biodegradation
72. International Journal of Biotechnology
73. International Journal of Phytoremediation
74. Journal of Applied Sciences and Environmental Management
75. Journal of Agriculture and Environmental Ethics
76. Journal Biological Control
77. Journal of Bacteriology
78. Journal of Chemical Technology & Biotechnology

79. Journal of Environmental Management
80. Journal of Food Science and Technology-Mysore
81. Journal of Hazardous Materials
82. Journal Indian Association Environment Management
83. Journal Indian Pollution Control
84. Journal of Indian Soil Science
85. Journal of Industrial Microbiology & Biotechnology
86. Journal of Scientific and Industrial Research
87. Microbial Review
88. Microbiological Research
89. Molecular Biotechnology
90. Mycological Research
91. Mycorrhizal Biology
92. Nature
93. Nature Biotechnology
94. New Biotechnology
95. Perspectives-in-Biotechnology
96. Pesticide research Journal
97. Pestology
98. Plants and Soil
99. Process Biochemistry
100. Pollution
101. Pollution Research
102. Reviews in Environmental Science and Biotechnology
103. Research Journal Chemistry & Environment
104. Sciences
105. Science & Culture
106. Shaspa
107. The Indian Forester
108. Trends in Biotechnology
109. Water, Air and Soil Pollution
110. World Journal of Biotechnology
111. World Journal of Microbiology and Biotechnology
112. Bio-metallurgy and Hydro-metallurgy

Authors Index

A. A. Romeh ^a	45
A. Deshpande ^a , G. Anitescu ^a , P.A. Rice ^a and L.L. Tavlarides ^a	146
A.J. Ward ^a and M.S. Kumar ^a	112
A.M.A. Morsy ^a , I.A. Ahmad ^b and A.M. Kamel ^c	132
A.R. Binupriya ^a , M. Sathishkumar ^b , K. Vijayaraghavan ^b and S.-I. Yun ^a	41
A.R. Khataee ^a , G. Dehghan ^b , A. Ebadi ^{a,b} , M. Zarei ^a and M. Pourhassan ^b	113
Abdul Rehman ¹ , S. Awais Butt and Shahida Hasnain	56
Ajay K. Manna ^a , Mou Sen ^a , Andrew R. Martin ^b and Parimal Pal ^a	23
Albert L. Juhasz ^a , Euan Smith ^a , Natasha Waller ^b , Richard Stewart ^c and John Weber ^a	63
Alei Geng ^{a,b} , Yanling He ^a , Changli Qian ^b , Xing Yan ^b and Zhihua Zhou ^b	142
Alessio Mengoni ¹ , Henk Schat ² and Jaco Vangronsveld ³	34
Ali Dehestani ^{1,2,3} , Kamal Kazemitabar ¹ , Gholamreza Ahmadian ² , Nadali Babaeian Jelodar ¹ , Ali Hatef Salmanian ³ , Mehdi Seyedi ³ , Heshmat Rahimian ⁴ and Seyedhadi Ghasemi ^{1,2}	75
Aloysio da S. Ferrão-Filho ^a , Maria Carolina S. Soares ^b , Valéria Freitas de Magalhães ^c and Sandra M.F.O. Azevedo ^c	124
Amit Bafana ^a , Kannan Krishnamurthi ^a , Mahendra Patil ^a and Tapan Chakrabarti ^a	40
Amparo Mauricio Gutierrez ^a ; Juan Jos Pea Cabriales ^a ; Mara Maldonado Vega ^b	44
Anal Chavan ¹ and Suparna Mukherji ¹	151
Andrea Dario ^a , Marc Schroeder ^a , Gibson S. Nyanhongo ^a , Gregor Englmaier ^b and Georg M. Guebitz ^a	103
Andrea Luna-Acosta ¹ , Paco Bustamante ¹ , Joachim Godefroy ¹ , Ingrid Fruitier-Arnaudin ¹ and HÃ©lÃ©ne Thomas-Guyon ¹	69
Anita Jemec ¹ , Damjana Drobne ² , Tatjana Tišler ¹ and Kristina Sepčić ²	70
Ann E. Hajek ¹ and Italo Delalibera Jr. ²	74
Anna KÃ©rrman ¹ , JosÃ© L. Domingo ² , Xavier Llebaria ³ , MartÃ© Nadal ² , Esther Bigas ³ , Bert van Bavel ¹ and Gunilla LindstrÃ©m ¹	125
Antonella Anastasi ^a , Federica Spina ^a , Valeria Prigione ^a , Valeria Tigini ^a , Pietro Giansanti ^b and Giovanna Cristina Varese ^a	53
Antonella Anastasi ^a , Valeria Prigione ^a and Giovanna Cristina Varese ^a	101
Ashish Bhatnagar ¹ , Monica Bhatnagar ² , Senthil Chinnasamy ¹ and K. C. Das ¹	137
Ashutosh Kumar Verma ¹ , Chandralata Raghukumar ¹ , Pankaj Verma ² , Yogesh S. Shouche ² and Chandrakant Govind Naik ¹	21

Awadhesh K. Shukla ^a , Pranjali Vishwakarma ^a , R.S. Singh ^b , S.N. Upadhyay ^b and Suresh K. Dubey ^a	111
B. Duarte ^a , M. Caetanoc, P.R. Almeida ^{a, b} , C. Valec and I. Caçador ^a	14
Bao-Yu Tian ¹ , Qin-Geng Huang ¹ , Yan Xu ¹ , Chun-Xiang Wang ¹ , Rui-Rui Lv ¹ and Jian-Zhong Huang ¹	107
Behzad Fatahi ^{a, b} , Hadi Khabbaz ^a and Buddhima Indraratna ^c	132
Boonsatien Boonsoong ¹ , Narumon Sangpradub ² , Michael T. Barbour ³ and Wijarn Simachaya ⁴	121
Bor-Yann Chen ^{a, d} , Meng-Meng Zhang ^b , Chang-Tang Chang ^{c, d} , Yongtao Ding ^b , Kae-Long Lin ^{c, d} , Chyow-San Chiou ^{c, d} , Chung-Chuan Hsueh ^a and Huizhong Xu ^b	141
Brent C. Christner ¹	56
Brian A. Pellerin ^{a, *} , Peter J. Hernes ^b , JohnFranco Saraceno ^a , Robert G. M. Spencer ^b and Brian A. Bergamaschi ^a	98
C. Forbes ^a , D. Hughes ^a , J. Fox ^a , P. Ryan ^a and E. Colleran ^a	109
C. Sivaraman ¹ , Anasuya Ganguly ¹ and Srikanth Mutnuri ¹	107
C.-H. Chen ^a , C.-F. Chang ^a and S.-M. Liu ^a	39
C.-H. Chen ^a , C.-F. Chang ^a and S.-M. Liu ^a	102
C.J. Wijesinghe ^a , R.S. Wilson Wijeratnam ^a , J.K.R.R. Samarasekara ^b and R.L.C. Wijesundera ^c	82
Carlos E. Rodríguez-Rodríguez ^a , Ernest Marco-Urrea ^b and Gloria Caminal ^a	113
Carolina Prado ^{a, 1} , Luisa Rodríguez-Montelongo ^b , Juan A. González ^c , Eduardo A. Pagano ^d , Mirna Hilal ^a and Fernando E. Prado ^a	37
Celine Justino ¹ , Ana Gabriela Marques ¹ , KÃ;tia Reis Duarte ¹ , Armando Costa Duarte ² , Ruth Pereira ³ , Teresa Rocha-Santos ¹ and Ana Cristina Freitas	96
Charles Hofer ^a , Frank J. Gallagher ^a and Claus Holzapfel ^b	13
Chen Zhang ^b , Le Jia ^c , Shenghui Wang ^a , Jie Qu ^a , Kang Li ^a , Lili Xu ^a , Yanhua Shi ^a and Yanchun Yan ^a	110
Christopher C. Rimmer ¹ , Eric K. Miller ² , Kent P. McFarland ¹ , Robert J. Taylor ³ and Steven D. Faccio ¹	17
Christos E. Papadopoulos ^a , Anastasia Lazaridou ^a , Asimina Koutsoumba ^a , Nikolaos Kokkinos ^a , Achilleas Christoforidis ^b and Nikolaos Nikolaou ^a	145
Chuloo Moon ^{1, 2} , Jae-Hyeong Ahn ¹ , Seung W. Kim ² , Byoung-In Sang ¹ and Youngsoon Um ¹	136
Chunyu Yang ¹ , Yang Li ¹ , Kun Zhang ¹ , Xia Wang ¹ , Cuiqing Ma ¹ , Hongzhi Tang ² and Ping Xu ^{1, 2}	86
Congying Wang ^{a, b} , Fang Wang ^a , Tao Wang ^a , Yongrong Bian ^a , Xinglun Yang ^a and Xin Jiang ^a	103
Cormac D. Murphy ¹	61

Cun-zheng ZHANG ^a , Xin-ming ZHANG ^d , Zi-hua TIAN ^b , Dan-jun HE ^c and Xian-jin LIU ^a	99
D.A. Hall ^{a, 1} , R.S. Gaster ^{b, c, 1} , T. Lin ^a , S.J. Osterfeld ^d , S. Han ^e , B. Murmann ^a and S.X. Wang ^{a, f}	127
D.A. Hall ^a , R.S. Gaster ^{b, c} , S.J. Osterfeld ^d , B. Murmann ^a and S.X. Wang ^{a, e}	128
D.K. Gupta ^a , H.G. Huang ^a , X.E. Yang ^a , B.H.N. Razafindrabe ^c and M. Inouhe ^b	36
Daisuke Sano ^a ; S. V. R. K. Prabhakar ^a	133
Daljit Singh Arora ¹ and Rakesh Kumar Sharma ¹	119
Dawen Gao ^{1,2} , Lina Du ¹ , Jiaoling Yang ¹ , Wei-Min Wu ³ , Hong Liang ¹	59
De-Wei Li ¹ and James LaMondia ¹	134
Dolors Sant ^a , Eva Casanova ^b , Guillem Segarra ^a , Manuel Avilés ^c , Màrio Reis ^d and M. Isabel Trillas ^a	83
Douglas I. Stewart ^a , Ian T. Burke ^b , Danielle V. Hughes-Berry ^b and Robert A. Whittleston ^b	63
E. Dinuccio ^a , P. Balsari ^a , F. Gioelli ^a and S. Menardo ^a	144
E. Komarova ^a , K. Reber ^a , M. Aldissi ^a and A. Bogomolova ^a	131
E. Kovač-Andrić ¹ , G. Šorgo ² , N. Kezele ² , T. Cvitaš ^{2, 3} and L. Klasinc ²	122
Ernest Marco-Urrea ^a , Miriam Pérez-Trujillo ^b , Paqui Blánquez ^a , Teresa Vicent ^a and Gloria Caminal ^c	112
Fang-Bo Yu ^{a, b, 1} , Shinawar Waseem Ali ^{b, 1} , Li-Bo Guan ^{a, b} , Shun-Peng Li ^b and Shan Zhou ^a	42
Fatih Duman ¹ , Fatma Ozturk ¹ and Zeki Aydin ²	25
Fatih Kalyoncu ¹	133
Fernando L. P. Pessoa ¹ , Shayane P. Magalhães ¹ and Pedro Wagner de Carvalho Falcão ²	135
Francesca Pagnanelli ^a , Carolina Cruz Viggi ^a and Luigi Toro ^a	53
G. Mascolo ^a , L. Balest ^a , D. Cassano ^a , G. Laera ^a , A. Lopez ^a , A. Pollice ^a and C. Salerno ^a ..	111
G. Mohanakrishna ^a , S. Venkata Mohan ^a and P.N. Sarma ^a	40
G. Ofori-Sarpong ^a , M. Tien ^b and K. Osseo-Asare ^{a, c}	60
G. Wultsch ¹ , D. Haas ² , H. Galler ² , G. Feierl ² , A. Melkes ² and F. F. Reinthaler ²	57
Geeta S. Nagvenkar ¹ and N. Ramaiah ¹	64
Ghulam Sarwar , Nazir Hussain , Fakhar Mujeeb , H. Schmeisky and Ghulam Hassan....	73
Grażyna A. Płaza ¹ , Grzegorz Nałęcz-Jawecki ² , Onruthai Pinyakong ³ , Paul Illmer ⁴ and Rosa Margesin ⁴	22
Hamed M. El-Mashad ^{a, b} and Ruihong Zhang ^b	141

Handan Uzun ^a , Ergun Yildiz ^a and Alper Nuhoglu ^a	111
Hao Chen ^a , Guoliang Dai ^a , Jie Zhao ^a , Aiguo Zhong ^a , Junyong Wu ^a and Hua Yan ^a	36
Hector Monclús ^a , Jan Sipma ^a , Giuliana Ferrero ^a , Ignasi Rodriguez-Roda ^{a, b} and Joaquim Comas ^a	52
Heidemarie Schaar ^a , Manfred Clara ^b , Oliver Gans ^b and Norbert Kreuzinger ^a	94
Héloïse Bastiaanse ^a , Luc de Lapeyre de Bellaire ^{b, c} , Ludivine Lassois ^a , Coralie Misson ^a and M. Haïssam Jijakli ^a	80
Hong-Yan Zeng ¹ , He Jiang ¹ , Kui Xia ¹ , Ya-Ju Wang ¹ and Yan Huang ¹	95
Hongyuan Wang ^{1, 2} , Zhenyao Shen ¹ , Junfeng Niu ¹ , Ya He ¹ , Qian Hong ¹ and Ying Wang ¹	122
Hosseini Farzaneh ^{1*} , Malekzadeh Fereidon ¹ , Amirmozafari Noor ² and Ghaemi Naser ³ ..	92, 117
Huda Mahmoud ¹ , Redha Al-Hasan ¹ , Majida Khanafer ¹ and Samir Radwan ¹	97
I. Del Toro ^a , K. Floyd ^b , J. Gardea-Torresdey ^c and D. Borrok ^d	13
Inés Infante ¹ , Maria A. Morel ¹ , Martha C. Ubalde ¹ , Cecilia Martínez-Rosales ¹ , Silvia Belvisi ² and Susana Castro-Sowinski ^{1, 3}	105
Itza Mendoza-Sanchez ¹ , Robin L. Autenrieth ² , Thomas J. McDonald ³ and Jeffrey A. Cunningham ⁴	90
Itzamná Baqueiro-Peña ^a , Gabriela Rodríguez-Serrano ^a , Eduardo González-Zamora ^b , Christopher Augur ^c , Octavio Loera ^a and Gerardo Saucedo-Castañeda ^a	65
J. Jacob Parnell ^{1, 4} Vincent J. Deneff ² , Joonhong Park ³ , Tamara Tsoi ¹ and James M. Tiedje ¹	88
J. K. Pell ¹ , J. J. Hannam ² and D. C. Steinkraus ²	75
J. Mann ¹ , J. L. Markham ² , P. Peiris ² , N. Nair ¹ , R. N. Spooner-Hart ¹ and P. Holford ¹	47
J.A. Post ^a , C.A. Kleinjan ^b , J.H. Hoffmann ^b and F.A.C. Impson ^{a, b}	79
J.W. Hong, J.Y. Park and G.M. Gadd	100
James M. Clomburg ¹ and Ramon Gonzalez ^{1, 2}	148
James P. Meador ¹ , Gina M. Ylitalo ² , Frank C. Sommers ¹ and Daryle T. Boyd ²	19
Jamil Anwar ^a , Umer Shafique ^a , Waheed-uz-Zaman ^a , Muhammad Salman ^a , Amara Dar ^a and Shafique Anwar ^b	55
Jan Kotyza ^{ab} ; Petr Soudek ^a ; Zdenk Kafka ^b ; Tom Vank ^a	45
Jason T. DeJong ^a , Brina M. Mortensen ^b , Brian C. Martinez ^b and Douglas C. Nelson ^c	95
Jasperien De Weert ^{1, 2} , Marc Viñas ³ , Tim Grotenhuis ² , Huub Rijnaarts ^{1, 2} and Alette Langenhoff ¹	115
Jennifer Read ^a ; Tim D. Fletcher ^b ; Tricia Wevill ^a ; Ana Deletic ^b	46
Jenny Rattfelt Nyholm ^a , Charlott Lundberg ^a and Patrik L. Andersson ^a	93
Jessica Amadio ¹ and Cormac D. Murphy ¹	67

Jessica M. Reichmuth ^a , Peddrick Weis ^b , and Judith S. Weis ^a	15
Jianzhong Chen ^{1,2} , Jian Dai ³ , Haiyang Zhang ¹ , Chenyi Wang ¹ , Guoqing Zhou ¹ , Zhiping Han ¹ and Zhili Liu ²	18
Jinshao Ye ^{a,b} , Hua Yin ^a , Bixian Mai ^b , Hui Peng ^a , Huaming Qin ^a , Baoyan He ^a and Na Zhang ^a	51
Johnson Kayode Adesodun ¹ , Mutiau O. Atayese ² , T. A. Agbaje ¹ , Bose A. Osadiaye ¹ , O. F. Mafe ¹ and Adeniyi A. Soretire ¹	49
Jung-Chun Chen ^a , Kai-Sung Wang ^{b,c} , Hsien Chen ^{b,c} , Chi-Yuan Lu ^{b,c} , Lung-Chiu Huang ^b , c, Heng-Ching Li ^{b,c} , Tzu-Huan Peng ^{b,c} and Shih-Hsien Chang ^{b,c}	53
Jyh-Ping Chen ¹ and Gen-Hsu Lin ¹	135
Jyoti P. Jadhav ¹ , Swapnil S. Phugare ¹ , Rhishikesh S. Dhanve ¹ and Shekhar B. Jadhav ¹ ..	91
K. Sarayu ¹ and S. Sandhya ¹	119
K. Tsekova ^a , D. Todorova ^a , V. Dencheva ^a and S. Ganeva ^b	55
Karel Komers ^a , František Skopal ^a and Alexander Čegan ^b	144
Keshetty Srisailam ¹ and Ciddi Veeresham ¹	62
Khalil I. Al-Mughrabi ^a	82
Kheirghadam Enayatzamir ^{1,2} , Hossein A. Alikhani ² , Bagher Yakhchali ³ , Fatemeh Tabandeh ³ and Susana RodrĂguez-Couto ^{1,4,5}	32
Kimberly S. Beltran ^{1,2} and Glorina N. Pocsidio ¹	69
Kristen N. Savage ^{1,2} , Lee R. Krumholz ² , Lisa M. Gieg ² , Victoria A. Parisi ² , Joseph M. Sufliata ² , Jon Allen ³ , R. Paul Philp ³ & Mostafa S. Elshahed ¹	94
Lei Yan ^a , Huanhuan Yin ^a , Shuang Zhang ^b , Feifan Leng ^a , Wenbin Nan ^a and Hongyu Li ^a ..	35
Leigh J. Pilkington ^a , Gerben Messelink ^b , Joop C. van Lenteren ^c and Kristian Le Mottee ^d	78
Li Kang ¹ , Wei Wang ¹ and Yoon Y. Lee ¹	92
Liaoyuan Zhang ^a , Yunlong Yang ^a , Jian'an Sun ^a , Yaling Shen ^a , Dongzhi Wei ^a , Jiawen Zhu ^b and Ju Chu ^c	147
Lin Qiao ^{a,b} and Jian-long Wang ^a	104
Lingxiangyu Li ^a , Zhenlan Xu ^a , Jianyang Wu ^a and Guangming Tian ^a	20
Liu Xingyu ^{a,b} , Wu Biao ^a , Chen Bowei ^a , Wen Jiankang ^a , Ruan Renman ^b , Yao Guocheng ^a and Wang Dianzuo ^c	46
Louisa Wessels Perelo ^a	38
Luis A. Sayavedra-Soto ¹ , Barbara Gvakharia ¹ , Peter J. Bottomley ² , Daniel J. Arp ¹ and Mark E. Dolan ³	114
Luisa F. González ^a , Victor Sarria ^b and Oscar F. Sánchez ^a	110

M. Dary ^a , M.A. Chamber-Pérez ^b , A.J. Palomares ^{a, b, 1} and E. Pajuelo ^a	39
M. Ruiz ¹ and J. Velasco ¹	19
M. S. Al-Masri ¹ , Y. Amin ¹ , B. Al-Akel ¹ and T. Al-Naama ¹	60
Mang Lu ^a , Zhongzhi Zhang ^a , Wei Qiao ^a , Xiaofang Wei ^{a, b} , Yueming Guan ^a , Qingxia Ma ^a and Yingchun Guan ^a	55
Mark A. Jackson ¹ , Christopher A. Dunlap ¹ and Stefan T. Jaronski ²	74
Mark L. Thompson ¹ , Ray Marriott ² , Adam Dowle ³ and Gideon Grogan ¹	66
Mathabatha Evodia Setati.....	117
Maurizio G. Paoletti ^a ; Alessandra D'Inc ^a ; Emanuele Tonin ^a ; Stefano Tonon ^a ; Carlo Migliorini ^b ; Giannantonio Petruzzelli ^c ; Beatrice Pezzarossa ^c ; Tiziano Gomiero ^a ; Daniele Sommaggio ^d	127
Md. Abul Kashem ¹ , Bal Ram Singh ² , Hiroshi Kubota ³ , Reiko Sugawara ³ , Nobuyuki Kitajima ³ , Toshihito Kondo ³ and Shigenao Kawai ¹	26
Md. Tofazzal Islam ^{1, 2}	76
Metka Udovic ¹ and Domen Lestan ¹	27
Michael Surgan ¹ , Madison Condon ¹ and Caroline Cox ²	124
Miguel E Vega-Sánchez ^{1, 2} and Pamela C Ronald ^{1, 2}	134
Minakshi Grover ¹ , Lata Nain ² , Shashi Bala Singh ³ and Anil Kumar Saxena ²	85
Mohammad Pourbafrani ^{a, b} , Gergely Forgács ^{a, b} , Ilona Sárvári Horváth ^b , Claes Niklasson ^a and Mohammad J. Taherzadeh ^b	143
Monika Dhote ^{1, 2} , Asha Juwarkar ¹ Anil Kumar ² , G. S. Kanade ¹ and Tapan Chakrabarti ¹	108
N. Suchkova ^{a, 1} , E. Darakas ^{b, 2} , and J. Ganoulis ^{b, 2}	24
Nai-Dong Chen ¹ , Jian Zhang ¹ , Ji-Hua Liu ² and Bo-Yang Yu ²	115
Naoki Harada ¹ , Kazuhiro Takagi ² , Koji Baba ² , Kunihiro Fujii ³ and Akio Iwasaki ³	91
Narendra Mohan Verma, Shakti Mehrotra*, Amitesh Shukla and Bhartendu Nath Mishra	149
Natalia N. Pozdnyakova ¹ , Svetlana V. Nikiforova ¹ , Oleg E. Makarov ¹ , Marina P. Chernyshova ¹ , Kirill E. Pankin ¹ and Olga V. Turkovskaya ¹	106
Nitu Sood ¹ , Sonali Patle ¹ and Banwari Lal ²	29
Nívea de Lima da Silva ¹ , Carlos Mario Garcia Santander ¹ , César Benedito Batistella ¹ , Rubens Maciel Filho ¹ and Maria Regina Wolf Maciel ¹	136
Ofelia Dolores Hernández ¹ , Ángel José Gutiérrez ¹ , Dailos González-Weller ^{2 *} , Gonzalo Lozano ³ , Enrique García Melón ⁴ , Carmen Rubio ¹ , Arturo Hardisson ¹	15
Oktay Erdogan ^a and Kemal Benlioglu ^b	79
P. C. Suryawanshi ¹ , A. B. Chaudhari ² , R. M. Kothari ¹	59
P.J. Strong ¹	48

P.R. Warman ^a and M.J. AngLopez ^a	72
Paola Pereira ^{a,1} , Andrea Nesci ^{a,2} , Carlos Castillo ^b and Miriam Etcheverry ^{a,2}	81
Patrizia CretÃ-1, Francesca Trinchella ² and Rosaria Scudiero ²	122
Pedro A. Isaza, Andrew J. Daugulis [*]	120
Peter O. Abioye ¹ , A. Abdul Aziz ² and P. Agamuthu ¹	108
Petra Bombach ^{1,2} , Hans H. Richnow ¹ , Matthias Kastner ³ and Anko Fischer ^{1,2}	116
Pietro Carlozzi ^a , Arianna Buccioni ^b , Sara Minieri ^b , Benjamin Pushparaj ^a , Raffaella Piccardi ^a , Alba Ena ^a and Cristina Pintucci ^a	145
Prabhat Kumar Rai ¹	22
Prasun K. Mukherjee ^{1,2} and Charles M. Kenerley ^{1*}	84
Prawit Kongjan ¹ , Sompong O-Thong ^{1,2} , Meher Kotay ¹ , Booki Min ^{1,3} , Irimi Angelidaki ¹	151
Priti N. Chaudhari ¹ , Bhushan L. Chaudhari ¹ and Sudhir B. Chincholkar ¹	61
Priyanshu Manab Sarma ¹ , Prem Duraja ² , Shilpanjali Deshpande ³ and Banwari Lal ⁴	86
Qayyum Husain ¹	34
Qin Lu ¹ , Zhenli L. He ¹ , Donald A. Graetz ² , Peter J. Stoffella ¹ and Xiaoe Yang ³	31
Qingqing Peng ^{a,b} , Yunguo Liu ^{a,b} , Guangming Zeng ^{a,b} , Weihua Xu ^{a,b} , Chunping Yang ^{a,b} and Jingjin Zhang ^{a,b}	38
Qun Yan ^{a,b} , Minking Zhao ^b , Hengfeng Miao ^{a,b} , Wenquan Ruan ^{a,b} and Rentao Song ^c	140
R. Schulz ¹² , H. Al-Najar ¹² , J. Breuer ¹³ and V. Romheld ¹²	73
Ragini Singh ^a , R.D. Tripathi ^a , Sanjay Dwivedi ^a , Amit Kumar ^a , P.K. Trivedi ^a and D. Chakrabarty ^a	20
Ram Chandra ¹ , Sangeeta Yadav ¹ and Ram Naresh Bharagava ¹	106
Rebecca M. Lennen, Drew J. Braden, Ryan M. West, James A. Dumesic, Brian F. Pflieger	150
Ren-bang ZHAO ^{a,b} , Hua-ying BAO ^a and Yuan-xia LIU ^a	99
Richard W. Eaton ¹ and Peter Sandusky ²	61
S.H. Hasan ^a , P. Srivastava ^a and M. Talat ^b	39
Sandro Froehner ¹ and Marcell Maceno ¹	13
San-Lang Wang ^{1,2} , Tao-Jen Chang ¹ and Tzu-Wen Liang ²	89
Sardar Alam Cheema ^{a,b} , Muhammad Imran Khan ^{a,b} , Chaofeng Shen ^a , Xianjin Tang ^a , Muhammad Farooq ^b , Lei Chen ^a , Congkai Zhang ^a and Yingxu Chen ^a	102
Senthil Chinnasamy ^a , Ashish Bhatnagar ^{a,b} , Ryan W. Hunt ^a and K.C. Das ^a	144

Shao Hong-Bo ^{1,2,3,4} , Chu Li-Ye ⁴ , Ruan Cheng-Jiang ³ , Li Hua ⁵ , Guo Dong-Gang ⁵ , Li Wei-Xiang ⁶	58
Sharon B. Velasquez-Orta ^{1, 2} , Ian M. Head ¹ , Thomas P. Curtis ¹ , Keith Scott ² , Jonathan R. Lloyd ³ and Harald von Canstein ^{3, 4}	147
Sheikh M. Basha ¹ , Hifza Mazhar ¹ and Hemanth K. N. Vasanthaiah ¹	59
Shuhe Wei ^a , Yunmeng Li ^{a, b} , Qixing Zhou ^a , Mrittunjai Srivastava ^c , Siuwai Chiu ^d , Jie Zhan ^e , Zhijie Wu ^a and Tieheng Sun ^a	72
Smita Raghuvanshi ¹ and B. V. Babu ²	85
Soma Roy ¹ , Kiranmayee Rao ² , Ch. Bhuvaneswari ² , Archana Giri ² and Lakshmi Narasu Mangamoori ²	77
Somayeh Niknam ¹ , Mohammad Ali Faramarzi ^{1, 2} , Khosrou Abdi ³ , Mojtaba Tabatabaei Yazdi ¹ , Mohsen Amini ³ and Hossein Rastegar ⁴	47
Stefanie Knauert ^a , Heinz Singer ^b , Juliane Hollender ^b and Katja Knauer ^c	24
Stephanie Lansing ^a , Jay F. Martin ^b , Raúl Botero Botero ^c , Tatiana Nogueira da Silva ^c and Ederson Dias da Silva ^c	139
Steven D. Frank ^a	77
Surekha K. Satpute ¹ , Arun G. Banpurkar ² , Prashant K. Dhakephalkar ³ , Ibrahim M. Banat ⁴ , Balu A. Chopade ¹	57
Sushanta Kumar Saha ^{a, b} , Palanisami Swaminathan ^a , C. Raghavan ^{a, c} , Lakshmanan Uma ^a and Gopalakrishnan Subramanian ^a	54
Ta-Chen Lin ^a , Po-Tsen Pan ^b and Sheng-Shung Cheng ^{b, c}	43
Tadashi Toyama ¹ , Noritaka Maeda ¹ , Manabu Murashita ¹ , Yong-Cheol Chang ¹ and Shintaro Kikuchi ¹	88
Tahseen Sayara ¹ , Montserrat Sarrà ¹ and Antoni Sánchez ¹	21
Tariq Mahmood* and Syed Tajammul Hussain	149
Tariq Mahmood ^{1*} , Syed Tajammul Hussain ¹ and Salman Akbar Malik ²	150
Tekin Şahan ^a , Hasan Ceylan ^b , Nurettin Şahiner ^c and Nahit Aktaş ^a	51
Teresa Manso ¹ , Carla Nunes ¹ , Sara Raposo ¹ and Maria Emília Lima-Costa ¹	76
Tobias Schulze ^a , Sara Weiss ^{a, c} , Emma Schymanski ^a , Peter Carsten von der Ohe ^a , Mechthild Schmitt-Jansen ^b , Rolf Altenburger ^b , Georg Streck ^a and Werner Brack ^a	62
Uwe Buczko ¹ and Rolf O. Kuchenbuch ²	123
V. Sivasankar ^a , T. Ramachandramoorthy ^b and A. Chandramohan ^c	42
Valery N. Konopsky ^a and Elena V. Alieva ^a	131
Victor Kraemer Wermelinger Sancho Araujo ^a , Silvio Hamacher ^a and Luiz Felipe Scavarda ^a	140
VladimĀr KoĀř ¹ , KlĀřra MocovĀř ¹ , Marie KulovanĀř ² and Simona VosĀřhlovĀř ¹	30
W. L. Ma ¹ , C. Y. Yan ^{1, 2} , J. H. Zhu ¹ , G. Y. Duan ¹ and R. M. Yu ¹	68

Wan Azlina Ahmad ^a , Zainul Akmar Zakaria ^a , Ali Reza Khasim ^a , Muhamad Anuar Alias ^a and Shaik Muhammad Hasbullah Shaik Ismail ^a	50
Wen-Teish Chang ¹ , Ming-Lun Chen ¹ and San-Lang Wang ²	105
Wu-ChungChan ^a and Yuan-Sheng Lin ^a	109
Wuxing Liu ^a ; Yongming Luo ^a ; Ying Teng ^a ; Zhen'gao Li ^a	44
Xavier Laffray ^a , Christophe Rose ^a and Jean-Pierre Garrec ^a	125
Xia Jiang ^a and Joo Hwa Tay ^a	114
Xiao Wu ^a , Wanying Yao ^b , Jun Zhu ^a and Curtis Miller ^a	143
Xiao-Zhang Yu ¹ , Xiao-Ying Peng ¹ and Li-Qun Xing ¹	25
Xin Cheng ¹ , Lin Huang ¹ , Xiao-rong Tu ¹ and Kun-tai Li ¹	87
Xinde Cao ^{1,3} , Lena Ma ³ , Aziz Shiralipour ² and Willie Harris ³	64
Xueling Wu ^a , Renxing Liang ^a , Qinyun Dai ^a , Decai Jin ^a , Yangyang Wang ^a and Weiliang Chao ^b	104
Xue-Qin Tao ¹ , Jie-Ping Liu ¹ , Gui-Ning Lu ^{2*} , Xiu Guo ¹ , Hui-Ping Jiang ¹ and Guan-Qiu Sun ¹	118
Y.F. Huang ^a , W.H. Kuan ^b , S.L. Lo ^a and C.F. Lin ^a	147
Y.-H. Percival Zhang ^{1 2 3 *}	68
Yan Xing ¹ , Zhuo Li ¹ , Yaoting Fan ¹ and Hongwei Hou ¹	137
Yanghui Xiong ¹ and Yu Liu ¹	83
Yanna Liang ¹ , Jemil Yesuf ¹ and Zisong Feng ¹	93
Yaohui Bai ¹ , Qinghua Sun ¹ , Cui Zhao ¹ , Donghui Wen ¹ and Xiaoyan Tang ¹	90
Yi Wang ^a , Chun-Jen Huang ^a , Ulrich Jonas ^{b, c} , Tianxin Wei ^d , Jakub Dostalek ^a and Wolfgang Knoll ^a	129
Yifei Wang ^a , Ting Yu ^a , Jindan Xia ^a , Dasheng Yu ^b , Jun Wang ^a and Xiaodong Zheng ^a	80
Yijun Dai ^a , Yinjuan Zhao ^{a, 1} , Wenjian Zhang ^a , Cigang Yu ^a , Weiwei Ji ^a , Wenping Xu ^a , Jueping Ni ^b and Sheng Yuan ^a	66
Ying Lua ^b , Fei Donga, Claire Deaconb, Huo-jun Chena, Andrea Raabb ^c and Andrew A. Mehargb.....	14
Ying Teng ^a , Yongming Luo ^a , Mingming Sun ^a , Zengjun Liu ^a , Zhengao Li ^a and Peter Christie ^b	52
Yin-Ming Kuo ^{1,2} , Maria S. Sep̃alveda ^{1,3} , Inez Hua ^{1,4} , Hugo G. Ochoa-Acũa ^{1,5} and Trent M. Sutton ⁶	16
Yin-Ming Kuo ^{1,2} , Maria S. Sep̃alveda ^{1,3} , Trent M. Sutton ⁵ , Hugo G. Ochoa-Acũa ^{1,6} , Andrew M. Muir ^{3,4} , Benjamin Miller ³ and Inez Hua ^{1,7}	17
Yinxi Huang ^a , Preeti Vikas Palkar ^a , Lain-Jong Li ^b , Hua Zhang ^b and Peng Chen ^a	130

Yong Wang^{a, b}, Fan Yang^a and Xiurong Yang^a	129
Yongming Zhang¹, Lei Wang¹ and Bruce E. Rittmann²	116
Yongyan Tan^a, Xiaoxia Guo^a, Jinghui Zhang^a and Jinqing Kan^a	130
Yong-Zhong Wang^a, Qiang Liao^a, Xun Zhu^a, Xin Tian^a and Chuan Zhang^a	142
Youngmin Lee^a and Woojin Lee^a	101
Yufeng Han^a, Chaoling Yu^a and Hong Liu^a	128
Zandra Arwidsson^{1, 2}, Emma Johansson¹, Thomas von Kronhelm², Bert Allard¹ and Patrick van Hees^{1, 3}	50
Zandra Arwidsson^{1, 2} and Bert Allard¹	48
Zhengbo Yue, Charles Teater, Yan Liu, James MacLellan, Wei Liao. *	120
Zhen-Ming Chi¹, Guanglei Liu¹, Shoufeng Zhao¹, Jing Li¹ and Ying Peng¹	84
Zhongfang Lei^a, Jiayi Chen^a, Zhenya Zhang^b and Norio Sugiura^b	139