



ENVIS CENTER

on

ENVIRONMENTAL BIOTECHNOLOGY



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ENVIS CENTRE
on
ENVIRONMENTAL BIOTECHNOLOGY

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BACKGROUND

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

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

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

This is the 22nd publication of Abstract Volume of this ENVIS Centre. This contains the abstracts of research papers collected from the various areas of Environmental Biotechnology from different journals published in last six months upto June, 2013. In this issue, various topics like Bioenergy, Bioengineering, Bio-degradation, Bio-remediation, Bio-transformation etc. have been covered. We are grateful to the various libraries and their staff for their cooperation extended to us during the collection of the articles.

Abstract Format

The format of the abstract is as follows:

Abstract: The abstracts are arranged in different subheads.

Author: Name of the authors are given in the order in which they appear in the original document. These names are given in succession.

Address of Authors: Address of the author is given in parenthesis at the end of the author's name. When the address of any other author is found, it is written after wards delimited by stop(.).

Locus: The name of the journal is followed by the volume number, issue number, year of publication and the page no.

GENERAL INFORMATION

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

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

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

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

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

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

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

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

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

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

1. Bacterial Biofertilizer
2. Algal Biofertilizer

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ABBREVIATIONS USED IN ADDRESSES AND CITED JOURNALS

Acad	Academy	Chem	Chemistry
Adm	Administration	Cheml	Chemical
Admn	Administrative	Clinl	Clinical
Adv	Advance	Co	Company
Agri	Agriculture	Coll	College
Agricl	Agricultural	Comm	Committee
Amer	American	Commn	Commission
An	Annual	Comp	Comparative
Analyt	Analytical	Conf	Conference
Anat	Anatomy	Conv	Convention
Anim	Animal	Conserv	Conservation
Ann	Annals	Contl	Control
Appl	Applied	Contam	Contamination
Arch	Archives	Corp	Corporation
Archaeo	Archaeology	Coun	Council
Archaeol	Archaeological	Cult	Culture
Architect	Architecture	Cultl	Cultural
Assoc	Association	Curr	Current
Asst	Assistant	Dept	Department
Atom	Atomic	Dev	Development
Bacterio	Bacteriology	Develop	Developmental
Bacteriol	Bacteriological	Dig	Digest
Bd	Board	Div	Division
Bio	Biology	Divl	Divisional
Biochem	Biochemistry	Dte	Directorate
Biocheml	Biochemical	Dy	Deputy
Bioengg	Bioengineering	Eco	Ecology
Biol	Biological	Ecol	Ecological
Biometeo	Biometeorology	Econ	Economics
Biophys	Biophysics	Ecosys	Ecosystem
Biometeol	Biometeorological	Ecotoxic	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	Ethnopharmac	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

Kateřina Kolařiková, Wolf von Tümpling, Peter Bartels. Bioaccumulation of HCH isomers in selected macroinvertebrates from the Elbe River: sources and environmental implications. *Environmental Monitoring and Assessment*, Volume 185 (5) (2013): 4333-4346

Sediments of the Elbe River have been extremely polluted by contaminants originating from previous large-scale hexachlorocyclohexane (HCH) production and the application of γ -HCH (lindane) in its catchment in the second half of the twentieth century. In order to gain knowledge on bioaccumulation processes at lower trophic levels, field investigations of HCHs in macroinvertebrates were carried out along the longitudinal profile of the Elbe and tributary. Among the sites studied, concentrations in macroinvertebrates ranged within five orders of magnitude (0.01–100 $\mu\text{g}/\text{kg}$). In general, lower values of HCH isomers were observed at all Czech sites (mostly $<1 \mu\text{g}/\text{kg}$) compared with those in Germany. At the most contaminated site, Spittelwasser brook (a tributary of the Mulde), extremely high concentrations were measured (up to 234 $\mu\text{g}/\text{kg}$ α -HCH and 587 $\mu\text{g}/\text{kg}$ β -HCH in Hydropsychidae). In contrast, the Obříství site, though also influenced by HCH production facilities, showed only negligibly elevated values (mostly $<1 \mu\text{g}/\text{kg}$). Results showed that fairly high levels of α -HCH and β -HCH compared to γ -HCH can still be detected in aquatic environments of the Elbe catchment, and these concentrations are decreasing over time to a lesser extent than γ -HCH. Higher HCH concentrations in sediments in the springtime are considered to be the result of erosion and transport processes during and after spring floods, and lower concentrations at sites downstream are thought to be caused by the time lapse involved in the transportation of contaminated particles from upstream. In addition, comparison with fish (bream) data from the literature revealed no increase in tissue concentrations between invertebrates and fish.

Aikaterini Sakellari, Sotirios Karavoltos, Dimitrios Theodorou, Manos Dassenakis, Michael Scoullas. Bioaccumulation of metals (Cd, Cu, Zn) by the marine bivalves *M. galloprovincialis*, *P. radiata*, *V. verrucosa* and *C. chione* in Mediterranean coastal microenvironments: association with metal bioavailability. *Environmental Monitoring and Assessment*, Volume 185 (4) (2013): 3383-3395

The concentrations of Cd, Cu and Zn in both the whole soft tissue and separate organs (gills, mantle, muscle and digestive gland) of wild bivalves (*Mytilus galloprovincialis*, *Pinctada radiata*, *Venus verrucosa* and *Callista chione*) from three different coastal microenvironments of Greece were monitored from 2003 to 2004. In parallel, by employing appropriate analytical protocols for metal partitioning, the labile fraction of the metals was determined in the dissolved phase, suspended particulate matter and sediments. Differences in the metal levels were detected both among the study areas as well as among the bivalves examined. Significant bioaccumulation was demonstrated regarding Zn in *M. galloprovincialis* specimens from the highly industrialized Gulf of Elefsis and Cd in *P. radiata* and *V. verrucosa* from the Maliakos Gulf, which is influenced by extended agricultural activity occurring at the neighbouring area and a river outflow. Data of the metal levels in the various environmental phases were correlated with their concentrations in bivalves' tissues. The clear relationships obtained in many cases

among the labile metal concentrations and the bioaccumulated concentrations in bivalves point out that the labile fraction of a metal is the most bioavailable. The lack of positive correlation for *C. chione* confirms the occurrence of effective mechanisms of internal regulation of metal concentrations.

I-Ting Hsieh, Hin-Kiu Mok, Fung-Chi Ko, S. Açik. Environmental assessment of trace element bioaccumulation in sipunculan from seagrass and wetland sediments. *Environmental Monitoring and Assessment*, Volume 185 (3) (2013): 2269-2279

This study is the first measurement of trace elements in sipunculan and their surrounding sediments. The bioaccumulation characteristics of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), and zinc (Zn) were analyzed and compared in two sipunculan species, *Sipuncula nudus* and *Siphonosoma vastum*, which were collected from seagrass beds and wetlands in Taiwan. The sipunculan and sediment samples were analyzed using an inductively coupled plasma mass spectrometer. Both sipunculan in the wetlands and seagrass beds had a high Cu bioaccumulation mechanism. Multivariate analysis, principle component analysis, and partial least squares for discriminant analysis of trace element levels and bioaccumulation factors were used to distinguish the element distributions that corresponded to the two habitats (seagrass beds and wetlands). Different levels of certain trace elements in these two sipunculan species may result not only from the environmental factors of various habitats but also from the accumulation characteristics of various species. The As, Cd, Cr, Cu, Hg, and Zn concentrations were markedly lower in sipunculan than in other invertebrates from the adjacent polluted regions. The public health issues regarding the consumption of sipunculan are also discussed.

M. E. Jara-Marini, J. N. Tapia-Alcaraz, J. A. Dumer-Gutiérrez, L. García-Rico, J. García-Hernández, F. Pérez-Osuna. Comparative bioaccumulation of trace metals using six filter feeder organisms in a coastal lagoon ecosystem (of the central-east Gulf of California). *Environmental Monitoring and Assessment*, Volume 185 (2) (2013): 1071-1085

The Tobarí Lagoon, located in the central-east coast of the Gulf of California, receives effluents from the Yaqui Valley, one of the most extensive agricultural areas of México. The Tobarí Lagoon also receives effluents from nearby shrimp farms and untreated municipal sewage. Surface sediment samples and six different species of filter feeders (*Crassostrea corteziensis*, *Crassostrea gigas*, *Chione gnidia*, *Anadara tuberculosa*, *Chione fluctifraga*, and *Fistulobalanus dentivarians*) were collected during the dry and the rainy seasons and analyzed to determine concentrations of cadmium (Cd), copper (Cu), mercury (Hg), lead (Pb), and zinc (Zn). Seasonal variations in metal concentrations in sediment were evident, especially for Cd, Cu, Hg, and Zn. The total and bioavailable concentrations of the five metals are not elevated in comparison to other areas around the world. The percentages of bioavailable respect to total concentrations of the metals varied from 0.6 % in Hg to 50.2 % for Cu. In the organisms, Hg showed the lowest concentrations (ranged from 0.22 to 0.65 µg/g) while Zn showed the highest (ranged from 36.6 to 1,702 µg/g). Linear correlations between the levels of Cu, Pb, and Zn in the soft tissues of *C. fluctifraga* and *C. gnidia*, and *A. tuberculosa* and *C. gnidia* were found. Seasonal and interspecies variations in the metal levels in filter feeders were found; *F. dentivarians*, *C. corteziensis*, and *C. gigas* exhibited the highest levels, could be used as biomonitors of metals contamination in this area.

Agoes Soegianto, Dwi Winarni, Usreg Sri Handayani, Hartati. (Department of Biology, Faculty of Sciences and Technology, Airlangga University, Kampus C, Jl. Mulyorejo,

Surabaya, 60115, Indonesia, Department of Chemistry, Faculty of Sciences and Technology, Airlangga University, Kampus C, Jl. Mulyorejo, Surabaya, 60115, Indonesia). Bioaccumulation, Elimination, and Toxic Effect of Cadmium on Structure of Gills and Hepatopancreas of Freshwater Prawn *Macrobrachium sintangese* (De Man, 1898). *Water, Air, & Soil Pollution*,(2013) (224) :1575

The objectives of this study were to determine the acute toxicity of cadmium and to examine the bioaccumulation and elimination of cadmium in different tissues of the freshwater prawn *Macrobrachium sintangese*. It also evaluated the structural damage of gills and hepatopancreas of *M. sintangese* when administered to sublethal cadmium concentration and when exposed prawns were transferred to cadmium-free media. According to the mortality data, the 96 h LC₅₀ value of Cd to *M. sintangese* was 86 µg/L. The highest cadmium accumulation was observed in gills, followed by the hepatopancreas, and the abdominal muscle. After being transferred to cadmium-free media, the highest cadmium elimination was observed in abdominal muscle, followed by the gills and hepatopancreas. The gills of prawns exposed to cadmium exhibited a severe hyperplasia, vacuolization, and multiple necroses which resulted to the swelling of lamellae. After transferring the cadmium-exposed prawns into the control media, the histopathological effects decreased. Severe alterations to the hepatopancreatic tissue were observed in prawns exposed to cadmium. The tubular epithelial cells were heavily vacuolated and even ruptured. The number of large vacuoles and R cells appeared in the tubular epithelial cells of the hepatopancreas. After transferring to the control media, the histological alterations of the hepatopancreas decreased. The tubular epithelial cells began to rearrange to the normal structure. The number of R cells and B cells were noted in the epithelial cells. The thickness of tubular epithelial cells was comparable to the controls. Due to the sensitivity of *M. sintangese* to cadmium, therefore this species potentially can be used as a testorganism in toxicity assays.

Rebeca Manzano, Jesús M. Peñalosa, Elvira Esteban. (Departamento de Química Agrícola, Universidad Autónoma de Madrid, Carretera de Colmenar Viejo km. 15, 28049, Madrid, Spain). Arsenic Accumulation and Tolerance of *Cytisus scoparius* Under Controlled Conditions. *Water, Air, & Soil Pollution*, (2012) (224) :1363

Cytisus scoparius is a native leguminous species which grows at a derelict arsenopyrite mine in NW Madrid, Spain. Among the species found in the area surrounding the mine, this plant has shown one of the highest arsenic bioaccumulation factors. For this reason, alongside with its ability to grow in a contaminated area and its high biomass, it was selected for an arsenate dose–response assay under controlled conditions in order to evaluate its potential resistance to arsenic. *C. scoparius* accumulated arsenic mainly in roots, and this had a negative effect on root phosphorous concentration. Stress indicators, such as glutathione and synthesis of phytochelatins, and the lack of evidence of an increase in malondialdehyde when arsenate was supplied indicate that *C. scoparius* has a certain resistance to arsenic. According to our results, *C. scoparius* would be a good candidate to revegetate arsenic-contaminated sites.

Diana Amaral Monteiro, Francisco Tadeu Rantin, Ana Lúcia Kalinin. (Department of Physiological Sciences, Federal University of São Carlos, UFSCar, Via Washington Luís km 235, São Carlos, São Paulo, 13565-905, Brazil). Dietary intake of inorganic mercury: bioaccumulation and oxidative stress parameters in the neotropical fish *Hoplias malabaricus*. *Ecotoxicology*, Volume 22 (3) (2013): 446-456

This study evaluated the effects of trophic and subchronic exposure to inorganic mercury (Hg) on the oxidative stress biomarkers and its bioaccumulation potential in the liver, gills, white muscle and heart of the freshwater top predator fish, *Hoplias malabaricus*, fed with contaminated live juveniles of matrinxã, *Brycon amazonicus*, as prey vehicle. Inorganic mercury increased superoxide dismutase, catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase, and glutathione reductase (GR) activities in the liver, white muscle and heart. Gills CAT activity remained unchanged while GPx and GR values showed a significant decrease. In the liver and gills, Hg induced significant increase in the reduced (GSH) and oxidized (GSSG) glutathione content, concomitantly with a significant decrease in [GSH]/[GSSG] ratio. Differently, in cardiac tissue, the Hg caused an increase in GSH level and increase in [GSH]/[GSSG] ratio. Lipid and protein oxidation and metallothionein levels were significantly higher after Hg trophic exposure in the liver, gills and heart, but remained at control values in the white muscle. Tissue-specific responses against oxidative stress were observed, and the liver and gills were the most sensitive organs, showing signs of redox homeostasis failure. At the end of the experiment, dietary inorganic mercury accumulated through food chain levels. In order, Hg bioaccumulation was: gills > liver » white muscle = heart. These results pointed out the potential of inorganic Hg to bioaccumulate in aquatic systems. Taken together, our findings suggest that Hg, even in the inorganic form and sublethal amounts, is a risk factor for aquatic biota.

Prachi Kaushik, Anushree Malik. (Applied Microbiology Laboratory, Centre for Rural Development and Technology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, 110016, India). Comparative performance evaluation of *Aspergillus lentulus* for dye removal through bioaccumulation and biosorption. Environmental Science and Pollution Research, Volume 20(5) (2013): 2882-2892

Dyes used in various industries are discharged into the environment and pose major environmental concern. In the present study, fungal isolate *Aspergillus lentulus* was utilized for the treatment of various dyes, dye mixtures and dye containing effluent in dual modes, bioaccumulation (employing growing biomass) and biosorption (employing pre-cultivated biomass). The effect of dye toxicity on the growth of the fungal isolate was studied through phase contrast and scanning electron microscopy. Dye biosorption was studied using first and second-order kinetic models. Effects of factors influencing adsorption and isotherm studies were also conducted. During bioaccumulation, good removal was obtained for anionic dyes (100 mg/l), viz. Acid Navy Blue, Fast Red A and Orange-HF dye (99.4 %, 98.8 % and 98.7 %, respectively) in 48 h. Cationic dyes (10 mg/l), viz. Rhodamine B and Methylene Blue, had low removal efficiency (80.3 % [48 h] and 92.7 % [144 h], respectively) as compared to anionic dyes. In addition to this, fungal isolate showed toxicity response towards Methylene Blue by producing larger aggregates of fungal pellets. To overcome the limitations of bioaccumulation, dye removal in biosorption mode was studied. In this mode, significant removal was observed for anionic (96.7–94.3 %) and cationic (35.4–90.9 %) dyes in 24 h. The removal of three anionic dyes and Rhodamine B followed first-order kinetic model whereas removal of Methylene Blue followed second-order kinetic model. Overall, fungal isolate could remove more than 90 % dye from different dye mixtures in bioaccumulation mode and more than 70 % dye in biosorption mode. Moreover, significant color removal from handmade paper unit effluent in bioaccumulation mode (86.4 %) as well as in biosorption mode (77.1 %) was obtained within 24 h. This study validates the potential of fungal isolate, *A. lentulus*, to be used as the primary organism for treating dye containing wastewater.

María Fernández-Sanjuan, Melissa Faria, Silvia Lacorte, Carlos Barata. (Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18, Barcelona, 08034, Spain). Bioaccumulation and effects of perfluorinated compounds (PFCs) in zebra mussels (*Dreissena polymorpha*). Environmental Science and Pollution Research, Volume 20(4) (2013): 2661-2669

Perfluorinated chemicals (PFCs) have been used for many years in numerous industrial products and are known to accumulate in organisms. A recent survey showed that tissue levels of PFCs in aquatic organisms varied among compounds and species being undetected in freshwater zebra mussels *Dreissena polymorpha*. Here we studied the bioaccumulation kinetics and effects of two major PFCs, perfluorooctane sulfonic acid compound (PFOS) and perfluorooctanoic acid (PFOA), in multixenobiotic transporter activity (MXR) and filtration and oxygen consumption rates in zebra mussel exposed to a range of concentrations of a PCF mixture (1–1,000 µg/L) during 10 days. Results indicate a low potential of the studied PFCs to bioaccumulate in zebra mussel tissues. PFCs altered mussel MXR transporter activity being inhibited at day 1 but not at day 10. Bioaccumulation kinetics of PFCs were inversely related with MXR transporter activity above 9 ng/g wet weight and unrelated at tissue concentration lower than 2 ng/g wet weight suggesting that at high tissue concentrations, these type of compounds may be effluxed out by MXR transporters and as a result have a low potential to be bioaccumulated in zebra mussels. Oxygen consumption rates but not filtering rates were increased in all exposure levels and periods indicating that at environmental relevant concentrations of 1 µg/L, the studied PFCs enhanced oxidative metabolism of mussels. Overall, the results obtained in this study confirm previous findings in the field indicating that an important fraction of PFC accumulated in mussel tissues is eliminated actively by MXR transporters or other processes that are metabolically costly.

Bioremediation

Xiang-Qun Chi^{a, b}, Jun-Jie Zhang^a, Shuo Zhao^{a, b}, Ning-Yi Zhou^a (a Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China, b Graduate School, Chinese Academy of Sciences, Beijing 100049, China). Bioaugmentation with a consortium of bacterial nitrophenol-degraders for remediation of soil contaminated with three nitrophenol isomers. Environmental Pollution, Volume 172(2013) : 33–41

A consortium consisting of *para*-nitrophenol utilizer *Pseudomonas* sp. strain WBC-3, *meta*-nitrophenol utilizer *Cupriavidus necator* JMP134 and *ortho*-nitrophenol utilizer *Alcaligenes* sp. strain NyZ215 was inoculated into soil contaminated with three nitrophenol isomers for bioaugmentation. Accelerated removal of all nitrophenols was achieved in inoculated soils compared to un-inoculated soils, with complete removal of nitrophenols in inoculated soils occurring between 2 and 16 days. Real-time polymerase chain reaction (PCR) targeting nitrophenol-degradation functional genes indicated that the three strains survived and were stable over the course of the incubation period. The abundance of total indigenous bacteria (measured by 16S rRNA gene real-time PCR) was slightly negatively impacted by the nitrophenol

contamination. Denaturing gradient gel electrophoresis profiles of total and group-specific indigenous community suggested a dynamic change in species richness occurred during the bioaugmentation process. Furthermore, Pareto–Lorenz curves and Community organization parameters indicated that the bioaugmentation process had little impact on species evenness within the microbial community.

Keywords: Bioaugmentation; Denaturing gradient gel electrophoresis; Nitrophenol isomers; Pareto–Lorenz curve; Real-time PCR

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Molecular tools in microbial community analysis give access to information on catabolic potential and diversity of microbes. Applied in bioremediation, they could provide a new dimension to improve pollution control. This concept has been demonstrated in the study using atrazine as model pollutant. Bioremediation of the herbicide, atrazine, was analyzed in microcosm studies by bioaugmentation, biostimulation and natural attenuation. Genes from the atrazine degrading pathway *atzA/B/C/D/E/F*, *trzN*, and *trzD* were monitored during the course of treatment and results demonstrated variation in *atzC*, *trzD* and *trzN* genes with time. Change in copy number of *trzN* gene under different treatment processes was demonstrated by real-time PCR. The amplified *trzN* gene was cloned and sequence data showed homology to genes reported in *Arthrobacter* and *Nocardioideis*. Results demonstrate that specific target genes can be monitored, quantified and correlated to degradation analysis which would help in predicting the outcome of any bioremediation strategy.

Keywords *atzA*; *trzN*; Atrazine; Bioaugmentation; Biostimulation; Molecular tools; Natural attenuation

Umar Farooq, Makshoof Athar, Misbahul Ain Khan, Janusz A. Kozinski. Biosorption of Pb(II) and Cr(III) from aqueous solutions: breakthrough curves and modeling studies. Environmental Monitoring and Assessment, Volume 185(1)(2013): 845-854

The sorption capacity parameters obtained for batch studies provide useful information about biosorption system. However, such data fail to explain the process under continuous-flow conditions. The present study is an attempt to explore the biosorption of Pb(II) and Cr(III) by straw from local wheat (*Triticum aestivum*). The biosorbent has been characterized by using Fourier transform infrared spectroscopy and surface area and elemental analyses and found to be porous and polyfunctional. S-shaped breakthrough curves were obtained at different column heights for the both metal ions. Various breakthrough parameters and saturation times have been determined. The column data have been successfully used to study the Bohart–Adams' bed depth service time (BDST) model and Yoon and Nelson's model. It was found that BDST model quite efficiently explained the whole column data whereas Yoon and Nelson model could explain it below 90 % breakthrough concentration. The predicted and calculated BDST parameters were in agreement with each other. Yoon and Nelson's constant decreased with an increase in the column

height for both metal ions. Effect of change in flow rate on the Pb(II) biosorption has also been discussed with respect to BDST approach.

Ondrej Uhlík^a, Mary-Cathrine Leewis^b, Michal Strejcek^a, Lucie Musilova^a, Martina Mackova^{a, 1}, Mary Beth Leigh^b, Tomas Macek^a. (^a Institute of Chemical Technology Prague, Faculty of Food and Biochemical Technology, Department of Biochemistry and Microbiology, Technicka 3, 166 28 Prague, Czech Republic, ^b Institute of Arctic Biology, University of Alaska Fairbanks, 902 N. Koyukuk Dr., Fairbanks, AK 99775-7000, USA). **Stable isotope probing in the metagenomics era: A bridge towards improved bioremediation. *Biotechnology Advances*, Volume 31(2) (2013):154–165**

Microbial biodegradation and biotransformation reactions are essential to most bioremediation processes, yet the specific organisms, genes, and mechanisms involved are often not well understood. Stable isotope probing (SIP) enables researchers to directly link microbial metabolic capability to phylogenetic and metagenomic information within a community context by tracking isotopically labeled substances into phylogenetically and functionally informative biomarkers. SIP is thus applicable as a tool for the identification of active members of the microbial community and associated genes integral to the community functional potential, such as biodegradative processes. The rapid evolution of SIP over the last decade and integration with metagenomics provide researchers with a much deeper insight into potential biodegradative genes, processes, and applications, thereby enabling an improved mechanistic understanding that can facilitate advances in the field of bioremediation.

Keywords: Bioremediation; Biodegradation; Stable isotope probing; Metagenomics; Sequence-based screening; Function-based screening; Gene-targeted metagenomics; High-throughput sequencing; High-throughput microarrays; Carbon flow

P.C. Abhilash^{a, b}, Bindu Singh^a, Pankaj Srivastava^a, Andreas Schaeffer^c, Nandita Singh^a. (^aEco-Auditing Group, National Botanical Research Institute (NBRI), Rana Pratap Marg, Lucknow 226 001, Uttar Pradesh, India, ^b Institute of Environment & Sustainable Development (IESD), Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India, ^cInstitute for Environmental Research (Biology V), RWTH Aachen University, Worringerweg 1, Germany). **Remediation of lindane by *Jatropha curcas* L: Utilization of multipurpose species for rhizoremediation. *Biomass and Bioenergy*, Volume 51(2013): 189–193**

In the present study we demonstrate the rhizoremediation potential of a biodiesel plant *Jatropha curcas* L. against lindane and discuss the field applicability of *Jatropha* based remediation techniques and future research prospects. Six different experimental approaches were conducted to evaluate the phytoremediation potential of *Jatropha* under glasshouse conditions. For this, *Jatropha* plants were grown in garden soil spiked with four increasing concentration of lindane (5, 10, 15 and 20 mg kg⁻¹) and harvested after 45, 180 and 300 d. One set of control plants were grown in lindane free soil and another set of spiked soils were kept without *Jatropha* plants. At every harvesting, plant growth, lindane accumulation in plant parts, residual lindane concentration in soil as well as percentage lindane dissipation from soil were calculated. After 300 d, the accumulation of lindane in *Jatropha* grown in four simulated soils reached up to 5.42, 10.83, 15.95 and 20.85 µg g⁻¹ plant dry matter, respectively. Correspondingly, the residual

lindane soil concentrations in the above four treatments were reduced to 89, 82, 77 and 72% with respect to the applied lindane amounts, respectively. We conclude that *Jatropha* enhances the dissipation of lindane in simulated soil and is useful for onsite remediation.

Keywords: Multipurpose species; Rhizoremediation; *Jatropha curcas* L.; Biodiesel plant; Lindane

Luís A. B. Novo, Emma F. Covelo, Luís González. (¹Department of Plant Biology and Soil Science, University of Vigo, As Lagoas, Marcosende, 36310, Vigo, Spain). **The Potential of *Salvia verbenaca* for Phytoremediation of Copper Mine Tailings Amended with Technosol and Compost. *Water, Air, & Soil Pollution*, 224 (2013):1513**

Unreclaimed mine tailings sites are a worldwide problem. This study evaluates the potential of *Salvia verbenaca* for phytoremediation of copper mine tailings treated with technosol and compost. Ecophysiological results reveal the species ability to thrive in the assessed range of conditions, while the hydrogen peroxide assays exhibit the plant's capacity to successfully respond to metal toxicity, supporting literature reports about its antioxidant capabilities. Furthermore, the results suggest a selective antioxidant response of *S. verbenaca* towards Cd, indicative of a protection mechanism against high concentrations of this element. Moderate concentrations of Cu in the roots, adequate translocation and bioconcentration factors, tolerance to metal toxicity, and ecophysiological characteristics classify *S. verbenaca* as a promising candidate for phytostabilization of mine tailings. The importance of the amendments in order to improve the overall phytostabilization performance is highlighted by the elevated correlations between the treatment properties and the extractable concentrations of trace metals.

Shabnam Khosravihaftkhany, Norhashimah Morad, Tjoon Tow Teng, Ahmad Zuhairi Abdullah, Ismail Norli. (¹School of Industrial Technology, Universiti Sains Malaysia, 11800, Penang, Malaysia, ²School of Chemical Engineering, Universiti Sains Malaysia, 14300, Nibong Tebal, Penang, Malaysia). **Biosorption of Pb(II) and Fe(III) from Aqueous Solutions Using Oil Palm Biomasses as Adsorbents. *Water, Air, & Soil Pollution*, 224(2013): 1455**

The removal of lead (II) and iron (III) from aqueous solutions using empty fruit bunch (EFB), oil palm leaves (OPL), oil palm frond (OPF), and oil palm bark (OPB) as biosorbents was investigated. The biosorbents were characterized through scanning electron microscopy, Brunauer–Emmett–Teller analysis, and Fourier transform infrared spectroscopy. Variables such as pH (2–12), biosorbent particle size (200–1,400 µm), adsorbent dosage (0.25–1.75 g/l), and agitation time (5–80 min) were investigated. The suitable pH range, particle size, adsorbent dosage, and agitation time for the removal of both metals were 5 to 6, 200 µm, 1 g/l, and 40 min, respectively. Under optimum conditions, OPB showed the highest adsorption efficiency of 80 % and 78 % for lead and iron, respectively. The adsorption equilibrium data were fitted to three adsorption isotherm models. The Langmuir isotherm showed the best result for both metals. The kinetics of the biosorption process was analyzed using pseudo-first-order and pseudo-second-order models. The latter showed a better fit for both metals. OPB biomass introduced the lowest chemical oxygen demand into the treated solution, with an average amount of 32.9 mg/l.

Ana Paula Meneghel, Affonso Celso Gonçalves Jr., Fernanda Rubio, Douglas Cardoso Dragunski, Cleber Antonio Lindino, Leonardo Strey. (¹Centro de Ciências Agrárias, Universidade Estadual do Oeste do Paraná, Unioeste, Rua Pernambuco, 1777, Marechal

Cândido Rondon, PR, 85960-000, Brazil, ²Universidade Paranaense, UNIPAR, Praça Mascarenhas de Moraes, s/n, Umuarama, PR, 87502-210, Brazil, ³Centro de Engenharias e Ciências Exatas, Universidade Estadual do Oeste do Paraná, Unioeste, Rua da Faculdade, 645, Jardim La Salle, Toledo, PR, 85903-000, Brazil). Biosorption of Cadmium from Water Using Moringa (*Moringa oleifera* Lam.) Seeds. *Water, Air, & Soil Pollution*, 224(2013):1383

This study aimed to evaluate the efficacy of using the byproduct of *Moringa oleifera* Lam. seeds as an adsorbent for removal of cadmium (Cd) from contaminated water. The material characterization was performed by scanning electron microscopy, infrared spectroscopy, and point of zero charge. The effects of the adsorbent mass, solution pH, contact time, and temperature were evaluated. In the preliminary studies, the mass of adsorbent (200–1200 mg) and pH conditions (5.0, 6.0, and 7.0) were varied. The time studies were performed at 20–180 min and the temperature studies at the range of 25–65 °C. The optimal conditions of adsorption obtained were 400 mg of adsorbent mass, 7.0 pH, and 160 min contact time with the adsorbent. The isotherms of adsorption were linearized according to Langmuir, Freundlich, and Dubinin–Radushkevich (D-R) models. The results showed better fit by the Freundlich and D-R models for Cd adsorption, describing a multilayer adsorption and, according to the value of the sorption energy (E), it has chemical nature. The maximum capacity of adsorption (Q_m) obtained was 7.864 mg g⁻¹. For a comparative study, the activated carbon (P.A.) was used applying the same optimal conditions used in the adsorption isotherms and desorption process for the biosorbent, obtaining a Q_m as 32.884 mg g⁻¹. The average desorption percentage showed that adsorbents have strong interaction with the metal. Based on these results, it was concluded that the biosorbent was effective in remediation of solutions containing Cd and thus the use of this alternative material is a viable option, since it has low cost and it is a byproduct which has not undergone previous treatment.

Renato C. Silva, José A. D. Rodrigues, Suzana M. Ratusznei, Marcelo Zaiat. (¹ Escola de Engenharia Mauá Instituto Mauá de Tecnologia (EEM/IMT), Praça Mauá 1, CEP 09.580-900, São Caetano do Sul, SP, Brazil, ² Departamento de Hidráulica e Saneamento–Escola de Engenharia de São Carlos, Universidade de São Paulo (SHS/EESC/USP), Av. Trabalhador São-Carlense 400, CEP 13.566-590, São Carlos, SP, Brazil). Anaerobic Treatment of Industrial Biodiesel Wastewater by an ASBR for Methane Production. *Applied Biochemistry and Biotechnology*, Volume 170 (1) (2013): 105-118

A mechanically stirred anaerobic sequencing batch reactor (5 L, 30 °C) containing granular biomass was used to treat the effluent of an industrial biodiesel production process with the purpose to produce methane. Process stability and efficiency were analyzed as a function of applied volumetric organic load (AVOL of 1,000 to 3,000 mgCOD/L), reactor feed time, and cycle length (8-h cycles with 10-min or 4-h feeding and 4-h cycles with 10-min or 2-h feeding). Batch operations (B) with 1,000 to 3,000 mgCOD/L involved 10-min feeding/discharge: (1) 1.0-L influent with 4-h cycle and (2) 2.0-L influent with 8-h cycle. Fed-batch operations (FB) with 1,000 to 3,000 mgCOD/L involved 10-min discharge and the following feeding: (1) 1.0-L influent in 2 h with 4-h cycle and (2) 2.0-L influent in 4 h with 8-h cycle. At 1,000 mgCOD/L (AVOL of 18 to 1.29 gCOD/L day), kinetic parameter values were 1.03 and 0.92 h⁻¹ at conditions B-1000-4 h and FB-1000-8/4 h, respectively. At both conditions, removal

efficiency was 88 %, and cycle length could be reduced to 3 h (B-1000-4 h) and 5 h (FB-1000-8/4 h). At 2,000 mgCOD/L (AVOL of 2.38 to 2.52 gCOD/L day), kinetic parameter values were 1.08 and 0.99 h⁻¹ at conditions B-2000-4/2 h and FB-2000-8/4 h, respectively, and removal efficiencies were 83 and 81 %. Cycle length could be reduced to 3 h (B-2000-4/2 h) and 6 h (FB-2000-8/4 h). At 3,000 mgCOD/L (AVOL of 3.71 to 3.89 gCOD/L day), conditions allowing stable operation were B-3000-4 h, FB-3000-8/4 h, and FB-3000-4/2 h. Stability could not be obtained at condition B-3000-8 h, and the best results were obtained at condition FB-3000-8/4 h. Specific methane production ranged from 41.1 to 93.7 NmLCH₄/gCOD, demonstrating reactor application potential and operation flexibility.

Sharrel Rebello, Aju K. Asok, Sunil V. Joseph, Biljo V. Joseph, Leny Jose, Sathish Mundayoor, Jisha M.S. (¹ School of Biosciences, Mahatma Gandhi University, Kottayam, India, ² Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India). **Bioconversion of Sodium Dodecyl Sulphate to Rhamnolipid by *Pseudomonas aeruginosa*: A Novel and Cost-Effective Production Strategy. Applied Biochemistry and Biotechnology, Volume 169 (2) (2013): 418-430**

The utility of rhamnolipids in industry is currently limited due to the high constraints in its economic production. In this scenario, the novel utility of sodium dodecyl sulphate (SDS) as carbon source could serve as promising cost-effective strategy. Screening of effective SDS biodegraders led to the isolation of *Pseudomonas aeruginosa* S15 capable of concomitant SDS degradation and biosurfactant synthesis. SDS-based rhamnolipid production was proved on SDS minimal agar plates using cetyl trimethylammonium bromide–methylene blue method and optimised in SDS-based minimal salt (SBS) medium. SDS proved to be an ideal carbon source for rhamnolipid synthesis with a high substrate to product conversion rate yielding 6.9 g/l of rhamnolipids from 1 g/l SDS in 5 days. Fast atom bombardment mass spectroscopy analysis of the purified biosurfactant proved the presence of mono- and di-rhamnolipids, viz., Rha-C₁₀-C₁₀, Rha-C₁₀-C₁₂ and Rha-Rha-C₁₀-C₁₀ with surface active properties. The secreted rhamnolipids were not utilised by S15 as a carbon source, but it caused a dispersion of bacterial biofilms in SBS medium. To the best of our knowledge, this is the first report on bioconversion of synthetic detergent to biodetergent. This SDS-based novel methodology presents a more economised mode of rhamnolipid synthesis utilising SDS as sole carbon source.

Abbas H. Sulaymon, Ahmed A. Mohammed, Tariq J. Al-Musawi. (¹ Environmental Engineering Department, College of Engineering, Baghdad University, Baghdad, Iraq). **Competitive biosorption of lead, cadmium, copper, and arsenic ions using algae. Environmental Science and Pollution Research, Volume 20 (5)(2013): 3011-3023**

The present study aims to evaluate the competitive biosorption of lead, cadmium, copper, and arsenic ions by using native algae. A series of experiments were carried out in a batch reactor to obtain equilibrium data for adsorption of single, binary, ternary, and quaternary metal solutions. The biosorption of these metals is based on ion exchange mechanism accompanied by the release of light metals such as calcium, magnesium, and sodium. Experimental parameters such as pH, initial metal concentrations, and temperature were studied. The optimum pH found for removal were 5 for Cd²⁺ and As³⁺ and 3 and 4 for Pb²⁺ and Cu²⁺, respectively. Fourier transformation infrared spectroscopy analysis was used to find the effects of functional groups of algae in biosorption process. The results showed that Pb²⁺ made a greater change in the functional groups of algal biomass due to high affinity to this metal. An ion exchange model was

found suitable for describing the biosorption process. The affinity constants sequence calculated for single system was $K_{Pb} > K_{Cu} > K_{Cd} > K_{As}$; these values reduced in binary, ternary, and quaternary systems. In addition, the experimental data showed that the biosorption of the four metals fitted well the pseudo-second-order kinetics model.

Chin Hong Neoh, Adibah Yahya, Robiah Adnan, Zaiton Abdul Majid, Zaharah Ibrahim. (¹ Department of Biological Sciences, Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia, ² Department of Industrial Biotechnology, Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia, ³ Department of Mathematics, Faculty of Sciences, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia, ⁴ Department of Chemistry, Faculty of Sciences, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia). **Optimization of decolorization of palm oil mill effluent (POME) by growing cultures of *Aspergillus fumigatus* using response surface methodology.** *Environmental Science and Pollution Research*, Volume 20 (5) (2013): 2912-2923

The conventional treatment process of palm oil mill effluent (POME) produces a highly colored effluent. Colored compounds in POME cause reduction in photosynthetic activities, produce carcinogenic by-products in drinking water, chelate with metal ions, and are toxic to aquatic biota. Thus, failure of conventional treatment methods to decolorize POME has become an important problem to be addressed as color has emerged as a critical water quality parameter for many countries such as Malaysia. *Aspergillus fumigatus* isolated from POME sludge was successfully grown in POME supplemented with glucose. Statistical optimization studies were conducted to evaluate the effects of the types and concentrations of carbon and nitrogen sources, pH, temperature, and size of the inoculum. Characterization of the fungus was performed using scanning electron microscopy, Fourier transform infrared (FTIR) spectroscopy, and Brunauer, Emmet, and Teller surface area analysis. Optimum conditions using response surface methods at pH 5.7, 35 °C, and 0.57 % w/v glucose with 2.5 % v/v inoculum size resulted in a successful removal of 71 % of the color (initial ADMI of 3,260); chemical oxygen demand, 71 %; ammoniacal nitrogen, 35 %; total polyphenolic compounds, 50 %; and lignin, 54 % after 5 days of treatment. The decolorization process was contributed mainly by biosorption involving pseudo-first-order kinetics. FTIR analysis revealed that the presence of hydroxyl, C–H alkane, amide carbonyl, nitro, and amine groups could combine intensively with the colored compounds in POME. This is the first reported work on the application of *A. fumigatus* for the decolorization of POME. The present investigation suggested that growing cultures of *A. fumigatus* has potential applications for the decolorization of POME through the biosorption and biodegradation processes.

C. Marisa R. Almeida, Izabela Reis, M. Nazaré Couto, Adriano. A. Bordalo, Ana P. Mucha. (¹ CIMAR/CIIMAR—Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123, Porto, Portugal, ³ Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 687, 4169-007, Porto, Portugal, ² Laboratório de Hidrobiologia, Instituto de Ciências Biomédicas, Universidade do Porto (ICBAS-UP), Largo Professor Abel Salazar, no. 2, 4099-003, Porto, Portugal). **Potential of the microbial community**

present in an unimpacted beach sediment to remediate petroleum hydrocarbons. Environmental Science and Pollution Research, Volume 20 (5) (2013): 3176-3184

The potential of the microbial communities present in the intertidal zone of an unimpacted beach (a beach that did not suffer any significant oil spill) to degrade hydrocarbons was investigated. For that, laboratory-based microcosms (50-ml flasks) were set up with sandy beach sediment spiked with crude oil and incubated with local seawater for 15 days in the dark. Three bioremediation treatments were tested (biostimulation (BS), autochthonous bioaugmentation (AB), and combined treatment of biostimulation + bioaugmentation (BS + AB)) and the results were compared with natural attenuation (NA). Visual inspection showed clearly an oil solubility increase (confirmed by a higher hydrocarbons concentration in supernatant solutions) for all tested treatments when compared to NA. Significant degradation of the oil, shown by different profiles of petroleum hydrocarbons, was also observed for the different treatments particularly for BS + AB. Therefore, the microbial community of this unimpacted beach sediment could respond to an oil spill, degrading hydrocarbons. But to increase the natural attenuation pace, obtained results indicated that BS + AB is an appropriate approach for the bioremediation of beaches recently impacted by an oil spill. The autochthonous microbial cultures can be obtained “before” or “after” the contamination of the target site, being inoculated into the site right after its contamination.

Dhia Al-Bader, Mayada K. Kansour, Rehab Rayan, Samir S. Radwan. (¹ Department of Biological Sciences, Faculty of Science, Kuwait University, PO Box 5969, Safat, 13060, Kuwait). Biofilm comprising phototrophic, diazotrophic, and hydrocarbon-utilizing bacteria: a promising consortium in the bioremediation of aquatic hydrocarbon pollutants. Environmental Science and Pollution Research, Volume 20 (5) (2013): 3252-3262

Biofilms harboring simultaneously anoxygenic and oxygenic phototrophic bacteria, diazotrophic bacteria, and hydrocarbon-utilizing bacteria were established on glass slides suspended in pristine and oily seawater. Via denaturing gradient gel electrophoresis analysis on PCR-amplified rRNA gene sequence fragments from the extracted DNA from biofilms, followed by band amplification, biofilm composition was determined. The biofilms contained anoxygenic phototrophs belonging to alphaproteobacteria; pico- and filamentous cyanobacteria (oxygenic phototrophs); two species of the diazotroph *Azospirillum*; and two hydrocarbon-utilizing gammaproteobacterial genera, *Cycloclasticus* and *Oleibacter*. The coexistence of all these microbial taxa with different physiologies in the biofilm makes the whole community nutritionally self-sufficient and adequately aerated, a condition quite suitable for the microbial biodegradation of aquatic pollutant hydrocarbons.

Muhammad Z. Iqbal, Ahmed A. Abdala. (¹ Department of Chemical Engineering, The Petroleum Institute, P.O. Box 2533, Abu Dhabi, United Arab Emirates). Oil spill cleanup using grapheme. Environmental Science and Pollution Research, Volume 20 (5) (2013): 3271-3279

In this article, we study the use of thermally reduced graphene (TRG) for oil spill cleanup. TRG was synthesized by thermal exfoliation of graphite oxide and characterized by X-ray diffraction, Raman spectroscopy, SEM, TEM, elemental analysis, and Brunauer–Emmett–Teller (BET) surface area measurement. Various aspects of the sorption process have been studied including the sorption capacity, the recovery of the adsorbed oil, and the recyclability of TRG. Our results show that TRG has a higher sorption capacity than any other carbon-based sorbents, with

sorption capacity as high as 131 g of oil per gram TRG. With recovery of the sorbed oil via filtration and reuse of TRG for up to six cycles, 1 g of TRG collectively removes approximately 300 g of crude oil. Moreover, the effects of TRG bulk density, pore volume, and carbon/oxygen ratio and the oil viscosity on the sorption process are also discussed.

V. K. Gupta, Deepak Pathania, Shilpi Agarwal, Shikha Sharma. (¹ Department of Chemistry, Indian Institute of Technology, Roorkee, 247667, India, ² Department of Chemistry, King Fahd University of Petroleum and Minerals, Dhahran, 31261, Saudi Arabia, ³ School of Chemistry, Shoolini University, Solan, 173212, Himachal Pradesh, India). **Removal of Cr(VI) onto *Ficus carica* biosorbent from water. Environmental Science and Pollution Research, Volume 20 (4) (2013): 2632-2644**

The utilization of sustainable and biodegradable lignocellulosic fiber to detoxify the noxious Cr(VI) from wastewater is considered a versatile approach to clean up a contaminated aquatic environment. The aim of the present research is to assess the proficiency and mechanism of biosorption on *Ficus carica* bast fiber via isotherm models (Langmuir, Freundlich, Temkin, Harkin's–Jura, and Dubinin–Radushkevich), kinetic models, and thermodynamic parameters. The biomass extracted from fig plant was characterized by scanning electron microscopy and Fourier-transform infrared spectroscopy. To optimize the maximum removal efficiency, different parameters like effect of initial concentration, effect of temperature, pH, and contact time were studied by batch method. The equilibrium data were best represented by the Langmuir isotherm model, and the maximum adsorption capacity of Cr(VI) onto biosorbent was found to be 19.68 mg/g. The pseudo-second-order kinetic model adequately described the kinetic data. The calculated values of thermodynamic parameters such as enthalpy change (ΔH^0), entropy change (ΔS^0), and free energy change (ΔG^0) were 21.55 kJ/mol, 76.24 J/mol K, and -1.55 kJ/mol, respectively, at 30 °C which accounted for spontaneous and endothermic processes. The study of adsorbent capacity for Cr(VI) removal in the presence of Na⁺, Mg²⁺, Ca²⁺, SO₄²⁻, HCO₃⁻ and Cl⁻ illustrated that the removal of Cr(VI) increased in the presence of HCO₃⁻ ions; the presence of Na⁺, SO₄²⁻ or Cl⁻ showed no significant influence on Cr(VI) adsorption, while Ca²⁺ and Mg²⁺ ions led to an insignificant decrease in Cr(VI) adsorption. Further, the desorption studies illustrated that 31.10 % of metal ions can be removed from an aqueous system, out of which 26.63 % of metal ions can be recovered by desorption in first cycle and the adsorbent can be reused. The results of the scale-up study show that the ecofriendly detoxification of Cr(VI) from aqueous systems was technologically feasible.

Baoguo Zhang, Ruimei Fan, Zhihui Bai, Shan Wang, Liang Wang, Jiping Shi. (¹ Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai, 201210, China, ² Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China). **Biosorption characteristics of *Bacillus gibsonii* S-2 waste biomass for removal of lead (II) from aqueous solution. Environmental Science and Pollution Research, Volume 20 (3) (2013): 1367-1373**

Lead (II) has been as one of the most toxic heavy metals because it is associated with many health hazards. Therefore, people are increasingly interested in discovering new methods for effectively and economically scavenging lead (II) from the aquatic system. Recent studies demonstrate biosorption is a promising technology for the treatment of pollutant streams. To

apply these techniques, suitable adsorbents with high efficiency and low cost are demanded. The waste biomass of *Bacillus gibsonii* S-2 biosorbent was used as low-cost biosorbent to remove metallic cations lead (II) from aqueous solution. To optimize the maximum removal efficiency, the effect of pH and temperature on the adsorption process was studied. The isotherm models, kinetic models and thermodynamic parameters were analysed to describe the adsorptive behaviour of *B. gibsonii* S-2 biosorbent. The mechanisms of lead (II) biosorption were also analysed by FTIR and EDX. The results showed that the optimum pH values for the biosorption at three different temperatures, i.e. 20, 30 and 40 °C, were determined as 4. The equilibrium data were well fitted to Langmuir model, with the maximum lead (II) uptake capacities of 333.3 mg g⁻¹. The kinetics for lead (II) biosorption followed the pseudo-second-order kinetic equation. The thermodynamic data showed that the biosorption process were endothermic ($\Delta G < 0$), spontaneous ($\Delta H > 0$) and irreversible ($\Delta S > 0$). The mechanism of lead (II) biosorption by the waste biomass of *B. gibsonii* S-2 biosorbent could be a combination of ion exchange and complexation with the functional groups present on the biosorbent surface. The application of the waste biomass of *B. gibsonii* S-2 for lead (II) adsorption, characterized with higher lead (II) sorption capacity and lower cost, may find potential application in industrial wastewater treatment.

Cristina Quintelas, Filomena Costa, Teresa Tavares. (¹IBB—Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal). **Bioremoval of diethylketone by the synergistic combination of microorganisms and clays: uptake, removal and kinetic studies.** *Environmental Science and Pollution Research*, Volume 20(3) (2013): 1374-1383

The performance of two bacteria, *Arthrobacter viscosus* and *Streptococcus equisimilis*, and the effect of the interaction of these bacteria with four different clays on the retention of diethylketone were investigated in batch experiments. The uptake, the removal percentages and the kinetics of the processes were determined. *S. equisimilis*, by itself, had the best performance in terms of removal percentage, for all the initial diethylketone concentrations tested: 200, 350 and 700 mg/L. The uptake values are similar for both bacteria. A possible mechanism to explain the removal of diethylketone includes its degradation by bacteria, followed by the adsorption of the intermediates/sub-products by the functional groups present on the cells' surfaces. The assays performed with bacteria and clays indicated that the uptake values are similar despite of the clay used, for the same microorganism and mass of clay, but in general, higher values are reached when *S. equisimilis* is used, compared to *A. viscosus*. Kinetic data were described by pseudo-first- and pseudo-second-order models.

Zhengkui Li, Yueming Wang, Ningmei Wu, Qichun Chen, Kai Wu. (¹ State Key Laboratory of Pollutant Control and Resource Reuse, Nanjing, 210046, People's Republic of China, ² School of the Environment, Nanjing University, 163 Xianlin Avenue, Nanjing, 210046, People's Republic of China). **Removal of heavy metal ions from wastewater by a novel HEA/AMPS copolymer hydrogel: preparation, characterization, and mechanism.** *Environmental Science and Pollution Research*, Volume 20 (3) (2013): 1511-1525

This study aims to synthesize 2-hydroxyethyl acrylate (HEA) and 2-acrylamido-2-methylpropane sulfonic (AMPS) acid-based hydrogels by gamma radiation and to investigate their swelling behavior and heavy metal ion adsorption capabilities. The copolymer hydrogels prepared were characterized via scanning electron microscopy, Fourier transformed infrared

spectra, thermal gravimetric analysis, and X-ray photoelectron spectroscopy. The research showed that the copolymer hydrogel was beneficial for permeation due to its porous structure. In addition, the experimental group A-2-d [70 % water volume ratio and $(n(\text{AMPS})/n(\text{HEA})) = 1:1$] was an optimal adsorbent. The optimal pH was 6.0 and the optimal temperature was 15 °C. Pb^{2+} , Cd^{2+} , Cu^{2+} , and Fe^{3+} achieved adsorption equilibria within 24 h, whereas Cr^{3+} reached equilibrium in 5 h. Pb^{2+} , Cd^{2+} , Cr^{3+} , and Fe^{3+} maximum load capacity was 1,000 mg L⁻¹, whereas the Cu^{2+} maximum capacity was 500 mg L⁻¹. The priority order in the multicomponent adsorption was $\text{Cr}^{3+} > \text{Fe}^{3+} > \text{Cu}^{2+} > \text{Cd}^{2+} > \text{Pb}^{2+}$. The adsorption process of the HEA/AMPS copolymer hydrogel for the heavy metal ions was mainly due to chemisorption, and was only partly due to physisorption, according to the pseudo-second-order equation and Langmuir adsorption isotherm analyses. The HEA/AMPS copolymer hydrogel was confirmed to be an effective adsorbent for heavy metal ion adsorption.

Anuprita D. Watharkar, Rahul V. Khandare, Apurva A. Kamble, Asma Y. Mulla, Sanjay P. Govindwar, Jyoti P. Jadhav. (¹Department of Biotechnology, Shivaji University, Kolhapur, 416 004, India, ²Department of Biochemistry, Shivaji University, Kolhapur, India). **Phytoremediation potential of *Petunia grandiflora* Juss., an ornamental plant to degrade a disperse, disulfonated triphenylmethane textile dye Brilliant Blue G.** *Environmental Science and Pollution Research*, Volume 20 (2) (2013): 939-949

Phytoremediation provides an ecofriendly alternative for the treatment of pollutants like textile dyes. The purpose of this study was to explore phytoremediation potential of *Petunia grandiflora* Juss. by using its wild as well as tissue-cultured plantlets to decolorize Brilliant Blue G (BBG) dye, a sample of dye mixture and a real textile effluent. In vitro cultures of *P. grandiflora* were obtained by seed culture method. The decolorization experiments were carried out using wild as well as tissue-cultured plants independently. The enzymatic analysis of the plant roots was performed before and after decolorization of BBG. Metabolites formed after dye degradation were analyzed using UV-vis spectroscopy, high-performance liquid chromatography, Fourier transform infrared spectroscopy, and gas chromatography-mass spectrometry. Phytotoxicity studies were performed. Characterization of dye mixture and textile effluent was also studied. The wild and tissue-cultured plants of *P. grandiflora* showed the decolorized BBG up to 86 %. Significant increase in the activities of lignin peroxidase, laccase, NADH-2,6-dichlorophenol-indophenol reductase, and tyrosinase was found in the roots of the plants. Three metabolites of BBG were identified as 3-[[ethyl(phenyl)amino]methyl]benzenesulfonic acid, 3-[[methyl(phenyl)amino]methyl]benzenesulfonic acid, and sodium-3-[(cyclohexa-2,5-dien-1-ylideneamino)methyl]benzenesulfonate. Textile effluent sample and a synthetic mixture of dyes were also decolorized by *P. grandiflora*. Phytotoxicity test revealed the nontoxic nature of metabolites. *P. grandiflora* showed the potential to decolorize and degrade BBG to nontoxic metabolites. The plant has efficiently treated a sample of dye mixture and textile effluent.

Mingming Sun, Yongming Luo, Ying Teng, Zhongjun Jia, Zhengao Li, Shiping Deng. (¹Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China, ²Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK, 74078, USA). **Remediation of polycyclic aromatic hydrocarbon and metal-contaminated soil by successive methyl-β-**

cyclodextrin-enhanced soil washing–microbial augmentation: a laboratory evaluation. Environmental Science and Pollution Research, Volume 20(2) (2013): 976-986

Polycyclic aromatic hydrocarbon (PAH) and metal-polluted sites caused by abandoned coking plants are receiving wide attention. To address the associated environmental concerns, innovative remediation technologies are urgently needed. This study was initiated to investigate the feasibility of a cleanup strategy that employed an initial phase, using methyl- β -cyclodextrin (MCD) solution to enhance *ex situ* soil washing for extracting PAHs and metals simultaneously, followed by the addition of PAH-degrading bacteria (*Paracoccus* sp. strain HPD-2) and supplemental nutrients to treat the residual soil-bound PAHs. Elevated temperature (50 °C) in combination with ultrasonication (35 kHz, 30 min) at 100 g MCD L⁻¹ was effective in extracting PAHs and metals to assist soil washing; 93 % of total PAHs, 72 % of Cd, 78 % of Ni, 93 % of Zn, 84 % of Cr, and 68 % of Pb were removed from soil after three successive washing cycles. Treating the residual soil-bound PAHs for 20 weeks led to maximum biodegradation rates of 34, 45, 36, and 32 % of the remaining total PAHs, 3-ring PAHs, 4-ring PAHs, and 5(+6)-ring PAHs after washing procedure, respectively. Based on BIOLOG Ecoplate assay, the combined treatment at least partially restored microbiological functions in the contaminated soil. The *ex situ* cleanup strategy through MCD-enhanced soil washing followed by microbial augmentation can be effective in remediating PAH and metal-contaminated soil.

Avinash A. Kadam, Jeevan D. Kamatkar, Rahul V. Khandare, Jyoti P. Jadhav, Sanjay P. Govindwar. (¹Department of Biotechnology, Shivaji University, Kolhapur, 416004, India, ²Department of Biochemistry, Shivaji University, Kolhapur, 416004, India). **Solid-state fermentation: tool for bioremediation of adsorbed textile dyestuff on distillery industry waste-yeast biomass using isolated *Bacillus cereus* strain EBT1. Environmental Science and Pollution Research, Volume 20 (2) (2013): 1009-1020**

Bioremediation of textile dyestuffs under solid-state fermentation (SSF) using industrial wastes as substrate pose an economically feasible, promising, and eco-friendly alternative. The purpose of this study was to adsorb Red M5B dye, a sample of dyes mixture and a real textile effluent on distillery industry waste-yeast biomass (DIW-YB) and its further bioremediation using *Bacillus cereus* EBT1 under SSF. Textile dyestuffs were allowed to adsorb on DIW-YB. DIW-YB adsorbed dyestuffs were decolorized under SSF by using *B. cereus*. Enzyme analysis was carried out to ensure decolorization of Red M5B. Metabolites after dye degradation were analyzed using UV-Vis spectroscopy, FTIR, HPLC, and GC-MS. DIW-YB showed adsorption of Red M5B, dyes mixture and a textile wastewater sample up to 87, 70, and 81 %, respectively. DIW-YB adsorbed Red M5B was decolorized up to 98 % by *B. cereus* in 36 h. Whereas *B. cereus* could effectively reduce American Dye Manufacture Institute value from DIW-YB adsorbed mixture of textile dyes and textile wastewater up to 70 and 100 %, respectively. Induction of extracellular enzymes such as laccase and azoreductase suggests their involvement in dye degradation. Repeated utilization of DIW-YB showed consistent adsorption and ADMI removal from textile wastewater up to seven cycles. HPLC and FTIR analysis confirms the biodegradation of Red M5B. GC-MS analysis revealed the formation of new metabolites. *B. cereus* has potential to bioremediate adsorbed textile dyestuffs on DIW-YB. *B. cereus* along with DIW-YB showed enhanced decolorization performance in tray bioreactor which suggests its potential for large-scale treatment procedures.

Shubha Nigam, Padma S. Vankar, Krishna Gopal. (¹CSIR-Indian Institute of Toxicology Research, M. G. Marg, Lucknow, 226 001, India, ²Indian Institute of Technology, Kanpur,

India). Biosorption of arsenic from aqueous solution using dye waste. Environmental Science and Pollution Research, Volume 20 (2) (2013):1161-1172

The purpose of this study is to examine on removal of arsenic from water by biosorption through potential application of herbal dye wastes. Four different flower dye residues (after extraction of natural dye) viz. *Hibiscus rosasinensis*, *Rosa rosa*, *Tagetes erecta*, and *Canna indica* were utilized successfully for the removal of arsenic from aqueous solution. Batch studies were carried out for various parameters viz. pH, sorbent dose, contact time, initial metal ion concentration, and temperature. Data were utilized for isothermal, kinetic, and thermodynamic studies. Scanningelectron microscopy (SEM), energy-dispersive x-ray spectroscopy (EDAX), and Fourier transform infrared (FTIR) analyses of biomass were performed. The results showed that 1 g/100 ml for 5.0–5.5 h contact time at pH 6.0–7.5 with agitation rate 150 rpm provided 98, 96, 92, and 85 % maximum absorption of arsenic by *R. rosa*, *H. rosasinensis*, *T. erecta*, and *C. indica*, respectively, at initial concentration of 500 ppb. Data followed Langmuir isotherm showing sorption to bemonolayer on heterogeneous surface of biosorbent. Negative values of ΔG° indicated spontaneous nature, whereas ΔH° indicates exothermic nature of system followed by pseudo-first-order adsorption kinetics. FTIR results showed apparent changes in functional group regions after metal chelation. SEM and EDAX analyses showed the changes in surface morphology of all test biosorbents. Herbal dye wastes, used as biosorbent, exhibited significant (85–98 %) removal of arsenic from aqueous solution. Hence, these biosorbents are cost-effective, easily available, eco-friendly, and comparatively more effective than other biosorbents already in use. These may be used to remove arsenic and other toxic metals from water.

Awatif Yateem. (¹Biotechnology Department, Kuwait Institute for Scientific Research, P.O. Box 24885, 13109, Safat, Kuwait). Rhizoremediation of oil-contaminated sites: a perspective on the Gulf War environmental catastrophe on the State of Kuwait. Environmental Science and Pollution Research, Volume 20(1) (2013): 100-107

The Gulf War brought about to the State of Kuwait some of the worst environmental pollution as a result of oil spill. Since 1995, research programs have been initiated to avoid further damage to theKuwaiti desert and marine environment and to restore and rehabilitate the polluted land, water, and air ecosystems. During the following 15 years, different bioremediation methods both on laboratory and small field scales were tested and evaluated. The findings of these studies were implemented to establish a bio-park in which ornamental shrubs and trees were grown in bioremediated soil. This review will focus on Kuwait's experience in rhizoremediation and its positive impacts on oil-contaminated sites.

Habib Khoudi, Yafa Maatar, Faiçal Brini, Amine Fourati, Najoua Ammar, Khaled Masmoudi. (¹Laboratory of Plant Protection and Improvement, Center of Biotechnology of Sfax (CBS), University of Sfax, Route Sidi Mansour Km. 6, B.P '1177', 3018, Sfax, Tunisia, ²Research Center on Phosphates and Phosphoric Acid: Groupe Chimique Tunisien (GCT), BP "S", 3003, Sfax, Tunisia). Phytoremediation potential of *Arabidopsis thaliana*, expressing ectopically a vacuolar proton pump, for the industrial waste phosphogypsum. Environmental Science and Pollution Research, Volume 20 (1) (2013): 270-280

Phosphogypsum (PG) is a by-product of the phosphorus–fertiliser industry and represents an environmental concern since it contains pollutants such as cadmium (Cd). We have recently

shown that the overexpression of a proton pump gene (*TaVPI*) in transgenic tobacco (*Nicotiana tabacum*) led to an enhanced Cd tolerance and accumulation. The aim of this study was to evaluate the potential of transgenic *Arabidopsis thaliana* plants harbouring the *TaVPI* gene to phytoremediate phosphogypsum. A pot experiment was carried out under greenhouse conditions. Transgenic *A. thaliana* plants harbouring the *TaVPI* gene were grown on various substrates containing phosphogypsum (0, 25, 50 and 100 %) for 40 days. At the end of the growth period, we examined the growth (germination, root length, fresh weight) and physiological parameters (chlorophyll and protein contents, catalase activity and proteolysis) as well as the cadmium, Mg, Ca, and P contents of the *A. thaliana* plants. In order to evaluate Cd tolerance of the *A. thaliana* lines harbouring the *TaVPI* gene, an in vitro experiment was also carried out. One week-old seedlings were transferred to Murashige and Skoog agar plates containing various concentrations of cadmium; the germination, total leaf area and root length were determined. The growth and physiological parameters of all *A. thaliana* plants were significantly altered by PG. The germination capacity, root growth and biomass production of wild-type (WT) plants were more severely inhibited by PG compared with the *TaVPI* transgenic *A. thaliana* lines. In addition, *TaVPI* transgenic *A. thaliana* plants maintained a higher antioxidant capacity than the WT. Interestingly, elemental analysis of leaf material derived from plants grown on PG revealed that the transgenic *A. thaliana* line accumulated up to ten times more Cd than WT. Despite its higher Cd content, the transgenic *A. thaliana* line performed better than the WT counterpart. In vitro evaluation of Cd tolerance showed that *TaVPI* transgenic *A. thaliana* lines were more Cd-tolerant than the WT plants. These results suggested that ectopic expression of a vacuolar proton pump in *A. thaliana* plants can lead to various biotechnological applications including the phytoremediation of industrial wastes.

Wendan Xiao, Huan Wang, Tingqiang Li, Zhiqiang Zhu, Jie Zhang, Zhenli He, Xiaoe Yang. (¹Ministry of Education Key Laboratory of Environmental Remediation and Ecological Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, 310058, China, ²Institute of Food and Agricultural Sciences, Indian River Research and Education Center, University of Florida, Fort Pierce, FL, 34945, USA). **Bioremediation of Cd and carbendazim co-contaminated soil by Cd-hyperaccumulator *Sedum alfredii* associated with carbendazim-degrading bacterial strains. Environmental Science and Pollution Research, Volume 20 (1) (2013): 380-389**

The objective of this study was to develop a bioremediation strategy for cadmium (Cd) and carbendazim co-contaminated soil using a hyperaccumulator plant (*Sedum alfredii*) combined with carbendazim-degrading bacterial strains (*Bacillus subtilis*, *Paracoccus* sp., *Flavobacterium* and *Pseudomonas* sp.). A pot experiment was conducted under greenhouse conditions for 180 days with *S. alfredii* and/or carbendazim-degrading strains grown in soil artificially polluted with two levels of contaminants (low level, 1 mg kg⁻¹ Cd and 21 mg kg⁻¹ carbendazim; high level, 6 mg kg⁻¹ Cd and 117 mg kg⁻¹ carbendazim). Cd removal efficiencies were 32.3–35.1 % and 7.8–8.2 % for the low and high contaminant level, respectively. Inoculation with carbendazim-degrading bacterial strains significantly ($P < 0.05$) increased Cd removal efficiencies at the low level. The carbendazim removal efficiencies increased by 32.1–42.5 % by the association of *S. alfredii* with carbendazim-degrading bacterial strains, as compared to control, regardless of contaminant level. Cultivation with *S. alfredii* and inoculation of carbendazim-degrading bacterial strains increased soil microbial biomass, dehydrogenase activities and microbial diversities by 46.2–121.3 %, 64.2–143.4 %, and 2.4–24.7 %, respectively. Polymerase chain reaction-denaturing gradient

gel electrophoresis (PCR-DGGE) analysis revealed that *S. alfredii* stimulated the activities of *Flavobacteria* and *Bradyrhizobiaceae*. The association of *S. alfredii* with carbendazim-degrading bacterial strains enhanced the degradation of carbendazim by changing microbial activity and community structure in the soil. The results demonstrated that association of *S. alfredii* with carbendazim-degrading bacterial strains is promising for remediation of Cd and carbendazim co-contaminated soil.

I. Vitte, R. Duran, G. Hernandez-Raquet, J. Mounier, R. Jézéquel, V. Bellet, P. Balaguer, P. Caumette, C. Cravo-Laureau. (¹Equipe Environnement et Microbiologie, IPREM UMR/CNRS 5254, Université de Pau, 64013, Pau Cedex, France, ⁶Département Biologie, Recherche et Développement, Laboratoire des Pyrénées, Rue des Ecoles, 64150, Lagor, France, ²UR050 Laboratoire de Biotechnologie de l'Environnement, INRA, Avenue des Etangs, Narbonne, 11100, France, ³Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, UMR5504, UMR792, CNRS, INRA, INSA, Avenue de Rangueil, 31077, Toulouse Cedex 4, France, ⁴Cedre Centre de Documentation, de Recherche et d'Expérimentations sur les pollutions accidentelles des eaux, 715 rue Alain Colas, CS 41836, 29818, Brest Cedex 2, France, ⁵IRCM - Institut de Recherche en Cancérologie de Montpellier INSERM U896 - UM1 - CRLC Val d'Aurelle - Parc Euromédecine, 34298, Montpellier Cedex 5, France). Dynamics of metabolically active bacterial communities involved in PAH and toxicity elimination from oil-contaminated sludge during anoxic/oxic oscillations. *Applied Microbiology and Biotechnology*, Volume 97(9) (2013): 4199-4211

The kinetics of polycyclic aromatic hydrocarbons (PAH) elimination from a contaminated sludge were determined in bioreactors under different conditions: continuously oxic, anoxic, and anoxic/oxic oscillations. The dynamics of metabolically active bacterial communities and their involvement in PAH degradation were followed by T-RFLP targeting 16S rRNA and ring hydroxylating dioxygenase (RHD) transcripts, respectively. PAH degradation was related to toxicity elimination using an aryl hydrocarbon receptor-responsive reporter cell line. Oxygen supply was identified as the main factor affecting the structure of bacterial communities and PAH removal. PAH-degrading bacterial communities were stable throughout the experiment in all conditions according to the presence of RHD transcripts, indicating that bacterial communities were well adapted to the presence of pollutants. Oxic and anoxic/oxic oscillating conditions showed similar levels of PAH removal at the end of the experiment despite several anoxic periods in oscillating conditions. These results highlight the role of dioxygenase activity after oxygen addition. Nevertheless, the higher toxicity elimination observed under oxic conditions suggests that some metabolites or other unidentified active compounds persisted under oscillating and anoxic conditions. Our results emphasize the importance of using complementary biological, chemical and toxicological approaches to implement efficient bioremediation strategies.

H. L. Eaton, J. M. Durringer, L. D. Murty, A. M. Craig. (¹Department of Microbiology, Oregon State University, Corvallis, OR, 97331, USA, ²Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, 97331, USA, ³Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR, 97331, USA, ⁴Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State

University, 101 Magruder Hall, Corvallis, OR, 97331, USA). Anaerobic bioremediation of RDX by ovine whole rumen fluid and pure culture isolates. Applied Microbiology and Biotechnology, Volume 97(8) (2013): 3699-3710

The ability of ruminal microbes to degrade the explosive compound hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in ovine whole rumen fluid (WRF) and as 24 bacterial isolates was examined under anaerobic conditions. Compound degradation was monitored by high-performance liquid chromatography analysis, followed by liquid chromatography–tandem mass spectrometry identification of metabolites. Organisms in WRF microcosms degraded 180 µM RDX within 4 h. Nitroso-intermediates hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) were present as early as 0.25 h and were detected throughout the 24-h incubation period, representing one reductive pathway of ring cleavage. Following reduction to MNX, peaks consistent with m/z 193 and 174 were also produced, which were unstable and resulted in rapid ring cleavage to a common metabolite consistent with an m/z of 149. These represent two additional reductive pathways for RDX degradation in ovine WRF, which have not been previously reported. The 24 ruminal isolates degraded RDX with varying efficiencies (0–96 %) over 120 h. Of the most efficient degraders identified, *Clostridium polysaccharolyticum* and *Desulfovibrio desulfuricans* subsp. *desulfuricans* degraded RDX when medium was supplemented with both nitrogen and carbon, while *Anaerovibrio lipolyticus*, *Prevotella ruminicola*, and *Streptococcus bovis* IFO utilized RDX as a sole source of nitrogen. This study showed that organisms in whole rumen fluid, as well as several ruminal isolates, have the ability to degrade RDX in vitro and, for the first time, delineated the metabolic pathway for its biodegradation.

Łukasz Ławniczak, Roman Marecik, Łukasz Chrzanowski. (¹Institute of Chemical Technology and Engineering, Poznan University of Technology, Pl. M. Skłodowskiej-Curie 2, 60-965, Poznań, Poland, ²Department of Biotechnology and Food Microbiology, University of Life Sciences in Poznań, Wojska Polskiego 48, 60-627, Poznań, Poland). Contributions of biosurfactants to natural or induced bioremediation. Applied Microbiology and Biotechnology, Volume 97(6) (2013): 2327-2339,

The number of studies dedicated to evaluating the influence of biosurfactants on bioremediation efficiency is constantly growing. Although significant progress regarding the explanation of mechanisms behind biosurfactant-induced effects could be observed, there are still many factors which are not sufficiently elucidated. This corresponds to the fact that although positive influence of biosurfactants is often reported, there are also numerous cases where no or negative effect was observed. This review summarizes the recent finding in the field of biosurfactant-amended bioremediation, focusing mainly on a critical approach towards potential limitations and causes of failure while investigating the effects of biosurfactants on the efficiency of biodegradation and phytoextraction processes. It also provides a summary of successive steps, which should be taken into consideration when designing biosurfactant-related treatment processes.

Xiao-Yan Fu, Wei Zhao, Ai-Sheng Xiong, Yong-Sheng Tian, Bo Zhu, Ri-He Peng, Quan-Hong Yao. (¹Shanghai Key Laboratory of Agricultural Genetics and Breeding, Biotechnology Research Institute, Shanghai Academy of Agricultural Sciences, 2901 Beidi Road, Shanghai, People's Republic of China). Phytoremediation of triphenylmethane dyes

by overexpressing a *Citrobacter* sp. triphenylmethane reductase in transgenic *Arabidopsis*. Applied Microbiology and Biotechnology, Volume 97 (4) (2013): 1799-1806

Triphenylmethane dyes are extensively utilized in textile industries, medicinal products, biological stains, and food processing industries, etc. They are generally considered as xenobiotic compounds, which are very recalcitrant to biodegradation. The widespread persistence of such compounds has generated concerns with regard to remediation of them because of their potential carcinogenicity, teratogenicity, and mutagenicity. In this study, we present a system of phytoremediation by *Arabidopsis* plants developed on the basis of overexpression of triphenylmethane reductase (TMR) from the *Citrobacter* sp. The morphology and growth of TMR transgenic *Arabidopsis* plants showed significantly enhanced tolerances to crystal violet (CV) and malachite green (MG). Further, HPLC and HPLC-MS analyses of samples before and after dye decolorization in culture media revealed that TMR transgenic plants exhibited strikingly higher capabilities of removing CV from their media and high efficiencies of converting CV to non-toxic leucocrystal violet (LCV). This work indicates that microbial degradative gene may be transgenically exploited in plants for bioremediation of triphenylmethane dyes in the environment.

Hong Zong, Bin Zhuge, Huiying Fang, Yanhui Cao, Lin Mu, Weilai Fu, Jian Song, Jian Zhuge. (¹The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, 214122, China, ²School of Chemical and Material Engineering, Jiangnan University, Wuxi, 214122, China). **Advances in the bioconversion mechanism of lovastatin to wuxistatin by *Amycolatopsis* sp. CGMCC 1149. Applied Microbiology and Biotechnology, Volume 97(2) (2013): 599-609**

Wuxistatin, a novel statin and more potent than lovastatin, was converted from lovastatin by *Amycolatopsis* sp. (CGMCC 1149). Product I, an intermediate product, was found in the fermentation broth, and the structure analysis showed that product I had an additional hydroxyl group at the methyl group attached to C3 compared to lovastatin, which indicates that product I is one isomer of wuxistatin. Isotope tracing experiment proved that hydroxyl group of wuxistatin was provided by product I and the reaction from product I to wuxistatin was an intramolecular transfer. Hydroxylation reaction established in a cell-free system could be inhibited by CO and enhanced by ATP, Fe²⁺, and ascorbic acid, which were consistent with the presumption that the hydroxylase was an induced cytochrome P450. Study on proteomics of *Amycolatopsis* sp. CGMCC 1149 suggested that three identified proteins, including integral membrane protein, Fe-S oxidoreductase, and GTP-binding protein YchF, were induced by lovastatin and required during hydroxylation reaction. In conclusion, bioconversion mechanism of wuxistatin by *Amycolatopsis* sp. CGMCC 1149 was proposed: lovastatin is firstly hydroxylated to product I by a hydroxylase, namely cytochrome P450, and then product I is rearranged to wuxistatin by isomerases.

Biotransformation

Carlos Huitrón, Rosalba Pérez, Luís Gutiérrez, Patricia Lappe, Pavel Petrosyan, Jesús Villegas, Cecilia Aguilar, Leticia Rocha-Zavaleta, Abel Blancas. **Bioconversion of *Agave tequilana* fructans by exo-inulinases from indigenous *Aspergillus niger* CH-A-2010**

enhances ethanol production from raw *Agave tequilana* juice. Journal of Industrial Microbiology & Biotechnology, Volume 40(1) (2013): 123-132

Agave tequilana fructans are the source of fermentable sugars for the production of tequila. Fructans are processed by acid hydrolysis or by cooking in ovens at high temperature. Enzymatic hydrolysis is considered an alternative for the bioconversion of fructans. We previously described the isolation of *Aspergillus niger* CH-A-2010, an indigenous strain that produces extracellular inulinases. Here we evaluated the potential application of *A. niger* CH-A-2010 inulinases for the bioconversion of *A. tequilana* fructans, and its impact on the production of ethanol. Inulinases were analyzed by Western blotting and thin layer chromatography. Optimal pH and temperature conditions for inulinase activity were determined. The efficiency of *A. niger* CH-A-2010 inulinases was compared with commercial enzymes and with acid hydrolysis. The hydrolysates obtained were subsequently fermented by *Saccharomyces cerevisiae* to determine the efficiency of ethanol production. Results indicate that *A. niger* CH-A-2010 predominantly produces an exo-inulinase activity. Optimal inulinase activity occurred at pH 5.0 and 50 °C. Hydrolysis of raw agave juice by CH-A-2010 inulinases yielded 33.5 g/l reducing sugars, compared with 27.3 g/l by Fructozyme® (Novozymes Corp, Bagsværd, Denmark) and 29.4 g/l by acid hydrolysis. After fermentation of hydrolysates, we observed that the conversion efficiency of sugars into ethanol was 97.5 % of the theoretical ethanol yield for enzymatically degraded agave juice, compared to 83.8 % for acid-hydrolyzed juice. These observations indicate that fructans from raw *Agave tequilana* juice can be efficiently hydrolyzed by using *A. niger* CH-A-2010 inulinases, and that this procedure impacts positively on the production of ethanol.

F. Xavier Simon, Elisabet Rudé, Joan Llorens, Sylvie Baig. (¹Department of Chemical Engineering, University of Barcelona, C/ Martí i Franquès 1, 08028, Barcelona, Spain, ²Degrémont SA, 183 avenue du 18 juin 1940, 92508, Rueil-Malmaison cedex, France). **Study of Seawater Biofiltration by Measuring Adenosine Triphosphate (ATP) and Turbidity. Water, Air, & Soil Pollution, Volume 224 (2013): 1568**

In the present study, we examined seawater biofiltration in terms of adenosine triphosphate (ATP) and turbidity. A pilot biofilter continuously fed with fresh seawater reduced both turbidity and biological activity measured by ATP. Experiments operated with an empty bed contact time (EBCT) of between 2 and 14 min resulted in cellular ATP removals of 32 to 60 % and turbidity removals of 38 to 75 %. Analysis of the water from backwashing the biofilter revealed that the first half of the biofilter concentrated around 80 % of the active biomass and colloidal material that produces turbidity. By reducing the EBCT, the biological activity measured as cellular ATP concentration moved from the first part of the biofilter to the end. Balances of cellular ATP and turbidity between consecutive backwashings indicated that the biological activity generated in the biofilter represented more than 90 % of the detached cellular ATP. In contrast, the effectively trapped ATP was less than 10 % of the overall cellular ATP detached during the backwashing process. Furthermore, the biological activity measured as cellular ATP generated in the biofilter seemed to be more dependent on the elapsed time than the volume filtered. In contrast, the turbidity trapped in the biofilter was proportional to the volume filtered, although a slightly higher amount of turbidity was found in the backwashing water; this was probably due to attrition of the bed medium. Finally, no correlations were found between turbidity and ATP, indicating that the two parameters focus on different matter. This suggests that turbidity should not be used as an alternative to cellular concentration.

Wangliang Li, Xia Jiang. (¹National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, 100190, People's Republic of China, ²Institute of Environmental Science and Engineering, Nanyang Technological University, Singapore, 63772, Singapore, ³College of Architecture and Environment, Sichuan University, No. 24, South Section 1, First Ring Road, Chengdu, 610065, Sichuan, People's Republic of China). Enhancement of bunker oil biodesulfurization by adding surfactant. *World Journal of Microbiology and Biotechnology*, Volume 29 (1) (2013): 103-108

Biodesulfurization (BDS) is a promising method to remove sulfur compounds from diesel and gasoline. However, the information on BDS of heavy oil is scanty, which might be due to their "undesirable" physical properties and more complicated sulfur diversities. In this study, the BDS of one kind of heavy oil, bunker oil MFO380 was investigated. The biocatalyst was obtained by the enrichment with oil sludge as the seed and using dibenzothiophene (DBT) as the sole sulfur source. The enriched biocatalyst (microbial mixed culture) could selectively remove sulfur from DBT and DBT was transformed into 2-hydroxybiphenyl, which indicates that the BDS process is beneficial to non-destructive carbon bonds and thus can maintain the calorific value. The bunker oil BDS results showed that after 7 days of incubation, the removal efficiency of sulfur in MFO380 was only 2.88 %, but this could be significantly improved by adding surfactants Triton X-100 or Tween 20. This effect could be attributed to greatly reduced viscosity of heavy oil and increased mass transfer of sulfur compounds in heavy oil into water. Adding Triton X-100 achieved the highest removal efficiency of sulfur, up to 51.7 % after 7 days of incubation. The optimal amount of Triton X-100 was 0.5 g/50 ml medium. When toluene was added as an organic solvent for MFO380, the BDS activity was improved, while lower than the effect of adding surfactants.

Shengnan Shi, Fang Ma, Tieheng Sun, Ang Li, Jiti Zhou, Yuanyuan Qu. (¹State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin, 150090, China, ²Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), School of Environmental Science and Technology, Dalian University of Technology, Dalian, 116024, China). Biotransformation of Indole to Indigo by the Whole Cells of Phenol Hydroxylase Engineered Strain in Biphase Systems. *Applied Biochemistry and Biotechnology*, Volume 169 (4) (2013): 1088-1097

Biotransformation of indole to indigo in liquid-liquid biphasic systems was performed in *Escherichia coli* cells expressing phenol hydroxylase. It was suggested that indole could inhibit the cell growth even at low concentration of 0.1 g/L. The critical Log *P* for strain PH_IND was about 5.0. Three different solvents, i.e., decane, dodecane, and dioctyl phthalate, were selected as organic phase in biphasic media. The results showed that dodecane gave the highest yield of indigo (176.4 mg/L), which was more than that of single phase (90.5 mg/L). The optimal conditions for biotransformation evaluated by response surface methodology were as follows: 540.26 mg/L of indole concentration, 42.27 % of organic phase ratio, and 200 r/min of stirrer speed; under these conditions, the maximal production of indigo was 243.51 mg/L. This study proved that the potential application of strain PH_IND in the biotransformation of indole to indigo using liquid-liquid biphasic systems.

Jun Zeng, Xiangui Lin, Jing Zhang, Hong Zhu, Hong Chen, Ming Hung Wong. (¹State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Beijing East Road, 71, Nanjing, 210008, People's Republic of China, ²Joint Open Laboratory of Soil and the Environment, Hong Kong Baptist University and Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, People's Republic of China, ⁶Department of Biology and Biochemistry, Institute of Soil Science, Chinese Academy of Sciences, Beijing East Road, 71, Nanjing, 210008, People's Republic of China, ³Graduate University of Chinese Academy of Sciences, Beijing, 100049, People's Republic of China, ⁴Soil and Environment Analysis Center, Institute of Soil Science, Chinese Academy of Science, Nanjing, People's Republic of China, ⁵Croucher Institute for Environmental Sciences, Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, People's Republic of China). **Successive transformation of benzo[*a*]pyrene by laccase of *Trametes versicolor* and pyrene-degrading *Mycobacterium* strains. *Applied Microbiology and Biotechnology*, Volume 97 (7) (2013): 3183-3194**

We previously hypothesized that polycyclic aromatic hydrocarbon (PAH)-degrading bacteria that produce laccase may enhance the degree of benzo[*a*]pyrene mineralization. However, whether the metabolites of benzo[*a*]pyrene oxidized by laccase can be further transformed by PAH degraders remains unknown. In this study, pyrene-degrading mycobacteria with diverse degradation properties were isolated and employed for investigating the subsequent transformation on the metabolites of benzo[*a*]pyrene oxidized by fungal laccase of *Trametes versicolor*. The results confirm the successive transformation of benzo[*a*]pyrene metabolites, 6-benzo[*a*]pyrenyl acetate, and quinones by *Mycobacterium* strains, and report the discovery of the involvement of a *O*-methylation mediated pathway in the process. In detail, the vast majority of metabolite 6-benzo[*a*]pyrenyl acetate was transformed into benzo[*a*]pyrene quinones or methoxybenzo[*a*]pyrene, via two distinct steps that were controlled by the catechol-*O*-methyltransferase mediated *O*-methylation, while quinones were reduced to dihydroxybenzo[*a*]pyrene and further transformed into dimethoxy derivatives.

Juan C. López, Guillermo Quijano, Theo S. O. Souza, José M. Estrada, Raquel Lebrero, Raúl Muñoz. (¹Department of Chemical Engineering and Environmental Technology, University of Valladolid, Dr. Mergelina s/n, 47011, Valladolid, Spain). **Biotechnologies for greenhouse gases (CH₄, N₂O, and CO₂) abatement: state of the art and challenges. *Applied Microbiology and Biotechnology*, Volume 97 (6) (2013): 2277-2303**

Today, methane (CH₄), nitrous oxide (N₂O), and carbon dioxide (CO₂) emissions represent approximately 98 % of the total greenhouse gas (GHG) inventory worldwide, and their share is expected to increase significantly in this twenty-first century. CO₂ represents the most important GHG with approximately 77 % of the total GHG emissions (considering its global warming potential) worldwide, while CH₄ and N₂O are emitted to a lesser extent (14 and 8 %, respectively) but exhibit global warming potentials 23 and 298 times higher than that of CO₂, respectively. Most members of the United Nations, based on the urgent need to maintain the global average temperature 2 °C above preindustrial levels, have committed themselves to significantly reduce their GHG emissions. In this context, an active abatement of these emissions will help to achieve these target emission cuts without compromising industrial growth. Nowadays, there are sufficient empirical evidence to support that biological technologies can become, if properly tailored, a low-cost and environmentally friendly alternative to physical/chemical methods for the abatement of GHGs. This study constitutes a state-of-the-art review of the microbiology (biochemistry, kinetics, and waste-to-value processes) and bioreactor

technology of CH₄, N₂O, and CO₂ abatement. The potential and limitations of biological GHG degradation processes are critically discussed, and the current knowledge gaps and technology niches in the field are identified.

Francisco J. Cervantes, Claudia M. Martínez, Jorge Gonzalez-Estrella, Arturo Márquez, Sonia Arriaga. (¹División de Ciencias Ambientales, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), Camino a la Presa San José 2055, Col. Lomas 4^a Sección, San Luis Potosí, 78216, Mexico, ²Department of Biotechnology, Norwegian University of Science and Technology (NTNU), 7491, Trondheim, Norway). **Kinetics during the redox biotransformation of pollutants mediated by immobilized and soluble humic acids. Applied Microbiology and Biotechnology, Volume 97 (6) (2013): 2671-2679**

The aim of this study was to elucidate the kinetic constraints during the redox biotransformation of the azo dye, Reactive Red 2 (RR2), and carbon tetrachloride (CT) mediated by soluble humic acids (HA_s) and immobilized humic acids (HA_i), as well as by the quinoid model compounds, anthraquinone-2,6-disulfonate (AQDS) and 1,2-naphthoquinone-4-sulfonate (NQS). The microbial reduction of both HA_s and HA_i by anaerobic granular sludge (AGS) was the rate-limiting step during decolorization of RR2 since the reduction of RR2 by reduced HA_i proceeded at more than three orders of magnitude faster than the electron-transferring rate observed during the microbial reduction of HA_i by AGS. Similarly, the reduction of RR2 by reduced AQDS proceeded 1.6- and 1.9-fold faster than the microbial reduction of AQDS by AGS when this redox mediator (RM) was supplied in soluble and immobilized form, respectively. In contrast, the reduction of NQS by AGS occurred 1.6- and 19.2-fold faster than the chemical reduction of RR2 by reduced NQS when this RM was supplied in soluble and immobilized form, respectively. The microbial reduction of HA_s and HA_i by a humus-reducing consortium proceeded 1,400- and 790-fold faster than the transfer of electrons from reduced HA_s and HA_i, respectively, to achieve the reductive dechlorination of CT to chloroform. Overall, the present study provides elucidation on the rate-limiting steps involved in the redox biotransformation of priority pollutants mediated by both HA_s and HA_i and offers technical suggestions to overcome the kinetic restrictions identified in the redox reactions evaluated.

Gustavo Molina, Mariana R. Pimentel, Gláucia M. Pastore. (¹Laboratory of Bioflavors, Department of Food Science, Faculty of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil). ***Pseudomonas*: a promising biocatalyst for the bioconversion of terpenes. Applied Microbiology and Biotechnology, Volume 97(5) (2013): 1851-1864**

The *Pseudomonas* genus is one of the most diverse and ecologically significant groups of known bacteria, and it includes species that have been isolated worldwide in all types of environments. The bacteria from this genus are characterized by an elevated metabolic versatility, which is due to the presence of a complex enzymatic system. Investigations since the early 1960s have demonstrated their potential as biocatalysts for the production of industrially relevant and value-added flavor compounds from terpenes. Although terpenes are often removed from essential oils as undesirable components, its synthetic oxy-functionalized derivatives have broad applications in flavors/fragrances and pharmaceutical industries. Hence, biotransformation appears to be an effective tool for the structural modification of terpene hydrocarbons and terpenoids to synthesize novel and high-valued compounds. This review highlights the potential

of *Pseudomonas* spp. as biocatalysts for the bioconversion of terpenes and summarizes the presently known bioflavors that are obtained from these processes.

S.S. Gauri^{1,2}, S.M. Mandal³, S. Atta⁴, S. Dey², B.R. Pati^{1,*}. (¹Department of Microbiology, Vidyasagar University, Midnapore, West Bengal, India, ²Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur, India, ³Central Research Facility, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal, India, ⁴Department of Chemistry, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal, India, * Correspondence author: Bikas R. Pati, HOD, Department of Microbiology, Vidyasagar University, Midnapore 721 102, West Bengal, India. E-mail: brpati@yahoo.com). **Novel route of tannic acid biotransformation and their effect on major biopolymer synthesis in *Azotobacter* sp. SSB81. Journal of Applied Microbiology, Volume 114(1) (2013): 84–95**

To examine tannic acid (TA) utilization capacity by nitrogen-fixing bacteria, *Azotobacter* sp. SSB81, and identify the intermediate products during biotransformation. Another aim of this work is to investigate the effects of TA on major biopolymers like extracellular polysaccharide (EPS) and polyhydroxybutyrate (PHB) synthesis.

Tannic acid utilization and tolerance capacity of the strain was determined according to CLSI method. Intermediate products were identified using high-performance liquid chromatography, LC-MS/MS and ¹H NMR analysis. Intermediates were quantified by multiple reactions monitoring using LC-MS/MS. The strain was able to tolerate a high level of TA and utilized through enzymatic system. Growth of *Azotobacter* in TA-supplemented medium was characterized by an extended lag phase and decreased growth rate. Presence of TA catalytic enzymes as tannase, polyphenol oxidase (PPO) and phenol decarboxylase was confirmed in cell lysate using their specific substrates. PPO activity was more prominent in TA-supplemented mineral medium after 48 h of growth when gallic to ellagic acid (EA) reversible reaction was remarkable. Phase contrast and scanning electron microscopic analysis revealed elongated and irregular size of *Azotobacter* cells in response to TA. ¹H NMR analysis indicated that TA was transformed into gallic acid (GA), EA and pyrogallol. Biopolymer (EPS and PHB) production was decreased several folds in the presence of TA compared with cells grown in only glucose medium.

This is the first evidence on the biotransformation of TA by *Azotobacter* and also elevated level of EA production from gallotannins. *Azotobacter* has developed the mechanism to utilize TA for their carbon and energy source.

The widespread occurrence and exploitation of *Azotobacter* sp. strain SSB81 in agricultural and forest soil have an additional advantage to utilize the soil-accumulated TA and detoxifies the allelopathic effect of constant accumulated TA in soil.

Keywords: *Azotobacter* sp; biotransformation; ellagic acid; tannic acid

Biomarker

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Department of Biosciences, Åbo Akademi University, Turku, Finland, ²Örebro Life Science Center, School of Science and Technology, Örebro University, Örebro, Sweden, ³Cefas Weymouth Laboratory, Weymouth, United Kingdom. Email: Tom Wiklund (twiklund@abo.fi). *Laboratory of Aquatic Pathobiology, Environmental and Marine Biology, Department of Biosciences, Åbo Akademi University, Turku, Finland). Assessment of reproductive biomarkers in three-spined stickleback (*Gasterosteus aculeatus*) from sewage effluent recipients. *Environmental Toxicology*, Volume 28 (4) (2013): 229–237

The aim of this study was to examine the occurrence of endocrine disruption close to sewage treatment plant effluent discharges along the Finnish Baltic Sea coast using a set of reproductive biomarkers present in adult three-spined stickleback (*Gasterosteus aculeatus*). Possible variation and sensitivity of the biomarkers during an entire reproductive period were also examined. The analysis of vitellogenin (VTG) for estrogenic activity and spiggin for androgenic activity, together with histopathological analysis indicated that sticklebacks were exposed to estrogenic loads sufficient to cause inappropriate production of VTG and to disrupt normal testicular structure in adult male sticklebacks. No androgenic disruption was observed. The results emphasize the need of a combination of several reproductive biomarkers in fish and repeated sampling for the detection of potential endocrine modulating substances under field condition.

Keywords: biomonitoring; endocrine disruption; histopathology; spiggin; three-spined stickleback; vitellogenin

Fang Wei¹, Jieping Yang², David T.W. Wong. (UCLA School of Dentistry, UCLA Dental Research Institute, 73-017 Center for Health Sciences, 10833 Le Conte Ave., University of California, Los Angeles, CA 90095-1668, USA). Detection of exosomal biomarker by electric field-induced release and measurement (EFIRM). *Biosensors and Bioelectronics*, Volume 44 (15) (2013): 115–121

Exosomes biomarkers mediating important biological process, especially in the systemic disease diagnostics and therapeutics, yet the protective exosomal vesicle structure hinders rapid, simple detection of the harbored molecules. We have established a new method, the electric field-induced release and measurement (EFIRM), which can simultaneously disrupt exosomes to release the contents and on-site monitoring the harbored exosomal RNA/proteins biomarkers. When exposed to a non-uniform electrical field, exosomal RNA and proteins are rapidly released. Bio-recognition of these biomolecules is carried out concurrently. We tested the hypothesis that the lung cancer cell line, H460 stably transfected with hCD63-GFP, would shed hCD63-GFP expressing exosomes that could be detected in serum and saliva. We confirmed *in vivo* that H460-CD63-GFP shed exosomes were transported to blood and saliva. This result demonstrates for the first time tumor-shed exosomes were detected in saliva, in addition to blood, presenting a new translational utility of exosome-based biomarker detection in saliva.

Keywords: Exosome; Electrochemical sensors; Tumor biomarkers; Lung cancer; Salivary diagnostics

Biofertilizer

Lata Nain¹, Anuj Rana¹, Monica Joshi¹, Shrikrishna D. Jadhav¹, Dinesh Kumar², Y. S. Shivay², Sangeeta Paul¹ and Radha Prasanna¹. (¹Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi, 110012, India, ²Division of Agronomy, Indian Agricultural Research Institute, New Delhi, 110012, India). Evaluation of synergistic effects of bacterial and cyanobacterial strains as biofertilizers for wheat. *Plant and Soil*, Volume 331(1-2) (2010): 217-230

An investigation was undertaken to screen, select and evaluate a set of bacterial and cyanobacterial isolates from the wheat rhizosphere for their role as biofertilizers in wheat. From an initial set of 23 cyanobacterial strains and 110 bacterial isolates from wheat rhizospheric soil, 3 bacterial and 3 cyanobacterial strains were selected based on their plant growth promoting potential under laboratory and controlled greenhouse conditions. In vitro compatibility studies revealed positive interactions among the six strains. Pot experiments were conducted with wheat variety *HD 2687*, with a total of 51 treatments, along with recommended fertilizer controls. Various combinations of the selected set of three bacterial (PW1, PW5 and PW7) and three cyanobacterial isolates (CW1, CW2 and CW3) were used along with 1/3 N and full dose of P and K fertilizers. Significant enhancement in the soil microbiological (Dehydrogenase activity, FDA hydrolase, Alkaline phosphatase and microbial biomass) and plant growth/yield parameters were recorded. Observations revealed a two-fold increase in panicle weight in selected combinations (PW1+PW7+CW3; PW1+ CW1+CW2/CW1+CW3; CW2+CW3), as compared to control treatment involving full dose of chemical fertilizers. Such combinations, which also provided N savings of 40–80 kg N/ha are being further evaluated in field experiments. This study for the first time illustrated the positive and dynamic interactions among bacterial and cyanobacterial strains and their promise in integrated nutrient management of wheat crop.

Biocomposting

S. Mariraj Mohan^{*}, K. Hafsa^{}. (*Assistant Professor in Civil Engineering, Government College of Engineering, Bodinayakanur, Tamilnadu, INDIA. Email: mari_sundar@yahoo.com.**PG Graduate, Environmental Engineering, A.C. College of Engg. & Tech., Karaikudi, INDIA). Biodegradation of food waste and raw vegetable peels through composting and vermicomposting using *sp. eudrilus eugeniae*. *Journal of Solid Waste Technology & Management*, Volume 39(1)(2013): 25**

Comparative study was performed to conclude the high efficient manure production from the food waste and vegetable waste by changing the mixing ratio in nine trays. Composting and vermicomposting was done simultaneously. Vermicomposting using *sp. Eudrilus eugenia* for 60 days is conducted after 5 days of pre composting. Three different ratios were employed such as 60:40 ; 70:30 and 80:20. For each ratio three trays were used. In 60:40 ratio ; first tray composed of cow dung and food waste ; second tray comprises of cow dung and vegetable waste; in third tray cow dung + food waste + vegetable waste were used as bedding material. This bedding was same for reaming ratios 70:30 and 80:20 . This bedding arrangement was same for composting and vermicomposting. The N P K value of the manure in each tray was estimated after the completion of the experiment in both composting and vermicomposting. The multiplication of earthworms in terms of number was calculated at the end of vermicomposting. The second type of bedding i.e. combination of cow dung and vegetable waste was suitable in vermicomposting.

The high cow dung composition (80:20 ratio) suited best for composting and vermicomposting. Vermicomposting was found to be efficient quick degradation method than composting.

Keywords: sp.*Eudrilus eugeniae*, Food waste, Vegetable waste, Cow dung, N P K, cocoons

Shivani Chaturvedi^{1,*}, Ashwani Kumar², Balraj Singh³, Lata Nain⁴, Monica Joshi⁴, Santosh Satya² (Department of Chemistry, Indian Institute of Technology, New Delhi, India, ²Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi, India, ³Centre for Protected Cultivation Technology, Indian Agricultural Research Institute, New Delhi, India, ⁴Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India. * Correspondence: Department of Chemistry, Indian Institute of Technology, New Delhi-110016, India, E-mail: shivani.d123@gmail.com; ashwaniitd@hotmail.com, Phone: +91 11 24121075, Fax: +91 11 26591121). **Bioaugmented composting of *Jatropha* de-oiled cake and vegetable waste under aerobic and partial anaerobic conditions. Journal of Basic Microbiology, Volume 53(4) (2013): 327–335**

This study was conducted to assess the effect of microbial inoculation in *Jatropha* cake composting with different vegetable waste. The microbial inoculums composed of fungal strains (*Aspergillus awamori*, *Aspergillus nidulans*, *Trichoderma viride*, *Phanerochaete chrysosporium*) and bacterial inoculums (*Pseudomonas striata* as phosphorus solublizer and *Azotobacter chroococcum* as nitrogen fixer) were added to the compost mixture after the thermophilic phase was over for bioaugmenting of *Jatropha* cake under aerobic and partial anaerobic conditions. Addition of both fungal and bacterial inoculum with mixed substrate (*Jatropha* cake + vegetable waste) during composting (aerobic and partial anaerobic) showed, better results as compared to compost with only fungal inoculants. Increased enzymatic activity initially, during composting (like dehydrogenase, alkaline phosphatase activity and FDA) proved role of inoculated microbes in rapid decomposition. Analysis of compost (with both bacterial and fungal inoculum) showed presence of high humus (12.7%), humic acid (0.5%), fulvic acid (5.68%), soluble protein content and low C/N ratio. Decreased in concentration of extractable metals (Cu, Fe and Mn) were recorded at maturity in all the substrate composts. The C/N ratio was significantly correlated to parameters like humic acid, humus, fulvic acid, protein and also microbial activity parameters. We conclude that the composting of de-oiled *Jatropha* cake with different vegetables waste could be feasible and sustainable approach in recycling of agricultural and industrial residues in huge quantities.

Keywords: *Jatropha*; Organic fertilization; Compost; Inoculants

Biopesticides

Eric. B. Brennan. (United States Department of Agriculture/Agricultural Research Service, U.S. Agricultural Research Station, 1636 East Alisal Street, Salinas, CA 93905, USA.). **Agronomic aspects of strip intercropping lettuce with alyssum for biological control of aphids. Biological Control Volume 65, (3), (2013):302–311.**

Organic lettuce growers in California typically use insectary strips of alyssum (*Lobularia maritima* (L.) Desv.) to attract hoverflies (Syrphidae) that provide biological control of aphids. A two year study with transplanted organic romaine lettuce in Salinas, California investigated agronomic aspects of lettuce monoculture and lettuce-alyssum strip intercropping on beds in replacement intercropping treatments where alyssum transplants replaced 2 to 8% of the lettuce transplants and in additive intercropping treatments where alyssum transplants were added to the standard lettuce density without displacing lettuce transplants. Alyssum and lettuce dry matter (DM) were determined at lettuce maturity. Alyssum transplants produced less shoot DM in the additive than in the replacement intercropping treatments. The number of open inflorescences of alyssum increased with alyssum DM, and among treatments ranged from 2 to 15 inflorescences per lettuce head. Compared with monoculture lettuce, lettuce heads on intercropped beds were slightly smaller and had lower nitrogen concentrations in the both additive treatments and in some replacement treatments. This research provides the first information on a novel additive intercropping approach to provide alyssum floral resources for biological control of lettuce aphids, and suggests that this approach may be a more land-efficient particularly for producing smaller lettuce heads for romaine hearts or for markets with less strict size requirements. Additional research is needed to determine if the increased competition between alyssum and lettuce in additive intercropping would reduce lettuce yields for wholesale markets with larger head size requirements. Practical aspects of implementing the various intercropping arrangements and alternatives are discussed.

Keywords: Intercropping; Organic farming; Biological control of aphids; Lettuce; Alyssum; Vegetable production;

Ivica Dimkića, Svetlana Živkovićb, Tanja Berića, Žarko Ivanovićb, Veljko Gavrilovićb, Slaviša Stankovića, Djordje Firać. (^a Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia. ^b Institute for Plant Protection and the Environment, Teodora Drajzera 9, 11000 Belgrade, Serbia.). **Characterization and evaluation of two *Bacillus* strains, SS-12.6 and SS-13.1, as potential agents for the control of phytopathogenic bacteria and fungi. *Biological Control* Volume 65 (3), (2013):312–321.**

Two strains of *Bacillus* sp., SS-12.6 and SS-13.1, showed very strong antibacterial and antifungal activity against phytopathogens. The PCR analysis showed that both strains have the genes for biosynthesis of iturin, bacillomycin and surfactin. Kinetics of production of antimicrobial substances in these strains showed that synthesis started at the beginning of exponential phase of growth. Maximum of activity was slowly reached at the beginning of stationary growth phase and was maintained until the end of observed period. Ethyl acetate extracts of cell-free supernatants of both strains were particularly active against several postharvest fungal pathogens, *in vitro* and *in vivo*, in the experiment with apple fruits. Mass spectrometry analysis of ethyl acetate extract of the supernatant of strain SS-12.6 confirmed the presence of antimicrobial lipopeptide surfactin.

Keywords: Bacillus; Lipopeptides; Phytopathogens; Biocontrol.

Qin Yua,¹ Zhu Liua,¹ Derun Lina, Wei Zhangb, Qun Sunc, Jianqing Zhud, Min Linb. (^aDepartment of Biology, Shantou University, Shantou 515063, PR China. ^b Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China. ^cCollege of Life Sciences, Sichuan University, Chengdu 610064, PR China. ^d Rice Research Institute, Sichuan Agricultural University, Wenjiang 611130, PR China). **Characterization**

and evaluation of *Staphylococcus* sp. strain LZ16 for the biological control of rice blast caused by *Magnaporthe oryzae*. *Biological Control*, Volume 65 (3), (2013):338–347.

Staphylococcus sp. strain LZ16 was isolated from seawater collected in the East China Sea. Both culture filtrate and cell lysate of LZ16 possessed strong growth inhibition activities against *Magnaporthe oryzae*. Morphological observations revealed that conidial germination, germ tube elongation, appressorium formation were significantly inhibited after the treatment with cell lysate of LZ16. The active substances remained stable at temperature from 7 to 45 °C, and pH from 6 to 8. One of the fractions conferring strong fungistatic activities was separated using Phenyl-Sepharose CL-4B and Sephacryl S-200 High resolution columns, and identified as purine nucleoside phosphorylase (PNP) by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry. The gene *deoD* encoding PNP was cloned and expressed in *Escherichia coli* for the verification of the antagonistic activity against *M. oryzae*. This is the first report that PNP was associated with fungistatic activity. Field experiments showed that the extract of the bacterial culture prevented and alleviated the disease severity of *M. oryzae* infection compared to the level in the untreated control. The acute toxicity study revealed the lethal oral dose was more than 5000 mg/kg, and the adverse effect was not observed on both male and female rats after treatment for 14 days. All the results suggested that strain LZ16 and its bioactive substances might be promising candidates for biopesticide development, or transgenic plant materials for the biocontrol of rice blast.

Keywords: *Staphylococcus* sp. strain LZ16; Antagonistic activity; Purine nucleoside phosphorylase; Rice blast.

Todd A. Ugine^a, Stephen P. Wraight^b, John P. Sanderson^a. (^a Cornell University, Department of Entomology, Ithaca, NY 14853, United States. ^b USDA-ARS Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853, United States.). Microbial biological control potential of three strains of *Beauveria bassiana* s. l. against greenhouse shore fly *Scatella tenuicosta*: Assessment of virulence, mass production capacity, and effects on shore fly reproduction. *Biological Control*, Volume 65 (3), (2013):348–356.

The microbial biological control potential of three strains of *Beauveria bassiana* sensu lato originally isolated from the shore fly *Scatella tenuicosta* (Diptera: Ephydriidae) was assessed in a series of laboratory bioassays. Comparisons were made to two commercially-available strains of *B. bassiana*. Two of the shore fly strains proved 27–67 times more virulent than the commercial strains in terms of LC₅₀ (14–17 vs. 458–942 conidia/mm²) and killed shore flies more rapidly. *B. bassiana* s. l. strain ST1 exhibited a mass production capacity comparable to the commercial *B. bassiana* strain GHA, producing 2.8×10^{12} conidia/kg barley-based solid substrate in ventilated mushroom spawn bags. The shore fly strains of *Beauveria* sporulated on a higher percentage of killed adult shore flies and produced substantially greater numbers of conidia per cadaver than the commercial strains, indicating that these pathogens are well adapted to this host. Female shore flies treated with strain ST1 survived for only 5 days, with longevity being reduced by 8–10 days compared to control insects. This reduction in survival had a large impact on total lifetime egg production, reducing it by 78–88%, depending on the time of treatment relative to the pre-oviposition period. However, fungal growth within infected female shore flies had no effect on egg production or egg viability until the day before the flies succumbed to mycosis (day 4 post-inoculation). As a consequence, the intrinsic rate of shore fly population increase and

population doubling time were little affected by fungal infection (0.4357 vs. 0.4152 and 1.6 vs. 1.7 days for control vs. *Beauveria*-treated populations, respectively). These findings underscore the challenges involved with use of slow-acting pathogens for control of highly fecund greenhouse pests and the fundamental necessity of integrating these agents into integrated pest management systems.

Keywords: Shore fly; *Scatella tenuicosta*; Fecundity; Entomopathogenic fungus; *Beauveria bassiana*; Virulence

Christopher D. Williams^a, Aoife B. Dillon^b, Robbie D. Girling^{a, 1}, Christine T. Griffin^a. (^aBehavioural Ecology and Biocontrol Laboratory, Department of Biology, National University of Ireland Maynooth, Co. Kildare, Ireland. ^b Coillte Teoranta, Newtownmountkennedy, Co. Wicklow, Ireland.). **Organic soils promote the efficacy of entomopathogenic nematodes, with different foraging strategies, in the control of a major forest pest: A meta-analysis of field trial data. Biological Control, Volume 65 (3), (2013):357–364.**

The large pine weevil, *Hylobius abietis*, is a serious pest of reforestation in northern Europe. However, weevils developing in stumps of felled trees can be killed by entomopathogenic nematodes applied to soil around the stumps and this method of control has been used at an operational level in the UK and Ireland. We investigated the factors affecting the efficacy of entomopathogenic nematodes in the control of the large pine weevil spanning 10 years of field experiments, by means of a meta-analysis of published studies and previously unpublished data. We investigated two species with different foraging strategies, the ‘ambusher’ *Steinernema carpocapsae*, the species most often used at an operational level, and the ‘cruiser’ *Heterorhabditis downesi*. Efficacy was measured both by percentage reduction in numbers of adults emerging relative to untreated controls and by percentage parasitism of developing weevils in the stump. Both measures were significantly higher with *H. downesi* compared to *S. carpocapsae*. General linear models were constructed for each nematode species separately, using substrate type (peat versus mineral soil) and tree species (pine versus spruce) as fixed factors, weevil abundance (from the mean of untreated stumps) as a covariate and percentage reduction or percentage parasitism as the response variable. For both nematode species, the most significant and parsimonious models showed that substrate type was consistently, but not always, the most significant variable, whether replicates were at a site or stump level, and that peaty soils significantly promote the efficacy of both species. Efficacy, in terms of percentage parasitism, was not density dependent.

Keywords: Forest pest management; Pine weevil; Entomopathogenic nematodes; Inundative biological control

P. Vanaclocha^a, D. Papacek^b, C. Monzó^c, M.J. Verdú^a, A. Urbaneja^a. (^a Unidad de Entomología, Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Carretera Moncada-Náquera Km. 4,5, 46113 Moncada, Valencia, Spain. ^b Bugs for Bugs, Bowen Street, Mundubbera 4626, Qld, Australia. ^cDepartment of Entomology and Nematology, University of Florida/IFAS, Southwest Florida Research and Education Center, 2685 SR 29N, Immokalee, FL 34142, USA). **Intra-guild interactions between the parasitoid *Aphytis lingnanensis* and the predator *Chilocorus circumdatus*: Implications for the biological control of armoured scales. Biological Control Volume 65 (2), (2013): 169–175.**

The parasitoid *Aphytis lingnanensis* and the predator *Chilocorus circumdatus* are released in different crops to control armoured scales. Both natural enemies compete, to some extent, for the same resource and therefore they can induce intraguild predation interactions (IGP). In the present work, the consequences of these interactions on the parasitism and predatory efficiency of these natural enemies were assessed under laboratory conditions by studying potential changes in their functional responses.

A type II functional response to host/prey density was observed in *A. lingnanensis* and *C. circumdatus* when acting alone. The predatory efficiency of *C. circumdatus* was not affected by the presence of *A. lingnanensis* in the same arena. Conversely, the parasitism efficiency of *A. lingnanensis* was affected by the presence of the predator. Due to IGP at low host densities there was a shift from functional response type II to type III. No changes in the handling time when the predator was present suggested that parasitism behavior was not influenced by the presence of the predator. *C. circumdatus* did not discriminate between parasitised and unparasitised scales. A recommended strategy in biological control programs could be the use of one of the two natural enemies at low infestation levels and to reinforce these releases with the other one at high densities of the pest.

Keywords: Biological control; Armoured scales; Functional response; Intraguild predation (IGP); Citrus.

Xinhua Fu^a, V. Benno Meyer-Rochow^{b, c}. (^aHubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, China. ^bJacobs University Bremen, Faculty of Engineering & Sciences, D-28725 Bremen, Germany. ^c Department of Biology, University of Oulu, SF-90014 Oulu, Finland). Larvae of the firefly *Pyrocoelia pectoralis* (Coleoptera: Lampyridae) as possible biological agents to control the land snail *Bradybaena ravida*. *Biological Control*, Volume 65 (2), (2013), 176–183.

Rearing experiments with the firefly *Pyrocoelia pectoralis* demonstrated that the species can be successfully bred under laboratory and field conditions and that there are two types of larva: overwintering and non-overwintering. Comparisons showed that the differentiation between the two larval types occurred after the third larval stage. In the field, non-overwintering larvae pupate in September, emerge in October and produce a second annual generation, while overwintering larvae begin to grow more slowly from the 3rd instar onward, then overwinter to ultimately reach a larger size than the non-overwintering larvae and to pupate in September. Adults emerge in October. Larval development at 15, 20, 25, 30 and 35 °C was investigated under a photoperiod of $L:D = 12:12$. At 15 °C all larvae died as 4th instars, but from 20 °C to 30 °C larval phases became increasingly shorter, while at 35 °C they lengthened again. Larval feeding capacity increased with higher temperature up to 30 °C, but decreased at 35 °C. Under three photoperiods, i.e., $L:D = 16:8$, $12:12$ and $8:16$ at 25 °C, the larval period was shortest under $L:D$ of $16:8$ and longest under $L:D = 8:16$. Feeding capacity of the larvae exhibited a positive correlation with the duration of the dark period. Larvae under longer periods of illumination pupated considerably earlier than those kept one under shorter periods of light exposure. No significant differences in the numbers of overwintering larvae were found in connection with different temperatures and photoperiods.

Keywords: Development; Feeding capacity; Land snails; Firefly; *Pyrocoelia pectoralis*

Sara Guiti Prado, Steven D. Frank. (Department of Entomology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695-7613, USA). Compact plants reduce biological control of *Myzus persicae* by *Aphidius colemani*. *Biological Control*, Volume 65 (2), (2013), 184–189.

Common horticultural practices, such as the use of plant growth regulators, may negatively influence the outcome of biological control programs. Plant growth regulators are applied to many ornamental and agricultural crops and can result in compact plants that have more branches and are bushier than untreated plants. Since plant architectural complexity can have strong effects on natural enemy foraging efficiency and pest suppression, our hypothesis was that the use of plant growth regulators would reduce aphid suppression by the parasitoid *Aphidius colemani*. In this study we investigated how the plant growth regulator paclobutrazol and the parasitic wasp *A. colemani* interact to affect the abundance and behavior of *Myzus persicae*. We found that paclobutrazol alone reduced aphid abundance compared to untreated plants. However, when parasitoids were present, paclobutrazol and associated changes in plant architecture reduced parasitism and increased aphid abundance compared to untreated plants. A likely mechanism for this result is that significantly more *M. persicae* fed in concealed locations on paclobutrazol-treated plants than on untreated plants. This study demonstrates that paclobutrazol reduced the efficacy of biological control by *A. colemani* and suggests that plant growth regulators could also affect biological control of other organisms.

Keywords: Foraging efficiency; Paclobutrazol; Plant growth regulator; Prey behavior

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Enhancing biological control of soil-dwelling insect pests is desirable, but due to the nature of the subterranean environment, it is challenging to elucidate interactions between pests and their natural enemies. Western corn rootworms (*Diabrotica virgifera virgifera*) are a major pest of maize, and the larvae can cause substantial damage to maize roots. Research has shown that numerous arthropods feed on rootworm immatures, and in some cases enhancing predator densities has led to rootworm suppression. We conducted a 2-yr field experiment and examined effects of releasing a predatory mite (*Gaeolaelaps aculeifer*) on densities of pest rootworms, root damage ratings, and plant growth parameters in conjunction with an insecticide control. In contrast to our expectations, larval rootworm incidence and root damage was generally higher in plots where mites were released. Differences in pest density and root damage did not translate into negative effects on plant biomass or grain yield at the end of the season, likely indicating that environmental conditions were favorable for plant compensation after rootworm damage. We also explored how rootworm and mite presence impacted densities of other soil invertebrates. Rootworm infestation had positive impacts on densities of carabid beetles and acarid mites during the time when rootworm eggs were hatching. Addition of predatory mites had both negative and positive effects on densities of soil invertebrates, with the latter potentially

due to disruption of naturally-occurring biocontrol. This research indicates that the soil invertebrate community can impact densities of rootworm larvae and subsequent root damage.

Keywords: Biological control; Generalist predator; Soil mite; *Gaeolaelaps aculeifer*; Rootworm; *Diabrotica*

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Four bacterial strains of *Bacillus* spp. which were antagonistic to the mango anthracnose pathogen were isolated and screened. Among them, TB09 and TB72 were identified by 16S rDNA sequence as *Bacillus pumilus* and *Bacillus thuringiensis*, respectively. *In vitro*, the anthracnose fungus showed 88.87% and 80.07% of mycelia growth inhibitions in presence of *B. pumilus* and *B. thuringiensis*, respectively and *in vivo*, the inhibitions of the disease were 94.28% and 87.06%, respectively. Based on the Gas Chromatography–Mass Spectrometer (GC–MS) analysis, 11 volatile compounds produced by *B. pumilus* and *B. thuringiensis* were identified. Among them, five volatiles showed better inhibition effects on the pathogens. 2-nonanone, β -benzeneethanamine, 2-decanone completely inhibited mycelia growth *in vitro* at a concentration of 100 $\mu\text{L L}^{-1}$, and thymol inhibited growth at concentrations of 50 mg L^{-1} and 100 mg L^{-1} . The inhibition rate of 40 $\mu\text{L L}^{-1}$ artificial mixture of 5 volatiles was 98.75% in the plate test. The results showed that the two screened antagonistic bacteria, and some of their produced volatiles and artificial mixtures could be promising control methods for anthracnose in harvested mango fruit.

Keywords: Volatile compounds; *Bacillus pumilus*; *Bacillus thuringiensis*; Bio-fumigation; *Colletotrichum gloeosporioides*; Mango fruit

Shuping Luo^{a, b}, J.P. Michaud^c, Jiancheng Li^d, Xiaoxia Liu^a, Qingwen Zhang^a. (^aDepartment of Entomology, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China, ^b State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China, ^c Department of Entomology, Kansas State University, Agricultural Research Center-Hays, Hays, USA, ^d Institute of Plant Protection, Hebei Academy of Agriculture and Forestry Sciences, Baoding 071001, China). Odor learning in *Microplitis mediator* (Hymenoptera: Braconidae) is mediated by sugar type and physiological state. *Biological Control*, Volume 65(2) (2013): 207–211

Parasitoids can be conditioned to respond to novel odors through associative learning, and learning can be sensitive to physiological state. This study examined the effects of various types

of sugar, and two physiological factors, mating status and oviposition experience, on odor learning in the parasitoid *Microplitis mediator* Haliday. Female *M. mediator* exhibited prolonged feeding periods on fructose, glucose and sucrose, whereas periods of feeding on raffinose, mannose and galactose were not different from water. Mating status did not affect feeding time on any sugars, but the conditioned response to eucalyptol was stronger in mated females than in virgins when the unconditioned stimulus was glucose. When females were conditioned to both food- and host-associated odors and then given a choice between them in a Y-tube olfactometer, hungry females preferred the former and satiated females, the latter, regardless of whether they had prior oviposition experience. However, oviposition experience shifted the preference of partially fed females in favor of the host-associated odor, whereas those without such experience preferred the food-associated odor. This finding suggests that parasitoid females in intermediate hunger states might be more responsive in tests of other experience effects than either starved or fully fed ones.

Keywords: Gustatory response; Sugar type; Physiological state; Learning; Hunger state; Ovipositional experience; Parasitoid

Robert S. Nofemela. (Insect Ecology Division, ARC-Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa, Department of Zoology & Entomology, University of Pretoria, Private Bag X20, Pretoria 0028, South Africa). The effect of obligate hyperparasitoids on biological control: Differential vulnerability of primary parasitoids to hyperparasitism can mitigate trophic cascades. *Biological Control*, Volume 65(2) (2013): 218–224

Obligate hyperparasitoids are widely considered an important ecological disturbance to biological control of insect pests, as they develop at the expense of primary parasitoids. However, supporting evidence is largely derived from direct trophic interactions in simple food webs. Yet, a multitude of insect pest populations simultaneously support development of several primary parasitoid species in horticultural and natural systems. Since primary parasitoid species in a community can differ in vulnerability to obligate hyperparasitoids, it is desirable to establish if the invulnerable primary parasitoids can take advantage of reduced competition from affected species by increasing their contribution to total primary parasitism levels thereby mitigating effects of hyperparasitism on biological control. To investigate this question, populations of the diamondback moth, *Plutella xylostella* (Linnaeus) (Plutellidae), its primary parasitoids and hyperparasitoids were monitored on unsprayed cabbage plots at weekly intervals over six consecutive years. *Cotesia vestalis* (Haliday) (Braconidae), a dominant primary parasitoid in this system, was a secondary host to three obligate hyperparasitoids: *Mesochorus* sp. (Ichneumonidae), *Eurytoma* sp. (Eurytomidae) and *Pteromalus* sp. (Pteromalidae). The higher efficiency of *C. vestalis* in utilizing younger host larvae at lower hyperparasitism levels limited host availability to other major primary parasitoids. But, as hyperparasitism levels increased and its populations declined, populations of *Oomyzus sokolowskii* (Kurdjumov) (Eulophidae) and *Diadromus collaris* (Gravenhorst) (Ichneumonidae) increased significantly as they parasitized a greater proportion of available hosts. As a consequence, the impact of hyperparasitoids did not result in trophic cascades, as their impact on total primary parasitism levels and infestation levels was insignificant. This study shows that primary parasitoid species that are invulnerable to hyperparasitism can take over the function of vulnerable ones in communities where interspecific interactions among species are strong. Thus, an approach that considers both direct and indirect effects of hyperparasitoids in primary parasitoid communities improves our understanding of the net impact of hyperparasitism on biological control of insect pests.

Keywords: *Plutella xylostella*; Infestation level; Primary parasitism; Hyperparasitism; Top-down effect; Developmental traits; Functional diversity

Shilpi K. Saikia, Sudeep Tiwari, Rakesh Pandey. (Microbial Technology and Nematology, CSIR-Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Lucknow 226015, India). Rhizospheric biological weapons for growth enhancement and *Meloidogyne incognita* management in *Withania somnifera* cv. Poshita. *Biological Control*, Volume 65(2) (2013): 225–234

Withania somnifera L. (Family Solanaceae) is an angiospermic medicinal herb, well recognized for the immense therapeutic potentials of its roots containing several withanolides. *W. somnifera* is also a susceptible host for southern root knot nematode, *Meloidogyne incognita*. The nematode infestation in roots causes serious crop losses in terms of yield and chemo-pharmaceutical quality of this medicinal herb. In the present study, influence of five rhizospheric microbes, namely *Bacillus megaterium* (ATCC No. 14581), *Pseudomonas fluorescens* (ATCC No. 13525), *Trichoderma viride* (MTCC No. 167), *Paecilomyces lilacinus* (PDBC PL55) and *Glomus intraradices* was studied for the management of *M. incognita* in *W. somnifera* cv. Poshita under greenhouse conditions. All rhizospheric microbes, except *G. intraradices*, displayed nematicidal potentials via ovicidal and larvicidal actions *in vitro* and, resulted in significant improvement in plant growth parameters. The rate of nematode damage to *W. somnifera* was directly proportional to *M. incognita* (number of J2) population.

Keywords: Rhizospheric microbes; *Meloidogyne incognita*; Ovicidal; Larvicidal; Root knot index

S.E. Jandricic^a, S.P. Wraight^b, D.R. Gillespie^c, J.P. Sanderson^a. (^aDepartment of Entomology, Comstock Hall, Cornell University, Ithaca, NY 14853, USA, ^b USDA-ARS Robert W. Holly Center for Agriculture and Health, Tower Road, Cornell University, Ithaca, NY 14853, USA, ^c Agriculture and Agri-Food Canada, Research Centre, Agassiz, BC, Canada V0M 1A0). Oviposition behavior of the biological control agent *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) in environments with multiple pest aphid species (Hemiptera: Aphididae). *Biological Control*, Volume 65(2) (2013): 235–245

We investigated the oviposition behavior of the aphidophagous midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) when faced with multiple prey choices, *i.e.* plants infested with *Myzus persicae* or *Aulacorthum solani* (Hemiptera: Aphididae). When within-plant location of aphid patches was controlled for, aphid density was a significant factor in *A. aphidimyza* oviposition, but species was not. When location was uncontrolled, aphid species and location of aphid patches on plants (and 2 and 3-way interactions with location) became significant, along with density. Aggregations of *M. persicae* on plant meristems received the largest number of *A. aphidimyza* eggs, while *A. solani*-infested plants received significantly fewer eggs (this aphid species being generally distributed among lower leaves). Upon giving *A. aphidimyza* a choice between two patch locations, aphid species was again unimportant in oviposition decisions, while a greater correlation with aphid density was seen in aphid colonies located on young plant tissue vs. old. These results suggest that, for *A. aphidimyza*, perceived quality of an aphid patch as an oviposition site is influenced more by density and location of the aphid patch on the plant than by the species of aphid within the patch. Given that within-plant distribution of pest aphid

species can differ, this oviposition behavior could have important implications for the efficacy of *A. aphidimyza* as a biocontrol agent for aphids in multi-species environments.

Keywords: Aphidoletes aphidimyza; Myzus persicae; Aulacorthum solani; Oviposition behavior; Biological control; Patch quality

J.M. Meyers¹, F.M. Stephen, L.J. Haavik, D.C. Steinkraus. (Department of Entomology, University of Arkansas, 319 Agriculture, Fayetteville, AR 72701, USA). Laboratory and field bioassays on the effects of *Beauveria bassiana* Vuillemin (Hypocreales: Cordycipitaceae) on red oak borer, *Enaphalodes rufulus* (Haldeman) (Coleoptera: Cerambycidae). *Biological Control*, Volume 65(2) (2013): 258–264

An unexpected outbreak of a native longhorned beetle, the red oak borer (*Enaphalodes rufulus* (Haldeman)), occurred in upland oak forests of Arkansas, Missouri and Oklahoma ca. 1999–2005. Few management tools exist for reducing *E. rufulus* populations. Laboratory bioassays were conducted to determine susceptibility of all *E. rufulus* life stages to the fungal pathogen *Beauveria bassiana* Vuillemin. Egg, neonate, early-instar, pupal, and adult stages of *E. rufulus* were all susceptible to a natural isolate of *B. bassiana* collected from *E. rufulus* (ARSEF 7404) or a commercial *B. bassiana* product, BotaniGard®. In July 2003, 10 living, *E. rufulus*-infested northern red oak (*Quercus rubra* L.) boles were sprayed with a 2 L BotaniGard® *B. bassiana* suspension of 1.3×10^{11} viable conidia per 2 m of each bole at a forested site in the Ozark National Forest, Franklin County, Arkansas. The following spring, trees were removed and sampled for *E. rufulus* larvae that had survived or died during the winter. Live *E. rufulus* larval density in untreated *Q. rubra* log samples was significantly greater than in treated log samples with means of 16.4 ± 1.8 (SE) and 4.4 ± 2.0 larvae per m² of bark for untreated and treated logs, respectively. Application of *B. bassiana* spray against early-instar *E. rufulus* would be most effective in mid-summer of odd-numbered years to target vulnerable early *E. rufulus* life stages and to reduce structural damage to trees caused by *E. rufulus* feeding.

Keywords: Biological control; Natural enemies; Wood borers; Fungal entomopathogen; BotaniGard®

Zhong Wei, Jianfeng Huang, Shiyong Tan, Xinlan Mei, Qirong Shen, Yangchun Xu. (Agricultural Ministry Key Lab of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, Jiangsu Key Lab and Engineering Center for Solid Organic Waste Utilization, Nanjing Agricultural University, 210095, China). The congeneric strain *Ralstonia pickettii* QL-A6 of *Ralstonia solanacearum* as an effective biocontrol agent for bacterial wilt of tomato. *Biological Control*, Volume 65(2) (2013): 278–285

The role of *Ralstonia pickettii* as a congeneric rhizobacterium of plant pathogenic *Ralstonia solanacearum* has not been investigated in biocontrol of bacterial wilt of tomato. Our preliminary study showed that the population of *R. pickettii* was significantly higher than that of *R. solanacearum* in the rhizosphere of healthy tomato plants in a heavily diseased field with bacterial wilt. Due to its good performances in inhibition of *R. solanacearum* *in vitro* and colonization of the rhizosphere soil and stem of tomato, *R. pickettii* QL-A6 was selected for suppression of *R. solanacearum* by use of the soil drench (SD) and stem injection (SI) methods in greenhouse. By the SI method, disease incidence was reduced by 71.2% on the average with an inoculation dosage of only about 10^5 cfu of *R. pickettii* QL-A6 per plant. By the SD method, disease incidence was reduced by 52.9% on the average but needed a higher inoculation dosage

of about 10^9 cfu of *R. pickettii* QL-A6 per plant. Thus, the SI method was chosen for further testing in field. The field disease incidence in *R. pickettii* QL-A6 treated plots was 8.8% at harvest time, while that in the sterilized water treated plots was as high as 33.1%. The population ratios of *R. pickettii* QL-A6 to *R. solanacearum* in the aboveground parts of field plants injected with *R. pickettii* QL-A6 ranged from 2 to 163. It is concluded that direct injection of *R. pickettii* QL-A6 in stem of tomato could be an alternative to chemical pesticides for biocontrol of *R. solanacearum*.

Keywords: *Ralstonia pickettii*; Colonization; Soil drench; Stem injection

A.L. Acebes, R.H. Messing. (University of Hawaii at Manoa, Kauai Agricultural Research Center, 7370 Kuamo'o Road, Kapaa, Hawaii 97646, USA). Comparative susceptibility to hyperparasitism of *Binodoxys communis* and *Aphidius colemani*, primary aphid parasitoids introduced to Hawaii. *Biological Control*, Volume 65(2) (2013): 286–292

Improving the success rate of introduced biological control agents requires analysis of possible causes when a new natural enemy fails to thrive. For aphid parasitoids, the impact of hyperparasites is a potential obstacle to establishment and reproductive success. We analyzed *Binodoxys communis* (Gahan), an aphid parasitoid newly introduced to Hawaii, and *Aphidius colemani* (Viereck), a previously introduced, well established parasitoid for their comparative susceptibility to attack by the established hyperparasitoid *Syrphophagus aphidovoratus* (Mayr). Our objective was to help explain the relatively low abundance of *B. communis* in the field in the months following its initial release, in contrast to the greater abundance of *A. colemani*, as a possible result of apparent competition mediated by the hyperparasitoid. Mummies of the two primary parasitoid species were exposed to adult female *S. aphidovoratus* under both choice and no-choice conditions. *B. communis* was susceptible to *S. aphidovoratus* attack, but *A. colemani* was the more suitable host, as evidenced by higher rates of hyperparasitism in choice tests, and more female-biased sex ratios among the resulting progeny. The greater suitability of *A. colemani* for hyperparasitoid development was likely due to its larger size, providing more resources for growth and development. Contrary to expectation, we found no evidence that apparent competition was unfavorably skewed against *B. communis*.

Keywords: Aphid; Parasitoid; Hyperparasitoid; *Aphidius colemani*; *Binodoxys communis*; Apparent competition

Adenirin Chabi-Olaye[‡], Nicholas M. Mwikya, Komi K.M. Fiaboe. (International Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772-00100, Nairobi, Kenya). Acceptability and suitability of three *Liriomyza* species as host for the endoparasitoid *Phaedrotoma scabriventris*: Implication for biological control of leafminers in the vegetable production system of Kenya. *Biological Control*, Volume 65(1) (2013): 1–5

The biological performance of the exotic solitary endoparasitoid *Phaedrotoma scabriventris* (Nixon) (Hymenoptera: Braconidae) was studied on three important *Liriomyza* hosts (*Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii*), found in the vegetable production system of Kenya. All *Liriomyza* species tested were successfully parasitized by *P. scabriventris*. But, *L. huidobrensis* was the most preferred host with 92.2% of the females tested ovipositing in *L. huidobrensis* vs. 58.9% in *L. sativae* and 60% in *L. trifolii*. Within 24 h of oviposition, *P.*

scabriventris laid 1.5 times more eggs in *L. huidobrensis* than *L. sativae* and *L. trifolii* and the average parasitoids emergence was 1.5 times higher in *L. huidobrensis* compared to *L. trifolii* and *L. sativae*. There was no difference in the developmental period of *P. scabriventris* reared on the three *Liriomyza* species, however, the parasitoid mortality was 11.6 times higher in *L. sativae* and *L. trifolii* compared to *L. huidobrensis*. Adult parasitoids reared on *L. huidobrensis*, were 1.3 times bigger than *P. scabriventris* reared on *L. sativae* and *L. trifolii*, indicating that host size affect parasitoid development and survival. But, our findings suggested that host quality will not be a limiting factor for the development and establishment of *P. scabriventris* in the vegetable production system of Kenya.

Keywords: Classical biological control; *Liriomyza*; *Phaenocarpa scabriventris*; Host suitability; Kenya

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Concern over non-target effects of imported parasitoids has resulted in much tighter regulations over their release in the United States. Specificity of candidate agents typically rely on small cage studies inside laboratories, which constrains prediction of their ecological host ranges. Few post-release field studies have examined non-target side effects of imported aphelinids (Hymenoptera), or their regional spread. *Eretmocerus* is one of the more important groups of aphelinids attacking whiteflies worldwide, and several species were imported into the United States for control of *Bemisia tabaci* (Hemiptera: Aleyrodidae) during the 1990s. Of five species released in California, only *Eretmocerus mundus* permanently established in the southern San Joaquin Valley. For 10 years a regional survey was conducted to determine their spread and possible alternate whitefly hosts. Over this period of time, *Eret. mundus* spread throughout the five counties surveyed and continued to be the only candidate that persisted, permanently establishing populations in this largely cotton growing region of central California. Eventually *Eret. mundus* displaced native *Eretmocerus* attacking *B. tabaci* on cotton, and now make up over 95% of the *Eretmocerus* attacking this pest. Except on one occasion, all *Eret. mundus* emerging from isolated whitefly nymphs came from *B. tabaci* ($n = 243$). Parasitism levels have varied from 7 to 35%, up from less than 1.4% when first measured. The percentage of cotton leaves infested by *B. tabaci* populations in the San Joaquin Valley has dropped from an average of 28–12.2% during the last three years measured. Most likely this change in infestation level is due to a combination of factors, including the introduction of more selective insecticides, drop in regional cotton acreage, and the permanent establishment of a more specific parasitoid.

Keywords: *Eretmocerus*; *Bemisia tabaci*; Non-target effects; Classical biological control

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Utrecht, The Netherlands, ^c Department of Plant Science, Faculty of Agriculture, University of Jiroft, P.O. Box 364, Jiroft, Iran). Induced systemic resistance in cucumber and *Arabidopsis thaliana* by the combination of *Trichoderma harzianum* Tr6 and *Pseudomonas* sp. Ps14. *Biological Control*, Volume 65(1) (2013): 14–23

Trichoderma species and fluorescent *Pseudomonas* spp. have been reported to induce systemic resistance in plants. In this study the effectiveness of a combination of these biological control agents on the efficacy of induced resistance was investigated in cucumber and the model plant *Arabidopsis thaliana*. *Trichoderma harzianum* Tr6, and *Pseudomonas* sp. Ps14, both isolated from the rhizosphere of cucumber, were tested as a single application and in combination for their abilities to elicit induced resistance in cucumber against *Fusarium oxysporum* f. sp. *radicis cucumerinum* and in *A. thaliana* against *Botrytis cinerea*. The combination of Tr6 and Ps14 induced a significantly higher level of resistance in cucumber, which was associated with the primed expression of a set of defense-related genes upon challenge with *Fusarium*. In *Arabidopsis* both Ps14 and Tr6 triggered ISR against *B. cinerea* but their combination did not show enhanced effects. In the induced systemic resistance-defective *Arabidopsis* mutant *myb72*, none of the treatments protected against *B. cinerea*, whereas in the SA-impaired mutant *sid2* all treatments were effective. Taken together, these results indicate that in *Arabidopsis* Ps14 and Tr6 activate the same signaling pathway and thus have no enhanced effect in combination. The enhanced protection in cucumber by the combination is most likely due to activation of different signaling pathways by the two biocontrol agents.

Keywords: *Arabidopsis thaliana*; *Botrytis cinerea*; Cucumber; Defense genes; *Fusarium oxysporum* f. sp. *radicis cucumerinum*; Induced systemic resistance; *Pseudomonas*; *Trichoderma*

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The rosy apple aphid, *Dysaphis plantaginea* Passerini, and the green apple aphid, *Aphis pomi* De Geer, are serious pests of apple and are commonly found together as pests in apple orchards. Both species establish mutualistic relationships with ants.

Two experiments manipulating the presence of the common black ant, *Lasius niger* (L.), visiting aphid colonies by ant-exclusion with sticky barriers, or ant-feeding with honey as an alternative sugar source, were conducted, one in an apple orchard in the United Kingdom and one in an apple orchard in Hungary to test if these methods can reduce aphid infestations on apple trees.

The exclusion of ants reduced *D. plantaginea* and *A. pomi* populations in both experiments. It caused significant increases in natural enemy pressure on both aphid species in the experiment in the UK and on *A. pomi* in the experiment in Hungary.

Ant-feeding with honey reduced the numbers of ants tending *D. plantaginea* colonies, and it consequently resulted in a reduction in *D. plantaginea* populations in both experiments. The supplementary feeding of ants was more effective in reducing *D. plantaginea* than *A. pomi*. This

was probably due to the greater intensity of ant attendance on *A. pomi* than on *D. plantaginea* colonies when both species were present together.

Our results showed that supplementary sugar feeding of ants is a successful method for supporting biocontrol of apple aphids through enhancing the effectiveness of their natural enemies. However, the species of aphid targeted and the design and position of the feeder could affect the efficacy of this biocontrol method.

Keywords: *Lasius niger*; *Dysaphis plantaginea*; *Aphis pomi*; Predators; Exclusion; Supplementary feeding

Muhammad Sarwar. (Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China, Pakistan Atomic Energy Commission, Nuclear Institute of Agriculture, Tandojam 70060, Sindh, Pakistan). Management of spider mite *Tetranychus cinnabarinus* (Boisduval) (Tetranychidae) infestation in cotton by releasing the predatory mite *Neoseiulus pseudolongispinosus* (Xin, Liang and Ke) (Phytoseiidae). *Biological Control*, Volume 65(1) (2013): 37–42

Biological control of the spider mite *Tetranychus cinnabarinus* (Boisduval) (Tetranychidae) in an open-field cotton crop (*Gossypium hirsutum* L.) by releasing the predatory mite *Neoseiulus pseudolongispinosus* (Xin, Liang and Ke) (Phytoseiidae) was investigated. The objectives were to determine the predatory efficiency of *N. pseudolongispinosus* released during different growth stages of the cotton and the different numbers of plants treated. The factors evaluated were release of predatory mites at a constant rate of five predators per plant using different plant numbers of cotton (every plant or every second or third plant treated) and timing of predator release (early, middle and late season releases). All predator released treatments were compared with a “no predator released” (control) trial. Based on treatment applications, the experimental data collected from biologically managed cotton fields and the untreated control showed significant differences in population densities of pest and predatory mites. Overall, the combined populations of both mites were not significantly different during early and mid-season releases, but varied significantly from late releases of predaceous mite. The results also showed that populations of both pests and predators were not significantly different when each cotton plant and every second plant was treated with predator but differed significantly when every third plant was treated, where increased numbers of *T. cinnabarinus* and decreased *N. pseudolongispinosus* were observed. Consequently, field release of the predaceous mite *N. pseudolongispinosus* to reduce the incidence of *T. cinnabarinus* at an early growth stage of cotton is a potentially useful pest management strategy if every plant is treated with predator.

Keywords: Mite release; Predation; *Neoseiulus*; *Tetranychus*; Cotton; Biological control

Chrysantus M. Tanga^{a, b}, Samira A. Mohamed^a, P. Govender^b, Sunday Ekesi^a. (^aInternational Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772-00100, Nairobi, Kenya, ^bDepartment of Zoology and Entomology, University of Pretoria, Pretoria, South Africa). Effect of host plant on bionomic and life history parameters of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), a parasitoid of the mango mealybug *Rastrococcus iceryoides* (Homoptera: Pseudococcidae). *Biological Control*, Volume 65(1) (2013): 43–52

Anagyrus pseudococci Girault is a solitary koinobiont endoparasitoid of several mealybug species. The effect of five host plants (*Mangifera indica* L., *Cucurbita moschata* Duchesne,

Parkinsonia aculeata L., *Cajanus cajan* L. and *Ficus benjamina* Roxb.) on host acceptability for oviposition and suitability for immature development of this parasitoid in the invasive mango mealybug *Rastrococcus iceryoides* Green were investigated. Effect of host plant on fitness traits (parasitoid size, egg load and longevity) and life table parameters were also assessed. Although *A. pseudococci* accepted the mealybug regardless of the host plant, the level of acceptability varied significantly. Percentage of parasitized nymphs was higher on *C. moschata*, followed by *P. aculeata* and *M. indica*, while it was lowest on *F. benjamina*. Host suitability was also strongly affected by the host plant and largely mirrored host acceptability for all the parameters evaluated. Female wasps reared on mealybugs maintained on *C. moschata* and *P. aculeata* were bigger and more fecund, while those reared from mealybugs maintained on *F. benjamina* were of inferior quality with regard to all fitness parameters evaluated. *A. pseudococci* achieved a greater intrinsic rate of natural increase (r_m), net reproductive rate (R_o) and finite rate of increase (λ) on mealybugs maintained on *C. moschata* and *P. aculeata*. In addition, the wasp had a shorter mean generation time (G) and population doubling time (T_d) on mealybugs maintained on *C. moschata*. The reverse was true for those maintained on *F. benjamina*. The findings are discussed in view of improvement of laboratory mass rearing, as well as field enhancement of the parasitoid performance.

Keywords: Host plants; *Anagyrus pseudococci*; Host acceptability; Host suitability; Life table parameters

Fritzi Grevstad^a, Richard Shaw^b, Robert Bouchier^c, Paolo Sanguankee^d, Ghislaine Cortat^e, Richard C. Reardon^f. (^aDepartment of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA, ^b CABI, Bakeham Lane, Egham, Surrey TW20 9TY, United Kingdom, ^c Agriculture and AgriFood Canada-Lethbridge Research Centre, Lethbridge, AB, Canada T1J 4B1, ^d Olympic Natural Resources Center, University of Washington, Forks, WA 98331, USA, ^e CABI, CH 2800 Delemont, Switzerland, ^f USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV 26505, USA). **Efficacy and host specificity compared between two populations of the psyllid *Aphalara itadori*, candidates for biological control of invasive knotweeds in North America. *Biological Control*, Volume 65(1) (2013): 53–62**

Invasive knotweeds are large perennial herbs in the Polygonaceae in the genus *Fallopia* that are native to Asia and invasive in North America. They include *Fallopia japonica* (Japanese knotweed), *F. sachalinensis* (giant knotweed), and a hybrid species *F. x bohemica* (Bohemian knotweed). Widespread throughout the continent and difficult to control by mechanical or chemical methods, these plants are good targets for classical biological control. We examined the suitability of two populations of the psyllid *Aphalara itadori* from Japan as biological control agents by comparing their impact on the target weeds and assessing their fundamental host ranges. Both populations were capable of halting knotweed plant growth and reducing both above and below ground biomass by more than 50% in just 50 days. Moreover, the psyllids caused mortality of several of the plants during this period. The two populations differed markedly in their reproductive potential on the different knotweed species. The Kyushu psyllid performed best on *F. japonica* and *F. bohemica* and the Hokkaido psyllid performed best on *F. sachalinensis*. Both were found to be specialized to knotweeds, with only very low occurrence of development on a small number of related non-target plant species. For the few non-target plant

species that supported development, choice tests and multi-generational tests were used to further evaluate the likelihood of non-target host use. We conclude that *A. itadori* would be both effective and low risk as a biological control agent for invasive knotweeds and that both the Kyushu and Hokkaido populations may be needed to effectively control the entire knotweed species complex.

Keywords: *Aphalara itadori*; *Fallopia sachalinensis*; *Fallopia japonica*; Host specificity; Knotweed biological control; North America

Timothy L. Widmer, Stephen C. Dodge. (Foreign Disease and Weed Science Research Unit, USDA-ARS, 1301 Ditto Avenue, Fort Detrick, MD 21702, USA). Can fungal epiphytes reduce disease symptoms caused by *Phytophthora ramorum*? *Biological Control*, Volume 65(1) (2013): 135–141

Leaf infection of ornamental species by *Phytophthora ramorum* has a significant impact on the spread of this disease. Fungicides have had limited success at controlling this disease. With increasing concerns that repeated fungicide applications will exacerbate the potential for fungicide resistance and mask symptoms, alternative control measures are desired. The potential of biological control has not been thoroughly examined. Fungi, isolated from soil, were screened in dual culture with *P. ramorum* for antagonistic activity. Three isolates, identified as *Penicillium daleae*, *Metarhizium anisopliae*, and *Penicillium herquei*, were selected for further testing on the aerial plant parts of rhododendrons. Different factors, including culture age, application timing, dose response, and additives in the formulation were studied to determine their effects on the antagonists to reduce leaf necrosis. Although responses were variable for the different antagonists, this study showed that fungi applied to the leaf surface could reduce necrosis caused by *P. ramorum*. The method developed can be used for screening potential antagonists *in planta*.

Keywords: Epiphytes; *Phytophthora ramorum*; Ornamentals; Ramorum blight; Sudden oak death

Karen L. Bailey^a, Stuart Falk^b, Jo-Anne Derby^a, Melody Melzer^{c, 1}, Greg J. Boland^c. (^aAgriculture & Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2, ^b The Scotts Company, 14310 Scottslawn Road, Marysville, OH 43041, USA, ^c School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1). The effect of fertilizers on the efficacy of the bioherbicide, *Phoma macrostoma*, to control dandelions in turfgrass. *Biological Control*, Volume 65(1) (2013): 147–151

Phoma macrostoma is registered as a bioherbicide in North America to control broadleaved weeds species in turfgrass. A study was conducted to examine the effect of nitrogen, phosphorus, potassium, lime, and commercial fertilizers with or without applications of the bioherbicide on the reduction of dandelion under greenhouse and field conditions. The bioherbicide provided 70–100% reduction of dandelion. The addition of nitrogen with the bioherbicide, in the form of urea (45-0-0), Scotts Turf Builder Pro (32-0-4 plus 2% Fe), and Scotts Lawn Pro (26-0-3, with no iron), significantly reduced dandelion more than in soil that was not amended with fertilizers in the greenhouse and field locations. Bioherbicide efficacy on dandelion was 10–20% better with these fertilizer treatments. Phosphate (0-46-0), potassium sulfate (0-0-42), and lime had either no effect or did not reduce dandelions under greenhouse conditions. This study showed that *P. macrostoma* retained bioherbicide efficacy on dandelion in conjunction with typical fertility

practices and the combination of the bioherbicide with nitrogen fertilizers improved bioherbicide efficacy, especially in low nitrogen soils.

Keywords: Bioherbicide; Mycoherbicide; Inorganic fertilizer; Turfgrass; Broadleaved weeds

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Conservation biological control (CBC), often described as the field of biological control with the greatest potential for use in developing world agriculture, has received only marginal, scattered research attention outside Western Europe or North America. As a consequence, pesticide overuse remains rampant in many cropping systems, while in others, a complete lack of safe, affordable and effective pest control options leaves farmers vulnerable in face of herbivore attack. In this study, we describe the current status of CBC research in a wide variety of agro-production systems outside North America, Australia, New Zealand, Japan and Western Europe. We summarize information on (1) a variety of CBC themes related to natural enemy biology and ecology, (2) factors that either disrupt or enhance natural enemy efficacy, and (3) field evaluation of CBC schemes. A total of 390 CBC-related literature records from 53 different crops were considered. Most records were from China, Brazil, or Cuba, while no CBC references were found from several developing countries. CBC research primarily focused on habitat management, with 71 records on general habitat manipulation and 80 records on the effects of inter-or cover-crops on natural enemy abundance or efficacy. The effects of deliberate modification of disturbance regimes, through alterations in pesticide use or tillage, on natural enemies were well-characterized in many cropping systems. For each of the CBC themes, research progress was assessed and opportunities were identified to translate current findings into practical solutions. On a crop level, most research was targeted at rice, maize and cotton. No CBC records were found for key staple crops such as yams, taro, sago or breadfruit; fruits such as papaya, pineapple and avocado; or forage crops. Also, millet, lentils, barley and plantain, all crops grown mainly in the developing world, received limited CBC research attention. CBC research has been done on myriad arthropod pests, including species with high levels of insecticide resistance such as *Chilo suppressalis* (Lepidoptera: Crambidae) and *Helicoverpa armigera* (Lepidoptera: Noctuidae). However, almost 70% of pests with high incidence of insecticide resistance have been overlooked. Lastly, we contrast country-specific CBC research advances with the national level of insecticide use and importation, and identify lucrative opportunities for countries to save funds through targeted research investment. Based upon our delineation of the current status of CBC, we indicate potential for well-orchestrated regional research projects to pursue higher levels of CBC integration into current pest management

schemes. This work constitutes a first step in drawing a roadmap for developing-world research that provides local farmers with safe, low-cost means to control damaging insect pests, safeguard harvests and secure their livelihoods.

Keywords: Conservation biological control; Natural enemies; Africa; Developing world; Poverty; Food security

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The evaluation of the potentiality of lactic acid bacteria (LAB) strains isolated from different origins to inhibit mould growth and to identify and characterize the antifungal metabolites were the aims of this study. From a total of ninety-one LAB strains tested, ten were selected due to their high inhibitory effect (>80%). The antifungal activity of the majority of the selected LAB strains was lost after the neutralization treatment determining the acidic nature of the antifungal metabolites. Lactic, acetic and phenyllactic (PLA) acids were identified as being responsible for antifungal effect in the 10 cell-free supernatants (CFS) evaluated. Amongst the strains evaluated, only *Lactobacillus fermentum* CRL 251 produced fungus inhibitory peptide/s, smaller than 10 kDa, thermostable, active in the pH range of 4–7 and sensitive to trypsin. This is the first report on antifungal peptide/s produced by a *L. fermentum* strain.

Keywords: Lactic acid bacteria; Antifungal activity; Phenyllactic acid; Antifungal peptide; *Lactobacillus fermentum*

Hui Sun^a, Eeva Terhonen^a, Kaisa Koskinen^b, Lars Paulin^b, Risto Kasanen^{a, c}, Fred O. Asiegbu^a. (^aDepartment of Forest Sciences, University of Helsinki, P.O. Box 27, FIN-00014 Helsinki, Finland, ^b DNA Sequencing and Genomics Lab, Institute of Biotechnology, University of Helsinki, P.O. Box 56, FIN-00014 Helsinki, Finland, ^c Finnish Forest Research Institute (Metla), Vantaa Research Unit, P.O. Box 18, FIN-01301 Vantaa, Finland). **The impacts of treatment with biocontrol fungus (*Phlebiopsis gigantea*) on bacterial diversity in Norway spruce stumps. *Biological Control*, Volume 64(3) (2013): 238–246**

The biocontrol agent *Phlebiopsis gigantea* has been intensively applied to the surface of *Picea abies* stumps to control *Heterobasidion* root rot. But little is known about the possible impact of this treatment on the resident bacteria community in the stumps. High throughput DNA bar-coded pyrosequencing was used to characterize the diversity of bacteria in the stumps of *P. abies* at 1, 6 and 13 years after treatment with *P. gigantea*. The sequences were classified into 12 phyla and 160 genera, of which *Proteobacteria* and *Acidobacteria* were the most abundant groups over time. Moreover, at the initial stages of decay, *Proteobacteria* were the most abundant whereas *Acidobacteria* were the most common at advanced stages of decay. Treatment with *P. gigantea* led to significant increase of the genus *Acidobacteria-Gp1* at 1 year after treatment. The analysis of observed and estimated operational taxonomic units (OTUs) as well as diversity indices revealed that *P. gigantea* treatment significantly decreased the initial bacterial richness in the

stumps, but the bacterial community gradually recovered and the negative effects of *P. gigantea* was attenuated. These results provide additional insight on the risk assessment as well as environmental impact on the long-term use of *P. gigantea* in the control of *Heterobasidion* root rot in conifer forests.

Keywords: *Phlebiopsis gigantea*; Bacteria diversity; Biological control; Pyrosequencing

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Biological control and soybean cultivars bred for increased resistance to the soybean aphid (*Aphis glycines*) are two approaches used to manage this serious pest of soybeans in North America. However, as with many other pest systems, the compatibility of these two pest management approaches has not been studied in detail. The aphidiine wasp *Aphidius colemani* is one of several candidate species for biological control of the soybean aphid in soybean in North America. Resistance to the soybean aphid in the USDA soybean cultivar Dowling is largely controlled by a single dominant gene *Rag1*, which is the focus of plant breeding programs directed against the soybean aphid. In this study, we measured developmental and behavioral differences in the parasitic wasp *A. colemani* when it attacked soybean aphids feeding on either the aphid-resistant Dowling or aphid-susceptible Glenwood cultivars of soybean. We used a combination of choice and no-choice experiments to examine the effects of host plant cultivar on the number of parasitized aphids formed and the sex ratio and body weights of adult offspring produced. Significantly more aphids were parasitized when they fed on Glenwood compared to Dowling and these offspring were larger when they developed in aphids that fed on Glenwood soybeans. To distinguish between effects on foraging decisions and offspring survivorship, we conducted an additional experiment that followed the oviposition decisions and fate of each parasitized aphid. Foraging female *A. colemani* spent less time handling individual aphids and encountered and attacked aphids at a higher rate when they fed on aphids feeding on Glenwood soybeans than aphids feeding on Dowling soybeans. Furthermore, wasp survivorship in aphids was greater on Glenwood than Dowling. Taken together, aphid-resistance in soybeans has negative effects on foraging behavior and offspring fitness of *A. colemani* raising concerns about the compatibility of these two pest management approaches.

Keywords: Parasitoid; Soybean aphid; *Aphis glycines*; *Rag1*; Dowling; Biological control; Host plant resistance; Tritrophic effects

Asad Shabbir^a, Kunjitapatham Dhileepan^b, Chris O'Donnell^a, Steve W. Adkins^a. (^aTropical and Sub-Tropical Weed Research Unit, School of Agriculture & Food Sciences, The University of Queensland, St. Lucia 4072, Brisbane, Australia, ^b Biosecurity Queensland, Department of Agriculture, Fisheries and Forestry, Ecosciences Precinct, Boggo Road, Brisbane, Australia). **Complementing biological control with plant suppression: Implications for improved management of parthenium weed (*Parthenium hysterophorus* L.). *Biological Control*, Volume 64(3) (2013): 270–275**

Parthenium hysterophorus L. is a weed of global significance that has become a major weed in Australia and many other parts of the world. A combined approach for the management of parthenium weed using biological control and plant suppression, was tested under field conditions over a two-year period in southern central Queensland. The six suppressive plant species, selected for their demonstrably suppressive ability in earlier glasshouse studies, worked synergistically with the biological control agents (*Epiblema strenuana* Walker, *Zygogramma bicolorata* Pallister, *Listronotus setosipennis* Hustache and *Puccinia abrupta* var. *partheniicola*) present in the field to reduce the growth (above ground biomass) of parthenium weed, by between 60–86% and 47–91%, in Years 1 and 2, respectively. The biomass of the suppressive plants was between 6% and 23% greater when biological control agents were present than when the biological control agents had been excluded. This shows that parthenium weed can be more effectively managed by combining the current biological control management strategy with selected sown suppressive plant species, both in Australia and elsewhere.

Keywords: Biocontrol; Suppressive plants; Asteraceae; Integrated weed management; Field study

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Ceratapion basicorne is a prospective biological control agent of yellow starthistle (*Centaurea solstitialis*), which is an invasive alien weed in the USA. Although the weevil has a strong preference for yellow starthistle, it has been reported to develop sometimes on safflower in larval transfer and no-choice experiments. Although safflower was not attacked by this insect in previous field experiments, a release permit was denied because of concern for risk to safflower. Adult *C. basicorne* were released in a field experiment in which two varieties of safflower were grown in solid blocks on either side of a small number of yellow starthistle plants. Plants were dissected at the time of weevil pupation. Immature insects were reared to adult stage on artificial diet or were preserved in acetone to identify by molecular genetic analysis. *C. basicorne* infested 54% of the yellow starthistle plants and 0% of 1021 safflower plants. A different weevil, *Ceratapion orientale*, infested 1.5% of the safflower plants. These results corroborate two other published field studies in which *C. basicorne* was not reared from safflower. The combined results of nine experiments provide a point estimate that the probability of attack is less than 0.00059, with 99.9% confidence that it is less than 0.0045. The consistency of results from field experiments in three countries and the absence of any report of this insect being reared from safflower in the field in the weevil’s native range support the conclusion that this insect poses no significant risk to safflower.

Keywords: Host plant specificity; Common garden; Risk assessment; Weed

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Agriculture and Rural Development, Brooks, AB, Canada, ^dJET Harvest Solutions, Longwood, FL, USA, ^eNational Fungal Identification Service, Agriculture and Agri-Food Canada, Ottawa, ON, Canada, ^fNew Brunswick Department of Agriculture and Aquaculture, Fredericton, NB, Canada). Efficacy of *Pseudomonas syringae* in the management of potato tuber diseases in storage. *Biological Control*, Volume 64(3) (2013): 315–322

Silver scurf caused by *Helminthosporium solani* and dry rot caused by *Fusarium* spp. are tuber diseases of economic importance in potato-growing areas worldwide. Recently, the two pathogens have developed resistance to thiabendazole (TBZ), a post-harvest fungicide commonly used for their control. Therefore, alternative disease control strategies are needed. The present study assessed the efficacy of the biopesticides Bio-Save 10LP (*Pseudomonas syringae*-strain ESC-10; *Ps*10) and Bio-Save 11LP (*P. syringae*-strain ESC-11; *Ps*11) against silver scurf and dry rot. Approximately 30 isolates representing the genus *Fusarium* were obtained from symptomatic potato specimens with dry rot from New Brunswick (NB), Nova Scotia (NS), Prince Edward Island (PE) and Alberta (AB), Canada. Species isolated were *Fusarium sambucinum*, *Fusarium tumidum*, *Fusarium coeruleum*, *Fusarium culmorum*, and *Fusarium avenaceum*. *H. solani* isolated from AB, NB and PE was included in the study as the causal agent of silver scurf. The efficacy of *P. syringae* against *F. sambucinum* and *H. solani* was tested *in vitro*. *Ps*10 and *Ps*11 inhibited the growth of *H. solani* up to 68% (NB isolate) and 73% (PE isolate), respectively and the inhibition was more or less comparable with that of TBZ. *F. sambucinum* was not significantly inhibited by *Ps*10; however *Ps*11 significantly inhibited AB, PE and NB isolates by 43%, 28% and 54%, respectively. Conversely, TBZ inhibited AB, PE and NB isolates of *Fusarium* spp. *in vitro* by 86%, 88% and 100%, respectively. TBZ in combination with either *Ps*10 or *Ps*11 did not always reduce the growth of *H. solani* or *Fusarium* spp. *in vitro* compared to that of TBZ alone. Storage trials conducted in NB and PE assessed the efficacy of *P. syringae* against *H. solani* or *Fusarium* spp. *in vivo* and confirmed that the application of *P. syringae* or TBZ alone or in combination significantly reduced the incidence and/or severity of silver scurf and *Fusarium* dry rot. *Ps*11 alone or in combination with TBZ was significantly more effective than *Ps*10 in controlling silver scurf disease severity. The reduction in disease severity of dry rot and silver scurf in storage due to *Ps*10, *Ps*11, or TBZ or their combinations was consistently comparable. Results indicate that the use of *P. syringae* (strains ESC-11 or ESC-10) as a post-harvest treatment can contribute to the management of both silver scurf and *Fusarium* dry rot in potato storages.

Keywords: Biopesticide; *Fusarium* dry rot; Postharvest disease control; Silver scurf; Thiabendazole

J. Megan Woltz, Douglas A. Landis. (Department of Entomology, Michigan State University, 578 Wilson Rd., Room 204, East Lansing, MI 48824, United States). Coccinellid immigration to infested host patches influences suppression of *Aphis glycines* in soybean. *Biological Control*, Volume 64(3) (2013): 330–337

Generalist natural enemies may be well adapted to annual crop systems in which pests and natural enemies re-colonize fields each year. In addition, for patchily-distributed pests, a natural enemy must disperse within a crop field to arrive at infested host patches. As they typically have longer generation times than their prey, theory suggests that generalist natural enemies need high

immigration rates to and within fields to effectively suppress pest populations. The soybean aphid, *Aphis glycines* Matsumura, is a pest of an annual crop and is predominantly controlled by coccinellids. To test if rates of coccinellid arrival at aphid-infested patches are crucial for soybean aphid control, we experimentally varied coccinellid immigration to 1 m² soybean patches using selective barriers and measured effects on *A. glycines* populations. In a year with low ambient aphid pressure, naturally-occurring levels of coccinellid immigration to host patches were sufficient to suppress aphid populations, while decreasing coccinellid immigration rates resulted in large increases in soybean aphid populations within infested patches. Activity of other predators was low in this year, suggesting that most of the differences in aphid population growth were due to changes in coccinellid immigration. Alternatively, in a year in which alate aphids continually colonized plots, aphid suppression was incomplete and increased activity of other predatory taxa contributed to adult coccinellid predation of *A. glycines*. Our results suggest that in a system in which natural enemy populations cannot track pest populations through reproduction, immigration of natural enemies to infested patches can compensate and result in pest control.

Keywords: Coccinellids; *Aphis glycines*; Immigration

Dany S.S.L. Amaral^a, Madelaine Venzon^b, Marcus V.A. Duarte^{a, b}, Fernanda F. Sousa^{a, b}, Angelo Pallini^b, James D. Harwood^c. (^aDepartment of Entomology, Federal University of Viçosa, Minas Gerais, Brazil, ^bAgriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), Viçosa, Minas Gerais, Brazil, ^cDepartment of Entomology, University of Kentucky, Lexington, KY 40546, USA). Non-crop vegetation associated with chili pepper agroecosystems promote the abundance and survival of aphid predators. *Biological Control*, Volume 64(3) (2013): 338–346

Habitat manipulation has long been used as strategy to enhance beneficial insects in agroecosystems. Non-crop weed strips have the potential of supplying food resources to natural enemies, even when pest densities are low. However, in tropical agroecosystems there is a paucity of information pertaining to the resources provided by non-crop weeds and their interactions with natural enemies. In this study we evaluated (a) whether weeds within chili pepper fields affect the diversity and abundance of aphidophagous species; (b) whether there are direct interactions between weeds and aphidophagous arthropods; and (c) the importance of weed floral resources for survival of a native and exotic coccinellid in chili pepper agroecosystems. In the field, aphidophagous arthropods were dominated by Coccinellidae, Syrphidae, Anthocoridae, Neuroptera and Araneae, and these natural enemies were readily observed preying on aphids, feeding on flowers or extrafloral nectaries, and using plant structures for oviposition and/or protection. Survival of native *Cycloneda sanguinea* (Coleoptera: Coccinellidae) differed between plant species, with significantly greater survival on *Ageratum conyzoides* and *Bidens pilosa*. However, no evidence was gathered to suggest that weed floral resources provided any nutritional benefit to the exotic *Harmonia axyridis* (Coleoptera: Coccinellidae). This research has provided evidence that naturally growing weeds in chili pepper agroecosystems can affect aphid natural enemy abundance and survival, highlighting the need for further research to fully characterize the structure and function of plant resources in these and other tropical agroecosystems.

Keywords: Aphidophagous species; Alternative food; Conservation biological control; Generalist predators; Coccinellidae; Syrphidae

Elisabeth M. Wood, Timothy D. Miles, Phillip S. Wharton. (Department of Plant, Soil, and Entomological Sciences, University of Idaho, Aberdeen, ID 83210, USA). The use of natural plant volatile compounds for the control of the potato postharvest diseases, black dot, silver scurf and soft rot. Biological Control, Volume 64(2) (2013): 152–159

Many naturally occurring plant volatile compounds are known for their anti-fungal properties. In this study, acetaldehyde and 2*E*-hexenal were chosen as prototype volatiles in order to investigate the use of volatile compounds for control of blemish pathogens in fresh-pack potato packaging. Pure cultures of the three main potato blemish pathogens, *Pectobacterium atrosepticum* (bacterial soft rot), *Colletotrichum coccodes* (black dot), and *Helminthosporium solani* (silver scurf), were used in the study. Pathogen cultures were exposed to the pure volatiles that were injected into the atmosphere of sealed jars for 4–8 days at 23 °C. Results showed that 2*E*-hexenal was the most effective of the two volatiles with 5 µL/L providing complete inhibition of growth for all three pathogens *in vitro*. Cytological studies showed that a concentration of 2.5 µL/L of 2*E*-hexenal was capable of inhibiting germination in both fungal pathogens. These results suggest that the primary mode of action of 2*E*-hexenal was inhibiting germination for fungi and suppressing bacterial growth. The quantities required to achieve pathogen inhibition are extremely low. This study suggests that these volatiles may be used to effectively manage potato postharvest blemish diseases in storage.

Keywords: *Colletotrichum coccodes*; *Helminthosporium solani*; *Pectobacterium atrosepticum*; *Solanum tuberosum*; Controlled atmospheric packaging; 2*E*-hexenal; Acetaldehyde

G. Sangeetha^a, R. Thangavelu^b, S. Usha Rani^a, A. Muthukumar^a. (^aDepartment of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram 608002, Tamil Nadu, India, ^bNational Research Centre for Banana, Thiruchirapalli 620102, Tamil Nadu, India). Antimicrobial activity of medicinal plants and induction of defense related compounds in banana fruits cv. Robusta against crown rot pathogens. Biological Control, Volume 64(1) (2013): 16–25

A total of 72 plant extracts were tested *in vitro* for their ability to inhibit the mycelial growth of *Lasiodiplodia theobromae* and *Colletotrichum musae* the causal agents of crown rot disease of banana. The results showed that the leaf extract of Zimmu (an interspecific hybrid of *Allium cepa* L. × *Allium sativum* L.) and tuber extract of *Zehneria scabra* recorded maximum inhibition of mycelial growth and spore germination of both the test pathogens. The dipping of banana fruits in Zimmu leaf extract at 25% conc. exhibited 100% inhibition of crown rot disease in cold storage (14 °C) up to 35 days and increased the shelf life to 64 days. However, at room storage (28 ± 2 °C), the same treatment exhibited 86% inhibition of crown rot disease up to 12 days. It was found that the treatment of banana fruits with Zimmu leaf extract did not alter the organoleptic properties of banana. The biochemical analysis of banana fruits treated with Zimmu leaf extract showed significant increase in phenylalanine ammonia-lyase (PAL), chitinase and β-1,3-glucanase activities and enhanced accumulation of phenolic compounds compared to other treatments. These findings suggest that the effect of Zimmu leaf extract on crown rot disease

may be associated with the direct fungi toxic property against the test pathogens and elicitation of defense related compounds in banana fruits.

Keywords: Banana; Medicinal plant extracts; Crown rot control; Defense enzymes

Sergio de los Santos-Villalobos^a, Doralinda A. Guzmán-Ortiz^a, Miguel A. Gómez-Lim^a, John P. Délano-Frier^a, Stefan de-Folter^a, Prometeo Sánchez-García^b, Juan J. Peña-Cabriales^a. (^aCentro de Investigación y de Estudios Avanzados-IPN, Unidad Irapuato, 36500 Irapuato, Guanajuato, Mexico, ^bColegio de Postgraduados, Campus Montecillo, Montecillo, Estado de Mexico, Mexico). **Potential use of *Trichoderma asperellum* (Samuels, Liechfeldt et Nirenberg) T8a as a biological control agent against anthracnose in mango (*Mangifera indica* L.). *Biological Control*, Volume 64(1) (2013): 37–44**

Twenty isolates of *Trichoderma* were obtained from orchards located in three main mango-producing States in Mexico: Chiapas, Oaxaca, and Michoacan, which represent different agronomical management practices and levels of soil fertility. Phylogenetic analysis showed that *Trichoderma* isolates belong to the following taxa: *Hypocrea lixii* (10 isolates), *Hypocrea jecorina* (four isolates), *Trichoderma asperellum* (three isolates), *Trichoderma spirale* (two isolates), and *Trichoderma brevicompactum* (one isolate). The genus *Hypocrea* is the teleomorph (sexual) stage of the genus *Trichoderma*, anamorph stage. Seventeen *Trichoderma* isolates showed at least 67% growth inhibition against the phytopathogenic fungus *Colletotrichum gloeosporioides* ATCC MYA 456 and three *Trichoderma* isolates showed complete overgrowth of this pathogen. One member of this group, identified as *T. asperellum* T8a, was able to control *C. gloeosporioides* ATCC MYA 456 *in vitro* and *in vivo*, as well as five *C. gloeosporioides* isolates obtained from mango orchards from the State of Oaxaca. Assay of the lytic enzymes involved suggest that cellulases of *T. asperellum* T8a play a role in biological control against *C. gloeosporioides* ATCC MYA 456 more than chitinase or glucanase. Thus, native *T. asperellum* T8a associated with mango trees can be used to enhance mango production, controlling anthracnose through cellulase activity.

Keywords: Antagonism; *Colletotrichum gloeosporioides*; Biological control; Lytic enzyme

Noemí Herrero Asensio, Salud Sánchez Márquez, Iñigo Zabalgogeoazcoa. (¹Fondazione Edmund Mach, IASMA Research and Innovation Centre, Via E. Mach 1, 38010, S. Michele all'Adige, TN, Italy, ²Department of Abiotic Stress, Instituto de Recursos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas (IRNASA-CSIC), Cordel de Merinas 40-52, 37008, Salamanca, Spain). **Mycovirus effect on the endophytic establishment of the entomopathogenic fungus *Tolypocladium cylindrosporum* in tomato and bean plants. *BioControl*, Volume 58(2) (2013): 225-232**

Two endophytic strains of the entomopathogenic fungus *Tolypocladium cylindrosporum*, originally isolated from the grass *Festuca rubra*, were artificially inoculated in tomato and bean plants. Strains 11-1L and 11-0BR were isolated from asymptomatic leaf fragments of both plant species at 3, 7, 14, 21, and 35 days after their inoculation. The percentage of leaf fragments infected by the fungus in inoculated leaves decreased at each sampling time, and no systemic colonization of the plants occurred. The two *T. cylindrosporum* strains tested were isogenic, differing in the infection by the victorivirus TcV1, harboured by strain 11-1L, but not by 11-0BR. The percentage of infected leaf fragments in leaves inoculated with the virus infected strain was greater in bean than in tomato plants, while the virus-free strain was

more successful in tomato than in bean plants. This result suggests that the mycovirus infection can affect the adaptation of *T. cylindrosporum* to particular host plants.

Gaofu Qi, XianFang Zhang, Xiuyun Zhao. (¹State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, 430070, People's Republic of China). **Endophytic *Bacillus subtilis* WH2 containing *Pinellia ternata* agglutinin showed insecticidal activity against whitebacked planthopper *Sogatella furcifera*.** *BioControl*, Volume 58(2) (2013): 233-246

Whitebacked planthopper (WBPH; *Sogatella furcifera*) has become a major threat to rice plants throughout Asia in recent years. In the study reported here, we attempted to biologically control WBPH by using endophytic bacterium strain WH2, which was isolated from rice seedlings and characterized as *Bacillus subtilis* based on its morphological, physiological, and biochemical characteristics and 16s rRNA gene sequence. Strain WH2 was genetically engineered to express anti-pest *Pinellia ternata* agglutinin (PTA). The PTA gene (*pta*) was cloned into plasmid pP43NMK and transformed to strain WH2 for expression, following which one positive transformant was selected by antibiotic resistance and PCR. This strain was denoted WH2::pta. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western blot analysis demonstrated that recombinant PTA protein was successfully expressed by recombinant strain WH2::pta. After inoculation, the recombinant WH2::pta was able to colonize rice plants and express secreted PTA protein. The results of the anti-planthopper activity assay showed that WH2::pta significantly decreased the survival and fecundity of WBPH fed on rice seedlings inoculated with WH2::pta ($p < 0.001$). On day 19 post-inoculation, the number of insects on individual rice plants in the control group was 104.7-fold higher than that on plants inoculated with recombinant WH2::pta and 58.2-fold higher than that on plants inoculated with wild-type WH2. By the end of the study period, all rice seedlings in the control group had died, but those inoculated with recombinant or wild-type WH2 were growing well. These results show that recombinant endophytic *B. subtilis* WH2 expressing PTA protein can endow its host rice plants with resistance against WBPH. This study presents an approach that may be applied in the future for biologically controlling planthopper using recombinant endophytes.

Changfeng Zhang, Kunsong Chen, Guoli Wang. (¹Shandong Key Laboratory of Storage and Transportation Technology of Agricultural Products, Shandong Institute of Commerce and Technology, 4516 Travel Road, Caishi, Licheng District, Jinan, 250103, Shandong, China, ²Laboratory of Fruit Quality Biology/The State Agriculture Ministry Laboratory of Horticultural Plant Growth, Development and Quality Improvement, Zhejiang University, Huajiachi Campus, Hangzhou, 310029, China, ³National Engineering Research Center for Agricultural Products Logistics, Jinan, 250103, China). **Combination of the biocontrol yeast *Cryptococcus laurentii* with UV-C treatment for control of postharvest diseases of tomato fruit.** *BioControl*, Volume 58(2) (2013): 269-281

Integrated management that combines different methods is being actively pursued in the control of postharvest disease. In this study, the biocontrol yeast *Cryptococcus laurentii* and ultraviolet-C (UV-C) treatment were evaluated for controlling infection following artificial inoculation with *Botrytis cinerea* or *Alternaria alternata*, and natural infection in tomato fruit. Applied separately, *C. laurentii* and UV-C (4 kJ m⁻²) effectively inhibited decay caused by *B. cinerea* or *A. alternata*, and natural infection. The combination of *C. laurentii* and UV-C showed

better control efficiency. UV-C treatment did not affect yeast growth in fruit wounds, while the treatment induced the transcript expression of β -1,3-glucanase, phenylalanine ammonia-lyase, peroxidase and superoxide dismutase based on real-time PCR analysis, as well as increased the activity of these enzymes in tomato fruit. Results indicate that the mechanism by which UV-C enhanced the biocontrol efficacy of *C. laurentii* may be associated with the elicitation of defense response in tomato fruit.

Qingyun Zhao, Wei Ran, Hui Wang, Xiang Li, Qirong Shen, Shengyuan Shen, Yangchun Xu. (¹Jiangsu Provincial Key Lab of Organic Solid Waste Utilization, Nanjing Agricultural University, Nanjing, 210095, China, ²Spice and Beverage Research Institute, China Academy of Tropical Agricultural Sciences, Haikou, 571533, China, ³Wujiang Agriculture and Technology Generalize Center, Wujiang, 215200, China). **Biocontrol of *Fusarium* wilt disease in muskmelon with *Bacillus subtilis* Y-IVI. *BioControl*, Volume 58(2): 283-292**

Muskmelon (*Cucumis melo* L.) wilt caused by *Fusarium oxysporum* f. sp. *melonis* leads to severe economic losses. A bio-organic fertilizer (BIO) fortified with an antagonistic strain of *Bacillus subtilis* Y-IVI was used to control this disease. Pot experiments were carried out to investigate the efficacy and to elucidate biocontrol mechanisms for the disease. BIO significantly reduced the disease incidence. Population of *F. oxysporum* in plant shoots of the BIO treatment were about 1000-fold lower than the control. Population of Y-IVI remained high in muskmelon rhizosphere of the BIO treatment during the experiment. Concentration of antifungal lipopeptides, iturin A, in the BIO treatment was significantly higher than other treatments. Ten days after transplantation, the salicylic acid content in BIO-treated plant leaves was significantly higher than control. In conclusion, BIO effectively controlled muskmelon wilt, possibly because the antagonistic microbes effectively colonize the plant rhizosphere and shoots to preclude pathogen invasion. Furthermore, Y-IVI produces antifungal lipopeptides in the rhizosphere.

Gerben J. Messelink, Chantal M. J. Bloemhard, Maurice W. Sabelis, Arne Janssen. (¹Wageningen UR Greenhouse Horticulture, P.O. Box 20, 2265 ZG, Bleiswijk, The Netherlands, ²IBED, Section Population Biology, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands). **Biological control of aphids in the presence of thrips and their enemies. *BioControl*, Volume 58(1) (2013): 45-55,**

Generalist predators are often used in biological control programs, although they can be detrimental for pest control through interference with other natural enemies. Here, we assess the effects of generalist natural enemies on the control of two major pest species in sweet pepper: the green peach aphid *Myzus persicae* (Sulzer) and the western flower thrips *Frankliniella occidentalis* (Pergande). In greenhouses, two commonly used specialist natural enemies of aphids, the parasitoid *Aphidius colemani* Viereck and the predatory midge *Aphidoletes aphidimyza* (Rondani), were released together with either *Neoseiulus cucumeris* Oudemans, a predator of thrips and a hyperpredator of *A. aphidimyza*, or *Orius majusculus* (Reuter), a predator of thrips and aphids and intraguild predator of both specialist natural enemies. The combined use of *O. majusculus*, predatory midges and parasitoids clearly enhanced the suppression of aphids and consequently decreased the number of honeydew-contaminated fruits. Although intraguild predation by *O. majusculus* on predatory midges and parasitoids will have affected control of aphids negatively, this was apparently offset by the consumption of aphids by *O. majusculus*. In contrast, the hyperpredator *N. cucumeris* does not prey upon aphids, but seemed to release aphids from control by consuming eggs of the midge.

Both *N. cucumeris* and *O. majusculus* did not affect rates of aphid parasitism by *A. colemani*. Thrips were also controlled effectively by *O. majusculus*. A laboratory experiment showed that adult predatory bugs feed on thrips as well as aphids and have no clear preference. Thus, the presence of thrips probably promoted the establishment of the predatory bugs and thereby the control of aphids. Our study shows that intraguild predation, which is potentially negative for biological control, may be more than compensated by positive effects of generalist predators, such as the control of multiple pests, and the establishment of natural enemies prior to pest invasions. Future work on biological control should focus on the impact of species interactions in communities of herbivorous arthropods and their enemies.

R. Castaño, C. Borrero, M. I. Trillas, M. Avilés. (¹Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica, University of Seville, Ctra. Utrera Km 1, 41013, Seville, Spain, ²Biocontrol Technologies S. L., Parc Científic de Barcelona, C/Baldiri Reixac, 15-21, 08028, Barcelona, Spain, ³Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 645, 08028, Barcelona, Spain). **Selection of biological control agents against tomato Fusarium wilt and evaluation in greenhouse conditions of two selected agents in three growing media. BioControl, Volume 58(1) (2013): 105-116**

Two biological control practices are the use of suppressive growing media and the application of biological control agents (BCAs). The goals of this study were: (i) to screen 584 potential BCAs obtained from Fusarium wilt (FW) suppressive growing media; (ii) to evaluate in greenhouse conditions selected BCAs in three growing media with different degrees of suppressiveness of tomato FW. Two isolates selected after screening were identified as *Fusarium solani* (305) and *Streptomyces* sp. (A19). Results showed that tomato FW was reduced and total production was improved when both BCAs were applied to a conducive medium (coir fiber). In highly suppressive growing medium (grape marc compost), A19 and 305 inoculations did not improve suppressiveness. In moderately suppressive growing medium (cork compost), only A19 improved this compost to natural grape marc compost suppressiveness level. Therefore, compost suppressiveness of tomato FW depended on the nature of the compost and on the isolates applied.

G. Cabrera Walsh, Y. Magalí Dalto, Federico M. Mattioli, Raymond I. Carruthers, Lars W. Anderson. (¹(Formerly USDA/ARS/South American Biological Control Laboratory), Fundación para el Estudio de Especies Invasivas (FUEDEI), Bolívar 1559, B1686EFA, Hurlingham, Buenos Aires, Argentina, ²Lab. de Microbiología y Biotecnología, CNEA, Centro Atómico Ezeiza, Ezeiza, Buenos Aires, Argentina, ³Monsanto S.A.I.C., Pergamino, Buenos Aires, Argentina, ⁴USDA-ARS-EIWRU (Exotic and Invasive Weed Research Unit), Albany, CA, USA, ⁵USDA-ARS-EIWRU, Davis, CA, USA). **Biology and ecology of Brazilian elodea (*Egeria densa*) and its specific herbivore, *Hydrellia* sp., in Argentina. BioControl, Volume 58(1) (2013): 133-147**

Egeria densa (Hydrocharitaceae) is a submerged macrophyte from South America that is a weed in several countries. It crowds out native plants and hinders water use, causing economic and environmental damage. The leafminer fly *Hydrellia* sp. 1 (Diptera: Ephydriidae), was found feeding in *E. densa* throughout its Argentine distribution, and is currently the only known specialist herbivore of *E. densa*. It was reared in the laboratory and tested on 25 plant species.

This herbivore can cause heavy defoliation in the laboratory and in the field. *Hydrellia* sp. 1 was found only on *E. densa*, but in the laboratory it also developed on two other Hydrocharitaceae species in the same family; *Egeria naias*, and *Elodea callitrichoides*. Significant oviposition and feeding were only observed on its primary natural host, and to a lesser degree on *E. naias*. Field studies indicate *Hydrellia* sp. 1 is present in the field year round, unless the host plant is prostrate for long periods, or covered by floating macrophytes. These results indicate *Hydrellia* sp. 1 may be a suitable biocontrol candidate for *E. densa*.

Junzhi Qiu,*Feifei Song,* Lihui Mao, Jie Tu, Xiong Guan. (Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, 350002 Fujian, People's Republic of China. Corresponding author: Xiong Guan, e-mail:guanxfafu@126.com). Time-dose-mortality data and modeling for the entomopathogenic fungus *Aschersonia placenta* against the whitefly *Bemisia tabaci*. *Canadian Journal of Microbiology*, 2013, 59(2): 97-101

The fungus *Aschersonia placenta* FJSM was evaluated for control of the sweet potato whitefly, *Bemisia tabaci*. *Bemisia tabaci* nymphs (1st–4th instars) on tomato plants in the greenhouse (25–27 °C, 70%–85% relative humidity) were sprayed with suspensions containing 0, 10⁴, 10⁵, 10⁶, 10⁷, or 10⁸ *A. placenta* FJSM conidia/mL. Mortality of fungus-treated 1st to 3rd instar nymphs ranged from 93% to 100% but was <25% for 4th instar nymphs; the fungus sporulated from 70% to 80% of the fungus-treated *B. tabaci* cadavers. LD₅₀ and LD₉₀ values decreased with time after treatment and increased with instar. LT₅₀ values decreased with conidial concentration. The data were then described with time-dose-mortality models. The results indicate that *A. placenta* FJSM has potential as a mycoinsecticide for control of *B. tabaci*.

Keywords: *Aschersonia placenta*, *Bemisia tabaci*, bioassay, time-dose-mortality (TDM) model

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This study is aimed at controlling eutrophication through converting the nutrients such as nitrogen and phosphorus into microbial protein and simultaneously inhibiting the growth of *Microcystis aeruginosa* by *Candida utilis*. *C. utilis* and *M. aeruginosa* (initial cell density was 2.25 × 10⁷ and 4.15 × 10⁷ cells·mL⁻¹) were cultured together in the absence or presence of a carbon source (glucose) during a 10-day experiment. In the absence of carbon source, the measured removal efficiencies of NH₄⁺-N and PO₄³⁻-P were 41.39 ± 2.19 % and 82.93 ± 3.95 %, respectively, at the second day, with the removal efficiency of 67.82 ± 2.29 % for *M. aeruginosa* at the fourth day. In contrast, the removal efficiencies of NH₄⁺-N and PO₄³⁻-P were increased to 87.45 ± 4.25 % and 83.73 ± 3.55 %, respectively, while the removal efficiency of *M. aeruginosa* decreased to 37.89 ± 8.41 % in the presence of the carbon source (C/N = 2:1). These results showed that the growth of *M. aeruginosa* was inhibited by *C. utilis*. Our finding sheds light on a novel potential approach for yeast to consume nutrients and control harmful algal during bloom events.

Noriaki Momma, Yuso Kobara, Seiji Uematsu, Nobuhiro Kita, Akinori Shinmura. (¹Institute for Horticultural Plant Breeding, 2-5-1 Kamishiki, Matsudo, Chiba, 270-2221, Japan, ²National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba, Ibaraki, 305-8604, Japan, ³Chiba Prefectural Agriculture and Forestry Research Center, Southern Horticultural Institute, 1762 Yamamoto, Tateyama, Chiba, 290-0014, Japan, ⁴Kanagawa Agricultural Technology Center, 1617 Kamikisawa, Hiratsuka, Kanagawa, 259-1204, Japan, ⁵Hokkaido Research Organization, Agriculture Research Department, Kamikawa Agricultural Experiment Station, Minami 1-5 Pippu, Kamikawa, Hokkaido, 078-0379, Japan). **Development of biological soil disinfestations in Japan. Applied Microbiology and Biotechnology, Volume 97(9) (2013): 3801-3809**

Biological soil disinfestations (BSDs) were developed separately in Japan and in The Netherlands as an alternative to chemical fumigations. In Japan, it was developed based on the knowledge of irrigated paddy rice and upland crop rotation system that was rather tolerant of soil-borne disease development. The methods consist of application of easily decomposable organic matter, irrigation, and covering the soil surface with plastic film, thereby inducing anaerobic (reductive) soil conditions and suppressing many soil-borne pests including fungi, bacteria, nematodes, and weeds. The methods are widely used by organic farmers in the area where residences and agricultural fields are intermingled. To note one advantage of these methods, maintenance of soil suppressiveness to Fusarium wilt of tomato was suggested, while soil treated with chloropicrin became conducive to the disease. Suppression of soil-borne fungal pathogens by BSDs might be attributed to anaerobicity and high temperature, organic acids generated, and metal ions released into soil water. Contributions of respective factors to suppression of respective pathogens might be diverse. Presumably, these factors might vary on the fungal community structure in BSD-treated soil. These factors also work in paddy fields. Therefore, the BSDs developed in Japan are probably a method to raise the efficacy of paddy–upland rotation through intensive organic matter application and through maintenance of a strongly anaerobic (reductive) soil condition.

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Agricultural productivity to meet growing demands of human population is a matter of great concern for all countries. Use of green compounds to achieve the sustainable agriculture is the present necessity. This review highlights the enormous use of harsh surfactants in agricultural soil and agrochemical industries. Biosurfactants which are reported to be produced by bacteria, yeasts, and fungi can serve as green surfactants. Biosurfactants are considered to be less toxic and eco-friendly and thus several types of biosurfactants have the potential to be commercially produced for extensive applications in pharmaceutical, cosmetics, and food industries. The biosurfactants synthesized by environmental isolates also has promising role in the agricultural industry. Many rhizosphere and plant associated microbes produce biosurfactant; these biomolecules play vital role in motility, signaling, and biofilm formation, indicating that biosurfactant governs plant–microbe interaction. In agriculture, biosurfactants can be used for plant pathogen elimination and for increasing the bioavailability of nutrient for beneficial plant

associated microbes. Biosurfactants can widely be applied for improving the agricultural soil quality by soil remediation. These biomolecules can replace the harsh surfactant presently being used in million dollar pesticide industries. Thus, exploring biosurfactants from environmental isolates for investigating their potential role in plant growth promotion and other related agricultural applications warrants details research. Conventional methods are followed for screening the microbial population for production of biosurfactant. However, molecular methods are fewer in reaching biosurfactants from diverse microbial population and there is need to explore novel biosurfactant from uncultured microbes in soil biosphere by using advanced methodologies like functional metagenomics.

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To analyse the biocidal efficacy of thermal sprayed copper surfaces.

Copper alloy sheet metals containing >60% copper have been shown to exhibit potent biocidal activity. Surface biocidal activity was assessed by epifluorescence microscopy. After 2-h exposure at 20°C in phosphate-buffered saline (PBS), contact killing of Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus epidermidis* by brass sheet metal and phosphor bronze was 3–4-times higher than that by stainless steel. SEM observations revealed that the surface membranes of both bacterial strains were slightly more irregular when exposed to brass sheet metal than stainless steel. However, when exposed to phosphor bronze coating, *E. coli* were 3–4 times larger with irregular membrane morphology. In addition, the majority of the cells were associated with spherical carbon-copper-phosphate crystalline nanostructures characteristic of nanoflowers. The membranes of many of the *S. epidermidis* exhibited blebbing, and a small subset was also associated with nanoflowers.

Our data indicate that increasing the surface roughness of copper alloys had a pronounced impact on the membrane integrity of Gram-positive and, to a lesser degree, Gram-negative bacteria. In the presence of PBS, carbon-copper-phosphate-containing nanoflowers were formed, likely nucleated by components derived from killed bacteria. The intimate association of the bacteria with the nanoflowers and phosphor bronze coating likely contributed to their nonreversible adhesion.

Thermal spraying of copper alloys provides a strategy for the rapid coating of three-dimensional organic and inorganic surfaces with biocidal copper alloys. Our study demonstrates that the macroscale surface roughness generated by the thermal spray process enhances the biocidal activity of copper alloys compared with the nanoscale surface roughness of copper sheet metals. Moreover, the coating surface topography provides conditions for the rapid formation of organic copper phosphate nanocrystals/nanoflowers.

Keywords: biocidal efficacy; copper alloys; *Escherichia coli*; nanoflowers; organic copper phosphate crystals; phosphorus bronze; *Staphylococcus epidermidis*; thermal spray

K. Sowndhararajan¹, S. Marimuthu², S. Manian^{1,*}. (¹Department of Botany, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India, ²R & D Centre, Parry Agro Industries Limited, Valparai, Tamil Nadu, India. *Correspondence: Sellamuthu Manian, Department of Botany, School of Life Sciences, Bharathiar University, Coimbatore – 641046, Tamil Nadu, India). **Biocontrol potential of phylloplane bacterium *Ochrobactrum anthropi* BMO-111 against blister blight disease of tea. Journal of Applied Microbiology, Volume 114(1) (2013): 209–218**

The present study was carried out to screen the phylloplane bacteria from tea for antagonism against grey blight caused by *Pestalotiopsis theae* and blister blight caused by *Exobasidium vexans* and to further evaluate the efficient isolates for disease control potential under field condition.

A total of 316 morphologically different phylloplane bacteria were isolated. Among the antagonists, the isolates designated as BMO-075, BMO-111 and BMO-147 exhibited maximum inhibitory activity against both the pathogens under *in vitro* conditions and hence were selected for further evaluation under microplot field trial. Foliar application of 36-h-old culture of BMO-111 (1×10^8 colony-forming units ml⁻¹) significantly reduced the blister blight disease incidence than the other isolates. The culture of BMO-111 as well as its culture filtrate effectively inhibited the mycelial growth of various fungal plant pathogens. The isolate BMO-111 was identified as *Ochrobactrum anthropi* based on the morphological and 16S rDNA sequence analyses.

It could be concluded that the biocontrol agent *O. anthropi* BMO-111 was effective against blister blight disease of tea.

Further study is required to demonstrate the mechanism of its action and formulation for the biocontrol potential against blister blight disease of tea.

Keywords: biocontrol; blister blight; *Exobasidium vexans*; *Ochrobactrum anthropi*; tea

Biodegradation

Simone Larcher, Viviane Yargeau. (Department of Chemical Engineering, McGill University, 3610 University Street, Montréal, Québec, Canada H3A 2B2). **Biodegradation of 17 α -ethinylestradiol by heterotrophic bacteria. Environmental Pollution, Volume 173(2013) : 17–22**

The presence of the synthetic estrogen 17 α -ethinylestradiol (EE2) in the environment is of increasing concern due to the endocrine disruption of aquatic organisms. Incomplete removal from wastewater (WW) is one of the main sources of EE2 in aquatic ecosystems, thus improving processes like biological WW treatment/activated sludge (AS) is becoming significantly important. There are opposing results regarding EE2 biodegradability by AS; one discrepancy is

the efficacy of heterotrophic bacteria. This research demonstrated the ability of heterotrophs commonly present in AS (*B. subtilis*, *P. aeruginosa*, *P. putida*, *R. equi*, *R. erythropolis*, *R. rhodochrous*, *R. zopfii*) to remove EE2. *R. rhodochrous* was the most successful with no detectable EE2 after 48 h; the other bacteria achieved 21%–61% EE2 removal. No additive or synergistic effects were observed due to the combination of the bacterial cultures with maximum EE2 removals of 43% after 300 h.

Keywords: 17 α -Ethinylestradiol (EE2); Estrogens; Biodegradation; Heterotrophic bacteria; *Rhodococcus*

Ingrid Hauser, Aslak Einbu, Kjetill Østgaard, Hallvard F. Svendsen, Francisco J. Cervantes. (Department of Biotechnology, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway, ²SINTEF Materials and Chemistry, 7465, Trondheim, Norway, ³Department of Chemical Engineering, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway, ⁴División de Ciencias Ambientales, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), Camino a la Presa San José 2055, Col. Lomas 4^a. Sección, C. P., 78216 San Luis Potosí, SLP, Mexico). **Biodegradation of amine waste generated from post-combustion CO₂ capture in a moving bed biofilm treatment system. *Biotechnology Letters*, Volume 35 (2) (2013): 219-224**

Nitrogen and organic matter removal from reclaimer waste of a monoethanolamine (MEA) based CO₂-capture plant was demonstrated in a pre-denitrification biofilm system. The reclaimer waste was generated from a 30 % (w/w) MEA solvent used for capturing CO₂ from flue gas from a coal-fired power plant. MEA, *N*-(2-hydroxyethyl)glycine (HEGly) and 2-hydroxyethylformamide (HEF) were the major contaminants treated. Hydrolysis of MEA to ammonia and further oxidation of organic intermediates readily occurred in the pre-denitrification system with a hydraulic retention time of 7 h. The biofilm system achieved 98 ± 1 % removal of MEA and 72 ± 16 % removal of total nitrogen. This is the first demonstration of efficient biodegradation of real amine waste from a post-combustion CO₂ capture facility by pre-denitrification without external electron donor.

Lichun Jiang, Qiping Ruan, Rulan Li, Tiandong Li*. (Key Laboratory for Molecular Biology and Biopharmaceutic, Mianyang Normal University, Mianyang, Sichuan, China. *Correspondence: Tiandong Li, Key Laboratory for Molecular Biology and Biopharmaceutic, Mianyang Normal University, Mianyang, Sichuan 621000, China. E-mail: tldli2010@163.com). **Biodegradation of phenol by using free and immobilized cells of *Acinetobacter* sp. BS8Y. *Journal of Basic Microbiology*, Volume 53(3) (2013): 224–230**

Strain BS8Y with high biodegradation activity and high tolerance of phenol was isolated from activated sludge in an insulating material plant of China. This strain was capable of removing 99.2% of the initial 600 mg/l phenol in liquid minimal medium within 24 h and tolerating phenol at concentrations of up to 1200 mg/ml. DNA sequencing and homologous analysis of the 16S rRNA gene identified that the strain BS8Y belonged to an *Acinetobacter* species. Polyvinyl alcohol was used as gel matrix to immobilize the strain BS8Y. The factors affecting the phenol degradation by immobilized cells and the phenol removal efficiency of free and immobilized cells were investigated; the stability of the immobilized cells is also reported. The results show that the immobilized cells could tolerate a higher phenol level and protected the bacteria much more effectively against changes in temperature and pH. The phenol

degradation efficiency was high at up to 96% within 30 h, with an initial concentration of 800 mg/l phenol, and the immobilized cells showed better performance than the suspended cells. Reusability tests revealed that the immobilized cells were stable enough even after reuse for ten times or storing at 4°C for 35 d. These results demonstrate that immobilized *Acinetobacter* sp. BS8Y possesses a good application potential in the treatment of phenol-containing wastewater.

Keywords: *Acinetobacter* sp. BS8Y; Phenol biodegradation; Free suspended cells; Immobilized cells; Polyvinyl alcohol

Ranjit G. Gurav, Jyoti P. Jadhav* (Department of Biotechnology, Shivaji University, Kolhapur, India. *Correspondence: Dr. (Mrs.) Jyoti P. Jadhav, Department of Biotechnology, Shivaji University, Kolhapur-416004, India, E-mail: jpbiochem@gmail.com). **Biodegradation of keratinous waste by *Chryseobacterium* sp. RBT isolated from soil contaminated with poultry waste. Journal of Basic Microbiology, Volume 53(2) (2013): 128–135**

In the present study, a feather degrading bacterial strain was isolated from poultry waste disposal site, Kolhapur, India. The bacterium was identified as *Chryseobacterium* sp. RBT using 16S rRNA gene sequence analysis. *Chryseobacterium* sp. RBT showed rapid hydrolysis of native feathers within 30 h and produced the highest level of keratinase activity (98.3 U/ml). Keratin containing wastes viz. silk, human hair, wool and chicken feathers were tested for keratin degrading ability of the bacterium. Amongst the tested substrates, the *Chryseobacterium* sp. RBT showed more specificity towards chicken feathers (98.6% degradation) with maximum keratinase activity (98.3 U/ml) and solubilized protein concentration (3.84 mg/ml). Effect of various physico-chemical parameters (temperature, pH, carbon and nitrogen sources) on keratinase production was monitored. The maximum keratinase activity was observed at pH (8.6) and temperature (50 °C). Molasses (1.0% w/v) acted as an inducer and enhanced the keratinolytic activity by two fold, while starch worked as an inhibitor. The goat skin when treated with crude keratinase enzyme (2% v/v), showed complete dehairing within 12 h. Hence, *Chryseobacterium* sp. RBT shows potential as a candidate for treating the keratinous waste in an ecofriendly manner.

Keywords: *Chryseobacterium* sp. RBT; Keratinase; Dehairing; Amino acids; Molasses

Ying Zhou, Jiashi Wei, Naimin Shao, Dongzhi Wei* (State Key Laboratory of Bioreactor Engineering, Newworld Institute of Biochemistry, East China University of Science and Technology, Shanghai, P.R. China. *Correspondence: Dongzhi Wei, State Key Laboratory of Bioreactor Engineering, Newworld Institute of Biochemistry, East China University of Science and Technology, Shanghai 200237, P.R. China. E-mail: dzhwei@ecust.edu.cn). **Construction of a genetically engineered microorganism for phenanthrene biodegradation. Journal of Basic Microbiology, Volume 53(2) (2013): 188–194**

The bacterium *Pseudomonas* sp. CGMCC2953, isolated from oil-polluted soil, was used as a recipient for a biodegradative gene encoding catechol 2,3-dioxygenase (C23O), which was successfully cloned into the plasmid pK4 derived from pRK415 with a broad host range. The

apparent phenanthrene biodegradation parameters of the recombinant microorganism (*Pseudomonas* sp. CGMCC2953-pK) were determined and compared with those of the wild type. As the key enzyme of phenanthrene degradation, C23O, could be expressed constitutively in the recombinant strain, *Pseudomonas* sp. CGMCC2953-pK showed an increased ability to degrade phenanthrene. The excessive production of C23O in *Pseudomonas* sp. CGMCC2953-pK could serve as an effective approach to construct genetically engineered microorganisms for the bioremediation of environmental contaminations.

Keywords: Genetically engineered microorganisms; pRK415; Biodegradation; Polycyclic aromatic hydrocarbons; Phenanthrene

N. M. Arif, S. A. Ahmad, M. A. Syed, M. Y. Shukor* (Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia. *Correspondence: Assoc. Prof. Dr. Mohd. Yunus Abd. Shukor, Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. E-mail: yunus@biotech.upm.edu.my). Isolation and characterization of a phenol-degrading *Rhodococcus* sp. strain AQ5NOL 2 KCTC 11961BP. *Journal of Basic Microbiology*, Volume 53(1) (2013): 9–19

In this work, we report on the isolation of a phenol-degrading *Rhodococcus* sp. with a high tolerance towards phenol. The isolate was identified as *Rhodococcus* sp. strain AQ5NOL 2, based on 16S rDNA analysis. The strain degraded phenol using the meta pathway, a trait shared by many phenol-degraders. In addition to phenol biodegradation, the strain was also capable of degrading diesel. Strain AQ5NOL 2 exhibited a broad optimum temperature for growth on phenol at between 20 °C and 35 °C. The best nitrogen sources were ammonium sulphate, glycine or phenylalanine, followed by proline, nitrate, leucine, and alanine (in decreasing efficiency). Strain AQ5NOL 2 showed a high tolerance and degradation capacity of phenol, for it was able to register growth in the presence of 2000 mg l⁻¹ phenol. The growth of this strain on phenol as sole carbon and energy source were modeled using Haldane kinetics with a maximal specific growth rate (μ_{max}) of 0.1102 hr⁻¹, a half-saturation constant (K_s) of 99.03 mg l⁻¹ or 1.05 mmol l⁻¹, and a substrate inhibition constant (K_i) of 354 mg l⁻¹ or 3.76 mmol l⁻¹. Aside from phenol, the strain could utilize diesel, 2,4-dinitrophenol and *p*-cresol as carbon sources for growth. Strain AQ5NOL 2 exhibited inhibition of phenol degradation by Zn²⁺, Cu²⁺, Cr⁶⁺, Ag⁺ and Hg²⁺ at 1 mg l⁻¹.

Keywords: *Rhodococcus* sp. strain AQ5NOL 2; Phenol; Biodegradation

Theresa Kauffhold¹, Marie Schmidt¹, Danuta Cichocka^{2,†}, Marcell Nikolausz^{3,4}, Ivonne Nijenhuis^{1,*}. (¹Department of Isotope Biogeochemistry, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany, ²Faculty of Bioscience Engineering, Division of Soil and Water Management, Catholic University of Leuven, Heverlee, Belgium, ³Department Environmental Biotechnology (formerly Bioremediation), Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany, ⁴Department of Bioenergy, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany, [†]University of Applied Sciences and Arts Northwestern Switzerland, School for Life Sciences, Institute for Ecopreneurship, Muttenz, Switzerland. *Correspondence: Ivonne Nijenhuis, Department of Isotope Biogeochemistry, Helmholtz Centre for Environmental

Research – UFZ, Permoserstrasse 15, 04318 Leipzig, Germany. Tel.: +49 (0) 341 235 1356; fax: +49 (0) 341 235 1443; e-mail: ivonne.nijenhuis@ufz.de). Dehalogenation of diverse halogenated substrates by a highly enriched *Dehalococcoides*-containing culture derived from the contaminated mega-site in Bitterfeld. FEMS Microbiology Ecology, Volume 83(1) (2013): 176–188

An enrichment culture dominated by one type of *Dehalococcoides* sp. (83% of clones) was characterised. This culture, originally derived from contaminated groundwater from the area of Bitterfeld-Wolfen (Saxony-Anhalt, Germany), dehalogenates chlorinated ethenes to ethene. Further, the culture also dehalogenated vinyl bromide (VB) and 1,2-dichloroethane (DCA) to ethene, 1,2,3,4- and 1,2,3,5-tetrachlorobenzene (TeCB), penta- and hexachlorobenzene (PeCB and HCB) to trichlorobenzenes (TCB), lindane to monochlorobenzene (MCB) and pentachlorophenol (PCP) to 2,3,4,6-tetrachlorophenol (TeCP). Growth was proven by quantitative PCR for all active cultures, except for those with TeCB, lindane and PCP. The growth yields obtained ranged from $(2.9 \pm 0.7) \times 10^7$ cells μmol^{-1} Br^- released on VB to $(34.8 \pm 5.4) \times 10^7$ cells μmol^{-1} Cl^- released on VC. Genes coding for nine putative reductive dehalogenases, the enzymes that mediate the respiratory process of dehalogenation, were identified. Phylogenetic analysis revealed eight reductive dehalogenases with similar sequences in other *Dehalococcoides* strains and one unique sequence.

Keywords: reductive dechlorination; chlorinated benzenes; vinyl bromide; lindane; chlorophenol; dichloroethane; *Dehalococcoides*; Bitterfeld

Xin Liu, Jin-Hong Fan, Lu-Ming Ma. (¹ National Engineering Research Center for Urban Pollution Control, State Key Laboratory of Pollution Control and Resources Reuse, Tongji University, Shanghai, 200092, China, email: jinhongfan@tongji.edu.cn). Oxidative Degradation of EDTA in Aqueous Solution by the Bimetallic Fe–Cu. Water, Air, & Soil Pollution, 224(2013): 1583

Oxidative degradation of ethylenediaminetetraacetic acid (EDTA) in aqueous solution at normal temperature and pressure by the bimetallic Fe–Cu was investigated in this work. The results showed that the removal efficiency of EDTA, total organic carbon (TOC), and total nitrogen (TN) could be about 95, 62.5, and 39 %, respectively, after 3-h reaction. The degradation of EDTA followed the pseudo-first-order reaction kinetics and would not be affected by the continuous use of bimetallic Fe–Cu. The degradation products were iminodiacetate, formate, and acetate determined by ion chromatogram. The effects of initial pH, initial concentration of EDTA, Cu content, Fe–Cu loading, and atmosphere were also investigated. Significantly, the bimetallic Fe–Cu process exhibited higher reactivity than ZEA process for the degradation of EDTA and it would not cause new heavy metal pollution in effluent. Reactive oxygen species (ROS) of OH was generated in situ. The evidence of oxidative degradation of EDTA was verified by electron spin resonance (ESR) spectroscopy and the product of *para*-hydroxybenzoic acid (*p*-HBA) by OH and benzoic acid (BA).

Xunan Yang, Jiaxin Ye, Limei Lyu, Qunhe Wu, Renduo Zhang. (¹ School of Environmental Science and Engineering, Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology, Sun Yat-sen University, Guangzhou, Guangdong, 510275, People's Republic of China). Anaerobic Biodegradation

of Pyrene by *Paracoccus denitrificans* Under Various Nitrate/Nitrite-Reducing Conditions. Water, Air, & Soil Pollution, 224(2013): 1578

As a polycyclic aromatic hydrocarbon (PAH), pyrene is one of hazardous persistent organic pollutants in the aquatic environment. The aim of this study was to investigate the influence of denitrifying conditions on pyrene degradation in a pure culture. With a strain isolated from petrol-contaminated river sediment, treatments of pyrene biodegradations were set up using various ratios of nitrate to nitrite ($\text{NO}_3^-/\text{NO}_2^-$). Results showed that various $\text{NO}_3^-/\text{NO}_2^-$ conditions significantly influenced the anaerobic pyrene degradation efficiency. Nitrite could induce the complete denitrification process so that NO_2^- acted as a key factor to promote high degradation efficiency. The low N treatment of NO_3^- and NO_2^- concentrations made the denitrifying-pyrene-degradation process more effective. Additionally, high C/N value stimulated high degradation rates. High concentrations of NO_3^- and NO_2^- as well as toxic intermediate product accumulation might inhibit the bacterial growth and biodegradation process. The information from this study should be useful to design bioremediation strategies of PAH.

Giovana Tommaso, Mercia Regina Domingues, Rogers Ribeiro, Maria Bernadete Amâncio Varesche, Marcelo Zaiat, Eugenio Foresti. (¹. Laboratório de Biotecnologia Ambiental (LBA), Departamento de Engenharia de Alimentos (ZEA), Faculdade de Zootecnia e Engenharia de Alimentos (FZEA), Universidade de São Paulo (USP), Rua Duque de Caxias Norte 225, 13635-900, Pirassununga, SP, Brazil, ². Laboratório de Processos Biológicos (LPB), Centro de Pesquisa, Desenvolvimento e Inovação em Engenharia Ambiental, Escola de Engenharia de São Carlos (EESC), Universidade de São Paulo (USP), Engenharia Ambiental, Bloco 4-F, Av. João Dagnone, 1100, Santa Angelina, 13.563-120, São Carlos, SP, Brazil). **Anaerobic Degradation of Protein: Simplified Kinetic Modelling and Microbial Dynamics. Water, Air, & Soil Pollution, 224(2013): 1554**

Data on the influence of substrate composition on the anaerobic degradation of peptone in a bench-scale packed-bed reactor are presented and discussed. The experiments were conducted in a horizontal-flow anaerobic immobilised biomass reactor operated with a hydraulic detention time of 4 h. Peptone was the sole carbon source in the first experiment (E1). In the second experiment (E2), the reactor was fed with peptone and carbohydrates, and in the third experiment (E3), lipids were also added. At end of each experiment, the samples were collected to obtain spatial profiles of the substrates and intermediary metabolites. A modified first-order kinetic expression fits well with the chemical oxygen demand data, allowing kinetic parameter inference in both E1 and E2. The presence of lipids in the E3 influent clearly disturbed the equilibrium of the process, which could be better represented by two first-order kinetic expressions in series. A kinetic model of irreversible first-order reactions (in series and in parallel) with two intermediate products was proposed for representing the entire process. Several modifications of the metabolic routes were clearly represented by the values of the model parameters. It was also possible to conclude that the adsorption of lipids in the fixed bed caused a decrease in the consumption rate of proteins and acetate. Microscopy examinations and fluorescence in situ hybridisation analyses corroborated the conclusions from the kinetic study. The frequencies of the microorganisms changed as the substrate composition was modified, indicating the capability of the microorganisms to adapt.

Fekadu Fufa, Esayas Alemayehu, Bernd Lennartz. (¹. Faculty of Agricultural and Environmental Sciences, Rostock University, Justus-Von-Liebig-Weg 6, 18059, Rostock, Germany, ². Jimma Institute of Technology, Jimma University, Jimma, Oromia, Ethiopia).

Defluoridation of Groundwater Using Termite Mound. Water, Air, & Soil Pollution, 224(2013): 1552

High competing anions was investigated. Equilibrium was achieved within 10 min of agitation time. A high percentage (~90 %) of fluoride removal was obtained in a wide pH range 3–8, which is important in the practical application. Kinetics data followed the pseudo-second-order model ($R^2 > 0.99$). The Dubinin–Radushkevich isotherm described most satisfactorily ($R^2 = 0.968$, $\chi^2 = 0.09$) the equilibrium adsorption, giving a sorption capacity of 2.70 mg/g. The obtained mean free energy ($E_{DR} = 11.62$ kJ/mol) suggested that chemisorption should be mainly responsible for fluoride adsorption. Fluoride removal was significantly decreased in the presence of carbonate and phosphate ions, whereas slightly increased in the presence of chloride, nitrate, and sulfate. The adsorbent reduced 7.56 mg/L fluoride content of groundwater to below 1.5 mg/L. The fluoride-loaded TM was successfully regenerated using calcined eggshell or NaOH solution with insignificant loss of metals. The adsorption efficiency of the regenerated TM was comparable to the fresh TM. The results obtained from this study could provide important information for evaluating the application of TM for defluoridation.

Zhiping Qiu, Qichang Yang, Wenke Liu. (¹ Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, 100081, China, ² Key Laboratory of Energy Conservation and Waste Management of Agricultural Structures, Ministry of Agriculture, Beijing, 100081, China). **Photocatalytic Degradation of Phytotoxic Substances in Waste Nutrient Solution by Various Immobilized Levels of Nano-TiO₂. Water, Air, & Soil Pollution, 224(2013):1461**

The photocatalytic degradation effectiveness of six selected typical phytotoxic substances (ferulic, benzoic, gallic, salicylic, tannic, and acetic acid) by two levels of 10 nm TiO₂ (11 and 22 g/m²) immobilized on tiles under 254 nm of UV light irradiation was investigated. The results showed that the immobilized nano-TiO₂ significantly degraded all phytotoxic substances dissolved in distilled water, and the cumulative degradation rates of ferulic, benzoic, gallic, salicylic, tannic, and acetic acid reached 22.2, 33.6, 48.2, 56.9, 57.5, and 76.0 % after 6 h of treatment, respectively. Furthermore, the cumulative degradation rates of six phytotoxic substances by immobilized nano-TiO₂ were different remarkably, i.e., salicylic acid > benzoic acid, gallic acid > ferulic acid, acetic acid > tannic acid. The maximal photocatalytic degradation efficiencies of all phytotoxic substances appeared at the first 2 h in the three experiments. During the 6-h treatment period, the photocatalytic degradation efficiency of all phytotoxic substances decreased gradually. There was no significant difference in the photocatalytic degradation of benzoic acid and ferulic acid between the two levels of immobilized nano-TiO₂ treatments, whereas a significant difference was found in the photocatalytic degradation of salicylic acid, gallic acid, tannic acid, and acetic acid. In a word, nano-TiO₂ photocatalysis is an effective method to degrade phytotoxic substances. And the photocatalytic degradation effectiveness of six typical phytotoxic substances may be related to their structures.

Mahendra Aryal, Maria Liakopoulou-Kyriakides. (¹ Faculty of Chemical Engineering, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece). **Biodegradation and Kinetics of Phenanthrene and Pyrene in the Presence of**

Nonionic Surfactants by *Arthrobacter* Strain Sphe3. Water, Air, & Soil Pollution, 224(2013):1426

Surfactant-enhanced solubilization and subsequent biodegradation of phenanthrene and pyrene from aqueous solutions by *Arthrobacter* strain Sphe3 was investigated. The results show that growth of *Arthrobacter* strain Sphe3 was increased upon increase in concentration of Tween 20 and Tween 80. Inhibition of bacterial growth was observed with increasing Triton X-100 concentrations, whereas sodium dodecyl sulfate (SDS) totally inhibited this bacterial growth. Phenanthrene and pyrene solubilization was enhanced in the presence of surfactants and found to be linearly proportional to their concentrations, above the critical micelle concentration (CMC). In addition, Tween 20 and Tween 80 enhanced the biodegradation of phenanthrene and pyrene. The high correlation coefficient (R^2) values obtained at all the concentrations studied, suggest that biodegradation kinetics of both phenanthrene and pyrene in the presence of Tween 20 and Tween 80 follow first-order kinetic equation model. Experimental results suggest that Tween 20 and Tween 80 may have great potential for applications in bioremediation of these polycyclic aromatic hydrocarbon (PAH) compounds using *Arthrobacter* strain Sphe3.

P. Rodríguez-Escales, E. Borràs, M. Sarra, A. Folch. (¹ D'Enginy Biorem, Carrer Madrazo 68, 08006, Barcelona, Spain, ² Unitat de Geodinàmica Externa i Hidrogeologia, Departament de Geologia, Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain, ³ Departament d'Enginyeria Química, Escola d'Enginyeria, Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain, ⁴ GHS, Departament d'Enginyeria del Terreny, Cartogràfica i Geofísica, Universitat Politècnica de Catalunya-BarcelonaTech, Jordi Girona 1-3, Modul D-2, 08034, Barcelona, Spain). Granulometry and Surfactants, Key Factors in Desorption and Biodegradation (*T.versicolor*) of PAHs in Soil and Groundwater. Water, Air, & Soil Pollution, 224(2013):1422

High hydrophobicity of polycyclic aromatic hydrocarbons (PAHs) is the most limiting factor for the remediation of polluted soils and aquifers. The present study analyzes the effect of three nonionic surfactants (Tween 80, BS-400, and Gold Crew) and the granulometry of soil (1 %, 5 %, 10 %, and 20 % of clay and silt) on desorption of a PAH mixture (fluorene, phenanthrene, anthracene, and pyrene). As a general trend, decrease of fine material content and increase of surfactant concentration raises desorption. However, some particularities have to be considered depending on granulometry together with the surfactant applied. Furthermore, increase of fine material content tends to reduce the importance of the PAH properties, e.g., K_{ow} and solubility, in desorption. To complete the remediation process, biodegradation by *Trametes versicolor* was tested with the surfactant Tween 80. Results indicate that a high concentration of surfactant does not affect the efficiency of fungus bioremediation. Nevertheless, high fine material content in soil/aquifer can reduce the degradation rate. Moreover, desorption and biodegradation used synergically guarantee better overall results in the remediation of soils polluted by PAH mixtures than other methods that separate desorption and remediation.

Gloria Sánchez-Galván, Francisco J. Mercado, Eugenia J. Olguín. (¹ Environmental Biotechnology Research Group, Institute of Ecology, Carretera Antigua a Coatepec # 351, El Haya, Xalapa, Veracruz, 91070, Mexico). Leaves and Roots of *Pistia stratiotes* as Sorbent Materials for the Removal of Crude Oil from Saline Solutions. Water, Air, & Soil Pollution, 224(2013):1421

The removal and sorption of oil from saline solutions by leaves (L) and roots (R) of *Pistia stratiotes* are described for the first time. The effects of biomass dose (0.5 and 1.0 g), contact time (30, 60, 90, and 120 min), and initial oil concentration (IOC = 979 ± 9.82 , $1,968 \pm 8.01$, $3,935 \pm 40.09$, $7,778 \pm 196.42$, and $15,694 \pm 196.41$ mg L⁻¹) on removal and sorption (q) were evaluated. Studies included physicochemical characterization of the biomass. High oil removal (L = 93.71 ± 0.18 % and R = 80.93 ± 0.11 %) and sorption values (L = $2,904.47 \pm 4.49$ mg g⁻¹ and R = $2,324.38 \pm 29.29$ mg g⁻¹) were found. Such a high sorption might be related to factors such as a high surface area (128.38 ± 0.61 and 112.62 ± 5.17 m² g⁻¹, for leaves and roots, respectively), a high degree of relative hydrophobicity in the case of the leaves (71.05 ± 0.71 %), and capillary action. A high correlation was found between IOC and sorption, suggesting that the biomass could adsorb oil at IOCs higher than $15,694 \pm 196.41$ mg L⁻¹. The Freundlich isotherm model was found to best describe crude oil sorption by leaves and roots of *P. stratiotes*. These sorbent materials could be good candidates to be used during an oil spill.

Waldemar Grzybowski, Jerzy Szydlowski. (¹Institute of Oceanography, Gdansk University, Al. Pilsudskiego 46, Gdynia, 81-378, Poland). **Photodegradation of Detergent Phosphorus in Aquatic Environment.** *Water, Air, & Soil Pollution*, 224(2013):1413

Eutrophication potential of anthropogenic phosphorus depends on its bioavailability. Here, we present the proof that the main component of washing powders, sodium triphosphate (also known as sodium tripolyphosphate—STPP), can be photodegraded to orthophosphate. The process was studied under simulated sunlight in coastal and river water samples, distilled water, and solution of humic substances. Irradiation of samples enriched with STPP (130 μM P) resulted in release of up to 1 μM of orthophosphate. The measurable effects were observed solely in presence of inorganic matrix. It may suggest that degradation of STPP is driven mainly by the direct energy transfer from photo-excited colored dissolved organic matter: both sodium triphosphate and humic substances are negatively charged at natural water pH and presence of cations abates electrostatic repulsion between them. In this case, absorption of the sample remains the main factor affecting efficiency of the degradation.

Xin-Chao Ruan, Rui Ai, Xiao Jin, Qing-Fu Zeng, Ze-Yu Yang. (¹The Research Center of Environmental Science, Wuhan Textile University, 1 Fangzhi Road, Hongshan District, Wuhan, 430073, China, ²Key laboratory of Catalysis and Materials Science of the State Ethnic Affairs Commission & Ministry of Education, South-Central University for Nationalities, 708 Minyuan Road, Hongshan District, Wuhan, 430074, China). **Photodegradation of Tri (2-chloroethyl) Phosphate in Aqueous Solution by UV/H₂O₂.** *Water, Air, & Soil Pollution*, 224(2013):1406

The photooxidation degradation of tri (2-chloroethyl) phosphate (TCEP) by combining UV with hydrogen peroxide as oxidant was primarily studied in the present study by evaluating various treatment parameters. The results suggested that light intensity, initial pH and concentration of TCEP and H₂O₂, and reaction time affected the degradation efficiency of TCEP. The total organic carbon (TOC) removal rates, and the yield rates of Cl⁻ and PO₄³⁻ reached up to 86 %, 94 % and 97 %, respectively, under the optimized conditions in the present study. The degradation process obeyed the pseudo-first-order kinetic reaction expressed as ln

$(C_t/C_0) = -0.0275 t$ with a R^2 of 0.9962. The addition of t-butanol indicated that hydroxyl radicals played an important role in the degradation of TCEP. The primary investigation of the degradation mechanism of TCEP suggested that TCEP molecules were attacked by hydroxyl radicals produced from H_2O_2 with the irradiation of UV light, PO_4^{3-} , Cl^- and chlorinated alcohol/aldehyde, and/or non-chlorinated aldehyde with small molecular weight were produced, these produced small organic molecules were further oxidized to acids, most of them were finally mineralized to CO_2 and H_2O . The present technology was successfully applied for degrading TCEP in simulated real wastewater, which shows a promising potential for treating similar contaminants using corresponding advanced oxidation technology.

E. K. Mitter, C. R. Corso. (¹ **Campus de Rio Claro, Instituto de Biociências, IB, Departamento de Bioquímica e Microbiologia, UNESP—Univ. Estadual Paulista, Av 24 A 1515 CEP 13506900, Bela Vista, Rio Claro, São Paulo, Brazil**). **Acid Dye Biodegradation Using *Saccharomyces cerevisiae* Immobilized with Polyethyleneimine-Treated Sugarcane Bagasse.** *Water, Air, & Soil Pollution*, 224(2013):1391

Chemical reagents used by the textile industry are very diverse in their composition, ranging from inorganic compounds to polymeric compounds. Strong color is the most notable characteristic of textile effluents, and a large number of processes have been employed for color removal. In recent years, attention has been directed toward various natural solid materials that are able to remove pollutants from contaminated water at low cost, such as sugarcane bagasse. Cell immobilization has emerged as an alternative that offers many advantages in the biodegradation process, including the reuse of immobilized cells and high mechanical strength, which enables metabolic processes to occur under adverse conditions of pH, sterility, and agitation. Support treatment also increases the number of charges on the surface, thereby facilitating cell immobilization processes through adsorption and ionic bonds. Polyethyleneimine (PEI) is a polycationic compound known to have a positive effect on enzyme activity and stability. The aim of the present study was to investigate a low-cost alternative for the biodegradation and bioremediation of textile dyes, analyzing *Saccharomyces cerevisiae* immobilization in activated bagasse for the promotion of Acid Black 48 dye biodegradation in an aqueous solution. A 1 % concentration of a *S. cerevisiae* suspension was evaluated to determine cell immobilization rates. Once immobilization was established, biodegradation assays with free and immobilized yeast in PEI-treated sugarcane bagasse were evaluated for 240 h using UV-vis spectrophotometry. The analysis revealed significant relative absorbance values, indicating the occurrence of biodegradation in both treatments. Therefore, *S. cerevisiae* immobilized in sugarcane bagasse is very attractive for use in biodegradation processes for the treatment of textile effluents.

M. T. Fernández-Pazos, B. Garrido-Rodríguez, J. C. Nóvoa-Muñoz, M. Arias-Estévez, M. J. Fernández-Sanjurjo, A. Núñez-Delgado, E. Álvarez. (¹ **Dep. Edafología y Química Agrícola, Escuela Politécnica Superior, Univ. Santiago de Compostela, Campus Universitario, 27002, Lugo, Spain,** ³ **Department of Soil Sciences and Agricultural Chemistry, University of Santiago de Compostela, Polytechnic School, Campus Universitario, 27002, Lugo, Spain,** ² **Área de Edafología y Química Agrícola, Dep. Biología Vegetal y Ciencia del Suelo, Fac. Ciencias, Univ. Vigo, Campus Universitario, 32004, Ourense, Spain,** ⁴ **Soil Sciences and Agricultural Chemistry, Dep. Biología Vegetal y Ciencia del Suelo, Fac. Ciencias, Campus Universitario, 32004, Ourense, Spain**). **Cr(VI)**

Adsorption and Desorption on Soils and Biosorbents. *Water, Air, & Soil Pollution*, 224(2013):1366

We study the adsorption and desorption of chromium on two soils (a forest soil and a vineyard soil), both individually or after being combined with ground mussel shell, and on various materials (mussel shell, pyritic material from a dump site, and slate processing fines). The adsorption capacity depends mainly on the initial Cr concentration, on the pH, and on the abundance of noncrystalline Fe. The highest adsorption percentage (94 %) corresponds to the pyritic material, which also shows very low desorption rates (1.4 %), has the lowest pH, and has the highest concentration of noncrystalline Fe. The adsorption isotherms in most cases fit the Freundlich and Lineal models, rather than the Langmuir model, with no easily predictable maximum for chromium adsorption.

M. D. Chengalroyen, E. R. Dabbs. (¹ Department of Genetics, University of Witwatersrand, P.O. Box 1038, Johannesburg, Braamfontein, South Africa). **The microbial degradation of azo dyes: minireview. *World Journal of Microbiology and Biotechnology*, Volume 29(3) (2013): 389-399**

The removal of dyes in wastewater treatment plants still involves physical or chemical processes. Yet numerous studies currently exist on degradation based on the use of microbes—which is a well-studied field. However progress in the use of biological methods to deal with this environmentally noxious waste is currently lacking. This review focuses on the largest dye class, that is azo dyes and their biodegradation. We summarize the bacteria identified thus far which have been implicated in dye decolorization and discuss the enzymes involved and mechanisms by which these colorants are broken down.

Víctor Emmanuel Herrera-González, Nora Ruiz-Ordaz, Juvencio Galíndez-Mayer, Cleotilde Juárez-Ramírez, Fortunata Santoyo-Tepole, Erick Marrón Montiel. (¹ Departamento de Ingeniería Bioquímica. Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Carpio y Plan de Ayala, Col. Santo Tomás, CP 11340, Mexico City, DF, Mexico). **Biodegradation of the herbicide propanil, and its 3,4-dichloroaniline by-product in a continuously operated biofilm reactor. *World Journal of Microbiology and Biotechnology*, Volume 29(3) (2013); 467-474**

The persistence of propanil in soil and aquatic environments along with the possible accumulation of toxic degradation products, such as chloroanilines, is of environmental concern. In this work, a continuous small-scale bioprocess to degrade the herbicide propanil, its main catabolic by-product, 3,4-dichloroaniline (3,4-DCA), and the herbicide adjuvants is carried out. A microbial consortium, constituted by nine bacterial genera, was selected. The isolated strains, identified by amplification and sequencing of their 16S rDNA, were: *Acidovorax* sp., *Luteibacter (rhizovicinus)*, *Xanthomonas* sp., *Flavobacterium* sp., *Variovorax* sp., *Acinetobacter (calcoaceticus)*, *Pseudomonas* sp., *Rhodococcus* sp., and *Kocuria* sp. The ability of the microbial consortium to degrade the herbicide was evaluated in a biofilm reactor at propanil loading rates ranging from 1.9 to 36.8 mg L⁻¹ h⁻¹. Complete removal of propanil, 3,4-DCA, chemical oxygen demand and total organic carbon was obtained at propanil loading rates up to 24.9 mg L⁻¹ h⁻¹. At higher loading rates, the removal efficiencies decayed. Four of the identified strains could grow individually in propanil, and 3,4-DCA: *Pseudomonas* sp., *Acinetobacter*

calcoaceticus, *Rhodococcus* sp., and *Xanthomonas* sp. The *Kocuria* strain grew on 3,4-DCA, but not on propanil. The first three bacteria have been related to biodegradation of phenyl urea herbicides or chlorinated anilines. Although some strains of the genera *Xanthomonas* and *Kocuria* have a role in the biodegradation of several xenobiotic compounds, as far as we know, there are no reports about degradation of propanil by *Xanthomonas* or 3,4-DCA by *Kocuria* species.

Jaseetha Abdul Salam, V. Lakshmi, Devlina Das, Nilanjana Das. (¹ School of Biosciences and Technology, VIT University, Vellore, 632014, Tamil Nadu, India). **Biodegradation of lindane using a novel yeast strain, *Rhodotorula* sp. VITJzN03 isolated from agricultural soil. World Journal of Microbiology and Biotechnology, Volume 29(3) (2013): 475-487**

Lindane is a notorious organochlorine pesticide due to its high toxicity, persistence in the environment and its tendency to bioaccumulate. A yeast strain isolated from sorghum cultivation field was able to use lindane as carbon and energy source under aerobic conditions. With molecular techniques, it was identified and named as *Rhodotorula* strain VITJzN03. The effects of nutritional and environmental factors on yeast growth and the biodegradation of lindane was investigated. The maximum production of yeast biomass along with 100 % lindane mineralization was noted at an initial lindane concentration of 600 mg l⁻¹ within a period of 10 days. Lindane concentration above 600 mg l⁻¹ inhibited the growth of yeast in liquid medium. A positive relationship was noted between the release of chloride ions and the increase of yeast biomass as well as degradation of lindane. The calculated degradation rate and half life of lindane were found to be 0.416 day⁻¹ and 1.66 days, respectively. The analysis of the metabolites using GC-MS identified the formation of seven intermediates including γ -pentachlorocyclohexane (γ -PCCH), 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCCHdiene), 1,2,4-trichlorobenzene (1,2,4 TCB), 1,4-dichlorobenzene (1,4 DCB), chloro-cis-1,2-dihydroxycyclohexadiene (CDCHdiene), 3-chlorocatechol (3-CC) and maleylacetate (MA) derivatives indicating that lindane degradation follows successive dechlorination and oxidation-reduction. Based on the results of the present study, the possible pathway for lindane degradation by *Rhodotorula* sp. VITJzN03 has been proposed. To the best of our knowledge, this is the first report on lindane degradation by yeast which can serve as a potential agent for in situ bioremediation of medium to high level lindane-contaminated sites.

Lerato M. Sekhohola, Eric E. Igbini, A. Keith Cowan. (¹ Institute for Environmental Biotechnology, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa). **Biological degradation and solubilisation of coal. Biodegradation, Volume 24(3) (2013): 305-318**

This review focuses on ligninolytic fungi, soil bacteria, plants and root exudates in the degradation and solubilisation of low grade and waste coal and the interaction between these mutualistic biocatalysts. Coal represents a considerable portion of the total global fossil fuel reserve and continued demand for, and supply of this resource generates vast quantities of spoil and low grade waste. Large scale bioremediation technologies for the beneficiation of waste coal have unfortunately not yet been realised despite the many discoveries of microorganisms capable of lignite, lignin, and humic acid breakdown. Even so, solubilisation and depolymerization of low grade coal appears to involve either ligninolytic enzyme action or the production of alkaline substances or both. While the precise mechanism of coal biosolubilisation is unclear, a model for the phyto-biodegradation of low rank coal by mutualistic interaction between ligninolytic

microorganisms and higher plants is proposed. Based on accumulated evidence this model suggests that solubilisation and degradation of lignite and waste coals commences upon plant root exudate and ligninolytic microorganism interaction, which is mutualistic, and includes soil bacteria and both mycorrhizal and non-mycorrhizal fungi. It is envisaged that this model and its further elaboration will aid in the development of functional technologies for commercial bioremediation of coal mine spoils, contribute to soil formation, and the overall biogeochemistry of organic carbon in the global ecosystem.

Débora Toledo Ramos, Márcio Luis Busi da Silva, Helen Simone Chiaranda, Pedro J. J. Alvarez, Henry Xavier Corseuil. (¹Department of Sanitary and Environmental Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil, ²EMBRAPA, BR153 Km 110, P.O. Box 21, Concórdia, SC, 89700-000, Brazil, ³Department of Civil and Environmental Engineering, Rice University, Houston, TX, USA). **Biostimulation of anaerobic BTEX biodegradation under fermentative methanogenic conditions at source-zone groundwater contaminated with a biodiesel blend (B20). Biodegradation, Volume 24(3)(2013): 333-341**

Field experiments were conducted to assess the potential for anaerobic biostimulation to enhance BTEX biodegradation under fermentative methanogenic conditions in groundwater impacted by abiodiesel blend (B20, consisting of 20 % v/v biodiesel and 80 % v/v diesel). B20 (100 L) was released at each of two plots through an area of 1 m² that was excavated down to the water table, 1.6 m below ground surface. One release was biostimulated with ammonium acetate, which was added weekly through injection wells near the source zone over 15 months. The other release was not biostimulated and served as a baseline control simulating natural attenuation. Ammonium acetate addition stimulated the development of strongly anaerobic conditions, as indicated by near-saturation methane concentrations. BTEX removal began within 8 months in the biostimulated source zone, but not in the natural attenuation control, where BTEX concentrations were still increasing (due to source dissolution) 2 years after the release. Phylogenetic analysis using quantitative PCR indicated an increase in concentration and relative abundance of Archaea (Crenarchaeota and Euryarchaeota), *Geobacteraceae* (*Geobacter* and *Pelobacter* spp.) and sulfate-reducing bacteria (*Desulfovibrio*, *Desulfomicrobium*, *Desulfuromusa*, and *Desulfuromonas*) in the biostimulated plot relative to the control. Apparently, biostimulation fortuitously enhanced the growth of putative anaerobic BTEX degraders and associated commensal microorganisms that consume acetate and H₂, and enhance the thermodynamic feasibility of BTEX fermentation. This is the first field study to suggest that anaerobic-methanogenic biostimulation could enhance source zone bioremediation of groundwater aquifers impacted by biodiesel blends.

Hirofumi Tsutsui, Yasutaka Anami, Masami Matsuda, Kurumi Hashimoto, Daisuke Inoue, Kazunari Sei, Satoshi Soda, Michihiko Ike. (¹ Division of Sustainable Energy and Environmental Engineering, Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita, Osaka, 565-0871, Japan, ² Agriculture Unit, Research and Education Faculty, Kochi University, Monobe Otsu 200, Nankoku, Kochi, 783-8502, Japan, ³ Department of Health Science, School of Allied Health Sciences, Kitasato University, 1-15-1 Kitasato, Sagami-hara-Minami, Kanagawa, 252-0373, Japan). **Plasmid-mediated bioaugmentation of sequencing batch reactors for enhancement of 2,4-**

dichlorophenoxyacetic acid removal in wastewater using plasmid pJP4. Biodegradation, Volume 24(3)(2013): 343-352

Plasmid-mediated bioaugmentation was demonstrated using sequencing batch reactors (SBRs) for enhancing 2,4-dichlorophenoxyacetic acid (2,4-D) removal by introducing *Cupriavidus necator*JMP134 and *Escherichia coli* HB101 harboring 2,4-D-degrading plasmid pJP4. *C. necator*JMP134(pJP4) can mineralize and grow on 2,4-D, while *E. coli* HB101(pJP4) cannot assimilate 2,4-D because it lacks the chromosomal genes to degrade the intermediates. The SBR with *C. necator*JMP134(pJP4) showed 100 % removal against 200 mg/l of 2,4-D just after its introduction, after which 2,4-D removal dropped to 0 % on day 7 with the decline in viability of the introduced strain. The SBR with *E. coli* HB101(pJP4) showed low 2,4-D removal, i.e., below 10 %, until day 7. Transconjugant strains of *Pseudomonas* and *Achromobacter* isolated on day 7 could not grow on 2,4-D. Both SBRs started removing 2,4-D at 100 % after day 16 with the appearance of 2,4-D-degrading transconjugants belonging to *Achromobacter*, *Burkholderia*, *Cupriavidus*, and *Pandoraea*. After the influent 2,4-D concentration was increased to 500 mg/l on day 65, the SBR with *E. coli* HB101(pJP4) maintained stable 2,4-D removal of more than 95 %. Although the SBR with *C. necator*JMP134(pJP4) showed a temporal depression of 2,4-D removal of 65 % on day 76, almost 100 % removal was achieved thereafter. During this period, transconjugants isolated from both SBRs were mainly *Achromobacter* with high 2,4-D-degrading capability. In conclusion, plasmid-mediated bioaugmentation can enhance the degradation capability of activated sludge regardless of the survival of introduced strains and their 2,4-D degradation capacity.

María F. Bergero, Gloria I. Lucchesi. (¹ Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, CPX5804BYA, Río Cuarto, Córdoba, Argentina). **Degradation of cationic surfactants using *Pseudomonas putida* A ATCC 12633 immobilized in calcium alginate beads. Biodegradation, Volume 24(3)(2013): 353-364**

In this study, the degradation of tetradecyltrimethylammonium bromide (TTAB) by freely suspended and alginate-entrapped cells from the bacteria *Pseudomonas putida* (*P. putida*) A ATCC 12633 was investigated in batch cultures. The optimal conditions to prepare beads for achieving a higher TTAB degradation rate were investigated by changing the concentration of sodium alginate, pH, temperature, agitation rate and initial concentration of TTAB. The results show that the optimal embedding conditions of calcium alginate beads are 4 % w/v of sodium alginate content and 2×10^8 cfu ml⁻¹ of *P. putida* A ATCC 12633 cells that had been previously grown in rich medium. The optimal degradation process was carried out in pH 7.4 buffered medium at 30 °C on a rotary shaker at 100 rpm. After 48 h of incubation, the free cells degraded 26 mg l⁻¹ of TTAB from an initial concentration of 50 mg l⁻¹ TTAB. When the initial TTAB concentration was increased to 100 mg l⁻¹, the free cells lost their degrading activity and were no longer viable. In contrast, when the cells were immobilized on alginate, they degraded 75 % of the TTAB after 24 h of incubation from an initial concentration of 330 mg l⁻¹ of TTAB. The immobilized cells can be stored at 4 °C for 25 days without loss of viability and can be reused without losing degrading capacity for three cycles.

Wannarak Nopcharoenkul, Parichat Netsakulnee, Onruthai Pinyakong. (¹ Inter-Department of Environmental Science, Graduate School, Chulalongkorn University, Bangkok, 10330, Thailand, ² Bioremediation Research Unit, Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand, ³ Center

of Excellence for Environmental and Hazardous Waste Management (EHWM), Bangkok, 10330, Thailand). Diesel oil removal by immobilized *Pseudoxanthomonas* sp. RN402. Biodegradation, Volume 24(3) (2013): 387-397

Pseudoxanthomonas sp. RN402 was capable of degrading diesel, crude oil, *n*-tetradecane and *n*-hexadecane. The RN402 cells were immobilized on the surface of high-density polyethylene plastic pellets at a maximum cell density of 10^8 most probable number (MPN) g^{-1} of plastic pellets. The immobilized cells not only showed a higher efficacy of diesel oil removal than free cells but could also degrade higher concentrations of diesel oil. The rate of diesel oil removal by immobilized RN402 cells in liquid culture was $1,050 \text{ mg l}^{-1} \text{ day}^{-1}$. Moreover, the immobilized cells could maintain high efficacy and viability throughout 70 cycles of bioremedial treatment of diesel-contaminated water. The stability of diesel oil degradation in the immobilized cells resulted from the ability of living RN402 cells to attach to material surfaces by biofilm formation, as was shown by CLSM imaging. These characteristics of the immobilized RN402 cells, including high degradative efficacy, stability and flotation, make them suitable for the purpose of continuous wastewater bioremediation.

Erin K. Field, Robin Gerlach, Sridhar Viamajala, Laura K. Jennings, Brent M. Peyton, William A. Apel. (¹ Department of Microbiology and Center for Biofilm Engineering, Montana State University, Bozeman, MT, 59717, USA, ² Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, 04544, USA, ³ Department of Chemical and Biological Engineering and Center for Biofilm Engineering, Montana State University, 366 EPS Building, Bozeman, MT, 59717, USA, ⁴ Department of Chemical and Environmental Engineering, The University of Toledo, Toledo, OH, 43606, USA, ⁵ Biological Systems Department, Idaho National Laboratory, Idaho Falls, ID, 83415, USA). **Hexavalent chromium reduction by *Cellulomonas* sp. strain ES6: the influence of carbon source, iron minerals, and electron shuttling compounds. Biodegradation, Volume 24(3) (2013): 437-450**

The reduction of hexavalent chromium, Cr(VI), to trivalent chromium, Cr(III), can be an important aspect of remediation processes at contaminated sites. *Cellulomonas* species are found at several Cr(VI) contaminated and uncontaminated locations at the Department of Energy site in Hanford, Washington. Members of this genus have demonstrated the ability to effectively reduce Cr(VI) to Cr(III) fermentatively and therefore play a potential role in Cr(VI) remediation at this site. Batch studies were conducted with *Cellulomonas* sp. strain ES6 to assess the influence of various carbon sources, iron minerals, and electron shuttling compounds on Cr(VI) reduction rates as these chemical species are likely to be present in, or added to, the environment during in situ bioremediation. Results indicated that the type of carbon source as well as the type of electron shuttle present influenced Cr(VI) reduction rates. Molasses stimulated Cr(VI) reduction more effectively than pure sucrose, presumably due to presence of more easily utilizable sugars, electron shuttling compounds or compounds with direct Cr(VI) reduction capabilities. Cr(VI) reduction rates increased with increasing concentration of anthraquinone-2,6-disulfonate (AQDS) regardless of the carbon source. The presence of iron minerals and their concentrations did not significantly influence Cr(VI) reduction rates. However, strain ES6 or AQDS could directly reduce surface-associated Fe(III) to Fe(II), which was capable of reducing Cr(VI) at a near instantaneous rate. These results suggest the rate limiting step in these systems was the

transfer of electrons from strain ES6 to the intermediate or terminal electron acceptor whether that was Cr(VI), Fe(III), or AQDS.

Xiaolong Geng, Michel C. Boufadel, Brian Wrenn. (¹Department of Civil and Environmental Engineering, Center for Natural Resources Development and Protection, New Jersey Institute of Technology, Newark, NJ, 07102, USA, ²Department of Mathematics, Anshan Normal University, Anshan, Liaoning, 114001, China). **Mathematical modeling of the biodegradation of residual hydrocarbon in a variably-saturated sand column. *Biodegradation*, Volume 24(2) (2013): 153-163**

The biodegradation of heptadecane in five sand columns was modeled using a multiplicative Monod approach. Each column contained 1.0 kg of sand and 2 g of heptadecane, and was supplied with an artificial seawater solution containing nutrients at a flow rate that resulted in unsaturated flow through the column. All nutrients were provided in excess with the exception of nitrate whose influent concentration was 0.1, 0.5, 1.0, 2.5, or 5.0 mg N/L. The experiment was run around 912 h until no measurable oxygen consumption or CO₂ production was observed. The residual mass of heptadecane was measured at the end of the experiments and the biodegradation was monitored based on oxygen consumption and CO₂ production. Biodegradation kinetic parameters were estimated by fitting the model to experimental data of oxygen, CO₂, and residual mass of heptadecane obtained from the two columns having influent nitrate-N concentration of 0.5 and 2.5 mg/L. Noting that the oxygen and CO₂ measurements leveled off at around 450 h, we fitted the model to these data for that range. The estimated parameters fell in within the range reported in the literature. In particular, the half-saturation constant for nitrate utilization, K_N , was estimated to be 0.45 mg N/L, and the yield coefficient was found to be 0.15 mg biomass/mg heptadecane. Using these values, the rest of experimental data from the five columns was predicted, and the model agreed with the observations. There were some consistent discrepancies at large times between the model simulation and observed data in the cases with higher nitrate concentration. One plausible explanation for these differences could be limitation of biodegradation by reduction of the heptadecane-water interfacial area in these columns while the model uses a constant interfacial area.

Eduardo Fernandez-Fontaina, Ines Pinho, Marta Carballa, Francisco Omil, Juan M. Lema. (¹Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Rúa Lope Gómez de Marzoa s/n, E-15782, Santiago de Compostela, Spain). **Biodegradation kinetic constants and sorption coefficients of micropollutants in membrane bioreactors. *Biodegradation*, Volume 24(2) (2013): 165-177**

In order to elucidate the capability of biomass developed in membrane bioreactors (MBR) to degrade and sorb emerging micropollutants, biodegradation (k_{biol}) and sorption (k_{sor}) kinetic constants as well as solid-liquid partition coefficients (K_d) of 13 selected pharmaceutical and personal care products (PPCPs) were determined with MBR heterotrophic biomass adding a pulse (100 ppb of each compound) and following the liquid and solid phase concentrations over time. The results obtained were compared to literature data referring to conventional activated sludge (CAS) systems. Two experiments were performed: one in the MBR itself and the second one in a batch reactor with the same type and concentration of biomass as in the MBR. Overall, both biodegradation and sorption coefficients were in the same range as previously reported by other studies in CAS systems, indicating that MBR biomass does not show better capabilities for the biological degradation and/or sorption of PPCPs compared to the biomass developed in CAS reactors. Therefore, the higher PPCPs removal efficiencies found in

MBRs are explained by the high biomass concentrations obtained at the long sludge retention times at which this type of reactors are usually operated.

Nicole Fahrenfeld, Jeffrey Zoeckler, Mark A. Widdowson, Amy Pruden. (¹Via Department of Civil and Environmental Engineering, 418 Durham Hall Virginia Tech, Blacksburg, VA, 24061, USA, ²US Army Corps of Engineers, Norfolk District, 803 Front Street, Norfolk, VA, 23510, USA). **Effect of biostimulants on 2,4,6-trinitrotoluene (TNT) degradation and bacterial community composition in contaminated aquifer sediment enrichments. *Biodegradation*, Volume 24(2) (2013): 179-190**

2,4,6-Trinitrotoluene (TNT) is a toxic and persistent explosive compound occurring as a contaminant at numerous sites worldwide. Knowledge of the microbial dynamics driving TNT biodegradation is limited, particularly in native aquifer sediments where it poses a threat to water resources. The purpose of this study was to quantify the effect of organic amendments on anaerobic TNT biodegradation rate and pathway in an enrichment culture obtained from historically contaminated aquifer sediment and to compare the bacterial community dynamics. TNT readily biodegraded in all microcosms, with the highest biodegradation rate obtained under the lactate amended condition followed by ethanol amended and naturally occurring organic matter (extracted from site sediment) amended conditions. Although a reductive pathway of TNT degradation was observed across all conditions, denaturing gradient gel electrophoresis (DGGE) analysis revealed distinct bacterial community compositions. In all microcosms, Gram-negative γ - or β -Proteobacteria and Gram-positive Negativicutes or Clostridia were observed. A *Pseudomonas* sp. in particular was observed to be stimulated under all conditions. According to non-metric multidimensional scaling analysis of DGGE profiles, the microcosm communities were most similar to heavily TNT-contaminated field site sediment, relative to moderately and uncontaminated sediments, suggesting that TNT contamination itself is a major driver of microbial community structure. Overall these results provide a new line of evidence of the key bacteria driving TNT degradation in aquifer sediments and their dynamics in response to organic carbon amendment, supporting this approach as a promising technology for stimulating in situ TNT bioremediation in the subsurface.

Yuka Ogata, Tadashi Toyama, Ning Yu, Xuan Wang, Kazunari Sei, Michihiko Ike. (¹Division of Sustainable Energy and Environmental Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka, 565-0871, Japan, ²Department of Research, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi, 400-8511, Japan, ³Department of Health Science, School of Allied Health Sciences, Kitasato University, 1-15-1 Kitasato, Sagami-hara-Minami, Kanagawa, 252-0373, Japan). **Occurrence of 4-*tert*-butylphenol (4-*t*-BP) biodegradation in an aquatic sample caused by the presence of *Spirodela polyrrhiza* and isolation of a 4-*t*-BP-utilizing bacterium. *Biodegradation*, Volume 24(2) (2013): 191-202**

Although 4-*tert*-butylphenol (4-*t*-BP) is a serious aquatic pollutant, its biodegradation in aquatic environments has not been well documented. In this study, 4-*t*-BP was obviously and repeatedly removed from water from four different environments in the presence of *Spirodela polyrrhiza*, giant duckweed, but 4-*t*-BP persisted in the environmental waters in the absence of *S. polyrrhiza*. Also, 4-*t*-BP was not removed from autoclaved pond water with sterilized *S. polyrrhiza*. These results suggest that the 4-*t*-BP removal from the environmental waters was

caused by biodegradation stimulated by the presence of *S. polyrrhiza* rather than by uptake by the plant. Moreover, *Spingobium fuliginis* OMI capable of utilizing 4-*t*-BP as a sole carbon and energy source was isolated from the *S. polyrrhiza* rhizosphere. Strain OMI degraded 4-*t*-BP via a *meta*-cleavage pathway, and also degraded a broad range of alkylphenols with linear or branched alkyl side chains containing two to nine carbon atoms. Root exudates of *S. polyrrhiza* stimulated 4-*t*-BP degradation and cell growth of strain OMI. Thus, the stimulating effects of *S. polyrrhiza* root exudates on 4-*t*-BP-degrading bacteria might have contributed to 4-*t*-BP removal in the environmental waters with *S. polyrrhiza*. These results demonstrate that the *S. polyrrhiza*–bacteria association may be applicable to the removal of highly persistent 4-*t*-BP from wastewaters or polluted aquatic environments.

Cécile Monard, Fabrice Martin-Laurent, Oscar Lima, Marion Devers-Lamrani, Françoise Binet. (¹UMR CNRS 6553 ‘EcoBio’—IFR2116/FR90 CAREN, Université de Rennes 1, 263 Avenue du Général Leclerc, Bat 14B, 35042, Rennes Cedex, France, ³Department of Forest Mycology and Plant Pathology, SLU, Box 7026, 750 07, Uppsala, Sweden, ²UMR 1347 Agroecologie, AgroSup/INRA/Université de Bourgogne, 17 rue Sully, BP 86510, 21065, Dijon Cedex, France). **Estimating the biodegradation of pesticide in soils by monitoring pesticide-degrading gene expression. *Biodegradation*, Volume 24(2) (2013): 203-213**

Assessing in situ microbial abilities of soils to degrade pesticides is of great interest giving insight in soil filtering capability, which is a key ecosystem function limiting pollution of groundwater. Quantification of pesticide-degrading gene expression by reverse transcription quantitative PCR (RT-qPCR) was tested as a suitable indicator to monitor pesticide biodegradation performances in soil. RNA extraction protocol was optimized to enhance the yield and quality of RNA recovered from soil samples to perform RT-qPCR assays. As a model, the activity of atrazine-degrading communities was monitored using RT-qPCRs to estimate the level of expression of *atzD* in five agricultural soils showing different atrazine mineralization abilities. Interestingly, the relative abundance of *atzD* mRNA copy numbers was positively correlated to the maximum rate and to the maximal amount of atrazine mineralized. Our findings indicate that the quantification of pesticide-degrading gene expression may be suitable to assess biodegradation performance in soil and monitor natural attenuation of pesticide.

Irmene Ortíz, Antonio Velasco, Sylvie Le Borgne, Sergio Revah. (¹Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana-Cuajimalpa, Artificios 40, Col. Hidalgo, Delegación Álvaro Obregón, 01120, Mexico, DF, Mexico, ²Centro Nacional de Investigación y Capacitación Ambiental, Instituto Nacional de Ecología, Mexico, DF, Mexico). **Biodegradation of DDT by stimulation of indigenous microbial populations in soil with cosubstrates. *Biodegradation*, Volume 24(2) (2013): 215-225**

Stimulation of native microbial populations in soil by the addition of small amounts of secondary carbon sources (cosubstrates) and its effect on the degradation and theoretical mineralization of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and its main metabolites, DDD and DDE, were evaluated. Microbial activity in soil polluted with DDT, DDE and DDD was increased by the presence of phenol, hexane and toluene as cosubstrates. The consumption of DDT was increased from 23 % in a control (without cosubstrate) to 67, 59 and 56 % in the presence of phenol, hexane and toluene, respectively. DDE was completely removed in all cases, and DDD removal was enhanced from 67 % in the control to ~86 % with all substrates tested, except for acetic acid and glucose substrates. In the latter cases, DDD removal was either

inhibited or unchanged from the control. The optimal amount of added cosubstrate was observed to be between 0.64 and 2.6 mg C g⁻¹ dry soil. The CO₂ produced was higher than the theoretical amount for complete cosubstrate mineralization indicating possible mineralization of DDT and its metabolites. Bacterial communities were evaluated by denaturing gradient gel electrophoresis, which indicated that native soil and the untreated control presented a low bacterial diversity. The detected bacteria were related to soil microorganisms and microorganisms with known biodegradative potential. In the presence of toluene a bacterium related to *Azoarcus*, a genus that includes species capable of growing at the expense of aromatic compounds such as toluene and halobenzoates under denitrifying conditions, was detected.

Irina S. Moreira, Catarina L. Amorim, Maria F. Carvalho, António C. Ferreira, Carlos M. Afonso, Paula M. L. Castro. (¹Centro de Biotecnologia e Química Fina (CBQF), Escola Superior de Biotecnologia, Centro Regional do Porto, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal, ²Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Rua Aníbal Cunha 164, 4050-047, Porto, Portugal). **Effect of the metals iron, copper and silver on fluorobenzene biodegradation by *Labrys portucalensis*. Biodegradation, Volume 24(2) (2013): 245-255**

Organic and metallic pollutants are ubiquitous in the environment. Many metals are reported to be toxic to microorganisms and to inhibit biodegradation. The effect of the metals iron, copper and silver on the metabolism of *Labrys portucalensis* F11 and on fluorobenzene (FB) biodegradation was examined. The results indicate that the addition of 1 mM of Fe²⁺ to the culture medium has a positive effect on bacterial growth and has no impact in the biodegradation of 1 and 2 mM of FB. The presence of 1 mM of Cu²⁺ was found to strongly inhibit the growth of F11 cultures and to reduce the biodegradation of 1 and 2 mM of FB to ca. 50 %, with 80 % of stoichiometrically expected fluoride released. In the experiments with resting cells, the FB degraded (from 2 mM supplied) was reduced ca. 20 % whereas the fluoride released was reduced to 45 % of that stoichiometrically expected. Ag⁺ was the most potent inhibitor of FB degradation. In experiments with growing cells, the addition of 1 mM of Ag⁺ to the culture medium containing 1 and 2 mM of FB resulted in no fluoride release, whereas FB degradation was only one third of that observed in control cultures. In the experiments with resting cells, the addition of Ag⁺ resulted in 25 % reduction in substrate degradation and fluoride release was only 20 % of that stoichiometrically expected. The accumulation of catechol and 4-fluorocatechol in cultures supplemented with Cu²⁺ or Ag⁺ suggest inhibition of the key enzyme of FB metabolism—catechol 1,2-dioxygenase.

S. Pradeep, P. Faseela, M. K. Sarath Josh, S. Balachandran, R. Sudha Devi, Sailas Benjamin. (¹Enzyme Technology Laboratory, Biotechnology Division, Department of Botany, University of Calicut, Malappuram, Kerala, 673 635, India, ²Department of Chemistry, Mahatma Gandhi College, Thiruvananthapuram, Kerala, 695 004, India). **Fungal biodegradation of phthalate plasticizer in situ. Biodegradation, Volume 24(2) (2013): 257-267**

This unique study describes how *Aspergillus japonicus*, *Penicillium brocae* and *Purpureocillium lilacinum*, three novel isolates of our laboratory from heavily plastics-contaminated soil completely utilized the plasticizer di(2-ethylhexyl)phthalate (DEHP) bound to PVC blood storage bags (BB) in simple basal salt medium (BSM) by static submerged growth (28 °C).

Initial quantification as well as percentage utilization of DEHP blended to BB were estimated periodically by extracting it into *n*-hexane. A two-stage cultivation strategy was employed for the complete mycoremediation of DEHP from BB in situ. During the first growth stage, about two-third parts of total (33.5 % w/w) DEHP bound to BB were utilized in two weeks, accompanied by increased fungal biomass (~0.15–0.32 g per g BB) and sharp declining (to ~3) of initial pH (7.2). At this stagnant growth state (low pH), spent medium was replaced by fresh BSM (pH, 7.2), and thus in the second stage the remaining DEHP (one-third) in BB was utilized completely. The ditches and furrows seen from the topology of the BB as seen by the 3D AFM image further confirmed the bioremediation of DEHP physically bound to BB in situ. Of the three mycelial fungi employed, *P. lilacinum* independently showed highest efficiency for the complete utilization of DEHP bound to BB, whose activity was comparable to that of the consortium comprising all the three fungi described herein. To sum up, the two-stage cultivation strategy demonstrated in this study shows that a batch process would efficiently remediate the phthalic acid esters blended in plastics on a large scale, and thus it offers potentials for the management of plastics wastes.

Rogers Ribeiro, Ivana Ribeiro de Nardi, Bruna Soares Fernandes, Eugenio Foresti, Marcelo Zaiat. (¹Laboratório de Biotecnologia Ambiental, Departamento de Engenharia de Alimentos, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Av. Duque de Caxias norte, 225, Pirassununga, SP, 13635-900, Brazil, ³Centro Universitário Central Paulista, São Carlos, Brazil, ²Laboratório de Processos Biológicos, Departamento de Hidráulica e Saneamento, Escola de Engenharia de São Carlos, Universidade de São Paulo, São Carlos, Brazil). **BTEX removal in a horizontal-flow anaerobic immobilized biomass reactor under denitrifying conditions. Biodegradation, Volume 24(2) (2013): 269-278**

Because benzene, toluene, ethylbenzene, and xylenes (BTEX) and ethanol are important contaminants present in Brazilian gasoline, it is essential to develop technology that can be used in the bioremediation of gasoline-contaminated aquifers. This paper evaluates the performance of a horizontal-flow anaerobic immobilized biomass (HAIB) reactor fed with water containing gasoline constituents under denitrifying conditions. Two HAIB reactors filled with polyurethane foam matrices (5 mm cubes, 23 kg/m³ density and 95 % porosity) for biomass attachment were assayed. The reactor fed with synthetic substrate containing protein, carbohydrates, sodium bicarbonate and BTEX solution in ethanol, at an Hydraulic retention time (HRT) of 13.5 h, presented hydrocarbon removal efficiencies of 99 % at the following initial concentrations: benzene 6.7 mg/L, toluene 4.9 mg/L, *m*-xylene and *p*-xylene 7.2 mg/L, ethylbenzene 3.7 mg/L, and nitrate 60 mg N/L. The HAIB reactor fed with gasoline-contaminated water at an HRT of 20 h showed hydrocarbon removal efficiencies of 96 % at the following initial concentrations: benzene, 4.9 mg/L; toluene, 7.2 mg/L; *m*-xylene, 3.7 mg/L; and nitrate 400 mg N/L. Microbiological observations along the length of the HAIB reactor fed with gasoline-contaminated water confirmed that in the first segment of the reactor, denitrifying metabolism predominated, whereas from the first sampling port on, the metabolism observed was predominantly methanogenic.

Murthy Kasi, Tanush Wadhawan, John McEvoy, G. Padmanabhan, Eakalak Khan. (¹Department of Civil Engineering, North Dakota State University, Fargo, ND, 58105, USA, ²Moore Engineering, Inc., West Fargo, ND, 58078, USA, ³Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, ND, 58105, USA).

Effect of carbon source during enrichment on BTEX degradation by anaerobic mixed bacterial cultures. Biodegradation, Volume 24(2) (2013): 279-293

A comprehensive study on the effects of different carbon sources during the bacterial enrichment on the removal performances of benzene, toluene, ethylbenzene, and xylenes (BTEX) compounds when present as a mixture was conducted. Batch BTEX removal kinetic experiments were performed using cultures enriched with individual BTEX compounds or BTEX as a mixture or benzoate alone or benzoate–BTEX mixture. An integrated Monod-type non-linear model was developed and a ratio between maximum growth rate (μ_{\max}) and half saturation constant (K_s) was used to fit the non-linear model. A higher μ_{\max}/K_s indicates a higher affinity to degrade BTEX compounds. Complete removal of BTEX mixture was observed by all the enriched cultures; however, the removal rates for individual compounds varied. Degradation rate and the type of removal kinetics were found to be dependent on the type of carbon source during the enrichment. Cultures enriched on toluene and those enriched on BTEX mixture were found to have the greatest μ_{\max}/K_s and cultures enriched on benzoate had the least μ_{\max}/K_s . Removal performances of the cultures enriched on all different carbon sources, including the ones enriched on benzoate or benzoate–BTEX mixture were also improved during a second exposure to BTEX. A molecular analysis showed that after each exposure to the BTEX mixture, the cultures enriched on benzoate and those enriched on benzoate–BTEX mixture had increased similarities to the culture enriched on BTEX mixture.

Xiangyu Cao, Chao Yang, Ruihua Liu, Qiang Li, Wei Zhang, Jianli Liu, Cunjiang Song, Chuanling Qiao, Ashok Mulchandani. (¹School of Life Science, Liaoning University, Shenyang, 110036, China, ²Department of Microbiology, College of Life Science, Nankai University, Tianjin, 300071, China, ³State Key Laboratory of Medicinal Chemical Biology and College of Pharmacy, Nankai University, Tianjin, 300071, China, ⁴State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China, ⁵Department of Chemical and Environmental Engineering, University of California, Riverside, Riverside, CA, 92521, USA). **Simultaneous degradation of organophosphate and organochlorine pesticides by *Sphingobium japonicum* UT26 with surface-displayed organophosphorus hydrolase. Biodegradation, Volume 24(2) (2013): 295-303**

A genetically engineered microorganism (GEM) capable of simultaneously degrading organophosphate and organochlorine pesticides was constructed for the first time by display of organophosphorus hydrolase (OPH) on the cell surface of a hexachlorocyclohexane (HCH)-degrading *Sphingobium japonicum* UT26. The GEM could potentially be used for removing the two classes of pesticides that may be present in mixtures at contaminated sites. A surface anchor system derived from the truncated ice nucleation protein (INPNC) from *Pseudomonas syringae* was used to target OPH onto the cell surface of UT26, reducing the potential substrate uptake limitation. The surface localization of INPNC–OPH fusion was verified by cell fractionation, western blot, proteinase accessibility, and immunofluorescence microscopy. Furthermore, the functionality of the surface-exposed OPH was demonstrated by OPH activity assays. Surface display of INPNC–OPH fusion (82 kDa) neither inhibited cell growth nor affected cell viability. The engineered UT26 could degrade parathion as well as γ -HCH rapidly in minimal salt medium. The removal of parathion and γ -HCH by engineered UT26 in sterile

and non-sterile soil was also studied. In both soil samples, a mixture of parathion (100 mg kg⁻¹) and γ -HCH (10 mg kg⁻¹) could be degraded completely within 15 days. Soil treatment results indicated that the engineered UT26 is a promising multifunctional bacterium that could be used for the bioremediation of multiple pesticide-contaminated environments.

Valentina Rivelli, Andrea Franzetti, Isabella Gandolfi, Sergio Cordoni, Giuseppina Bestetti. (¹Department of Environmental Sciences, University of Milano-Bicocca, Piazza della Scienza 1, 20126, Milan, Italy, ² ioEco srl, Via Leonardo Da Vinci 13, 20090, Segrate, MI, Italy). **Persistence and degrading activity of free and immobilised allochthonous bacteria during bioremediation of hydrocarbon-contaminated soils. Biodegradation, Volume 24(1) (2013): 1-11**

Rhodococcus sp. and *Pseudomonas* sp. bioremediation experiments were carried out using free and immobilized cells on natural carrier material (corn cob powder) in order to evaluate the feasibility of its use in the bioremediation of hydrocarbon-contaminated soils. Terminal restriction fragment length polymorphism analysis was performed on the 16S rRNA gene as molecular fingerprinting method in order to assess the persistence of inoculated strains in the soil over time. Immobilized *Pseudomonas* cells degraded hydrocarbons more efficiently in the short term compared to the free ones. Immobilization seemed also to increase cell growth and stability in the soil. Free and immobilized *Rhodococcus* cells showed comparable degradation percentages, probably due to the peculiarity of *Rhodococcus* cells to aggregate into irregular clusters in the presence of hydrocarbons as sole carbon source. It is likely that the cells were not properly adsorbed on the porous matrix as a result of the small size of its pores. When *Rhodococcus* and *Pseudomonas* cells were co-immobilized on the matrix, a competition established between the two strains, that probably ended in the exclusion of *Pseudomonas* cells from the pores. The organic matrix might act as protective agent, but it also possibly limited cell density. Nevertheless, when the cells were properly adsorbed on the porous matrix, the immobilization became a suitable bioremediation strategy.

Marie Bank Nielsen, Kjeld Ingvorsen. (¹Department of Bioscience, Microbiology, Aarhus University, Ny Munkegade 114-116, Building 1540, 8000, Aarhus C, Denmark). **Biodegradation of *para*-nitrophenol by *Citricoccus nitrophenolicus* strain PNP1^T at high pH. Biodegradation, Volume 24(1) (2013): 79-87**

A gram-positive bacterium *Citricoccus nitrophenolicus* (strain PNP1^T, DSM 23311^T, CCUG 59571^T) isolated from a waste water treatment plant was capable of effectively degrading *p*-nitrophenol (pNP) as a source of carbon, nitrogen and energy for growth. Degradation of pNP required oxygen and resulted in the stoichiometric release of nitrite. Strain PNP1^T also degraded 4-chlorophenol, phenol and salicylate. pNP was degraded at pH values between 6.8 and 10.0 and at temperatures between 15–32 °C. pNP at concentrations up to 150 mg L⁻¹ were degraded during growth in media at pH ≤ 10, whereas 200 mg L⁻¹ was completely inhibitory to growth. When incubated in an NH₄Cl-free medium (pH 10) containing both pNP and acetate, pNP is degraded with concomitant release of nitrite which was subsequently assimilated during acetate degradation. Intact cells of strain PNP1^T suspended in NaHCO₃/Na₂CO₃ buffer were able to continuously degrade 200 mg L⁻¹ pNP over a 40 day period at pH 10.

Hyun Jeong Jeon, Mal Nam Kim. (¹Department of Life Science, Sangmyung University, Seoul, 110-743, Republic of Korea). **Isolation of a thermophilic bacterium capable of low-molecular-weight polyethylene degradation. Biodegradation, Volume 24(1) (2013): 89-98**

A thermophilic bacterium capable of low-molecular-weight polyethylene (LMWPE) degradation was isolated from a compost sample, and was identified as *Chelatococcus* sp. E1, through sequencing of the 16S rRNA gene. LMWPE was prepared by thermal degradation of commercial PE in a strict nitrogen atmosphere. LMWPE with a weight-average-molecular-weight (Mw) in the range of 1,700–23,700 was noticeably mineralized into CO₂ by the bacterium. The biodegradability of LMWPE decreased as the Mw increased. The low molecular weight fraction of LMWPE decreased significantly as a result of the degradation process, and thereby both the number-average-molecular-weight and Mw increased after biodegradation. The polydispersity of LMWPE was either narrowed or widened, depending on the initial Mw of LMWPE, due to the preferential elimination of the low molecular weight fraction, in comparison to the high molecular weight portion. LMWPE free from an extremely low molecular weight fraction was also mineralized by the strain at a remarkable rate, and FTIR peaks assignable to C–O stretching appeared as a result of microbial action. The FTIR peaks corresponding to alkenes also became more intense, indicating that dehydrogenations occurred concomitantly with microbial induced oxidation.

Raquel Almeida, Ana P. Mucha, Catarina Teixeira, Adriano A. Bordalo, C. Marisa R. Almeida. (¹CIMAR/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123, Porto, Portugal, ²Laboratório de Hidrobiologia, Instituto de Ciências Biomédicas, Universidade do Porto (ICBAS-UP), Largo Professor Abel Salazar, No. 2, 4099-003, Porto, Portugal). **Biodegradation of petroleum hydrocarbons in estuarine sediments: metal influence. Biodegradation, Volume 24(1) (2013): 111-123**

In this work, the potential effect of metals, such as Cd, Cu and Pb, on the biodegradation of petroleum hydrocarbons in estuarine sediments was investigated under laboratory conditions. Sandy and muddy non-vegetated sediments were collected in the Lima River estuary (NW Portugal) and spiked with crude oil and each of the metals. Spiked sediments were left in the dark under constant shaking for 15 days, after which crude oil biodegradation was evaluated. To estimate microbial abundance, total cell counts were obtained by DAPI staining and microbial community structure was characterized by ARISA. Culturable hydrocarbon degraders were determined using a modified most probable number protocol. Total petroleum hydrocarbons concentrations were analysed by Fourier Transform Infrared Spectroscopy after their extraction by sonication, and metal contents were determined by atomic absorption spectrometry. The results obtained showed that microbial communities had the potential to degrade petroleum hydrocarbons, with a maximum of 32 % degradation obtained for sandy sediments. Both crude oil and metals changed the microbial community structure, being the higher effect observed for Cu. Also, among the studied metals, only Cu displayed measurable deleterious effect on the hydrocarbons degradation process, as shown by a decrease in the hydrocarbon degrading microorganisms abundance and in the hydrocarbon degradation rates. Both degradation potential and metal influence varied with sediment characteristics probably due to differences in contaminant bioavailability, a feature that should be taken into account in developing bioremediation strategies for co-contaminated estuarine sites.

Hong Li, Robert Muir, Neil R. McFarlane, Richard J. Soilleux, Xiaohong Yu, Ian P. Thompson, Simon A. Jackman. (¹Natural Environmental Research Council, Centre for

Ecology and Hydrology, Mansfield Road, Oxford, OX1 3SR, UK, ⁵Natural Environmental Research Council, Centre for Ecology and Hydrology-Lancaster, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK, ²Defence Science and Technology Laboratory, Porton Down, Salisbury, Wilts, SP4 0JQ, UK, ³Department of Engineering Science, University of Oxford, Parks Road, Oxford, OX1 3PJ, UK, ⁴Department of Earth Sciences, University of Oxford, Parks Road, Oxford, OX1 3PR, UK). Soil biotransformation of thiodiglycol, the hydrolysis product of mustard gas: understanding the factors governing remediation of mustard gas contaminated soil. *Biodegradation*, Volume 24(1) (2013): 125-135

Thiodiglycol (TDG) is both the precursor for chemical synthesis of mustard gas and the product of mustard gas hydrolysis. TDG can also react with intermediates of mustard gas degradation to form more toxic and/or persistent aggregates, or reverse the pathway of mustard gas degradation. The persistence of TDG have been observed in soils and in the groundwater at sites contaminated by mustard gas 60 years ago. The biotransformation of TDG has been demonstrated in three soils not previously exposed to the chemical. TDG biotransformation occurred via the oxidative pathway with an optimum rate at pH 8.25. In contrast with bacteria isolated from historically contaminated soil, which could degrade TDG individually, a consortium of three bacterial strains isolated from the soil never contaminated by mustard gas was able to grow on TDG in minimal medium and in hydrolysate derived from an historical mustard gas bomb. Exposure to TDG had little impacts on the soil microbial physiology or on community structure. Therefore, the persistency of TDG in soils historically contaminated by mustard gas might be attributed to the toxicity of mustard gas to microorganisms and the impact to soil chemistry during the hydrolysis. TDG biodegradation may form part of a remediation strategy for mustard gas contaminated sites, and may be enhanced by pH adjustment and aeration.

Yen-Hui Lin, Wen-Fan Lin, Kai-Ning Jhang, Pei-Yu Lin, Mong-Chuan Lee. (¹Department of Safety, Health and Environmental Engineering, Central Taiwan University of Science and Technology, 666, Bu-zih Road, Bei-tun District, Taichung, 40601, Taiwan, ²Institute of Life Sciences, Central Taiwan University of Science and Technology, 666, Bu-zih Road, Bei-tun District, Taichung, 40601, Taiwan). Adsorption with biodegradation for decolorization of reactive black 5 by *Funalia trogii* 200800 on a fly ash-chitosan medium in a fluidized bed bioreactor-kinetic model and reactor performance. *Biodegradation*, Volume 24(1) (2013): 137-152

A non-steady-state mathematical model system for the kinetics of adsorption and biodegradation of reactive black 5 (RB5) by *Funalia trogii* (*F. trogii*) ATCC 200800 biofilm on fly ash-chitosan bead in the fluidized bed process was derived. The mechanisms in the model system included adsorption by fly ash-chitosan beads, biodegradation by *F. trogii* cells and mass transport diffusion. Batch kinetic tests were independently performed to determine surface diffusivity of RB5, adsorption parameters for RB5 and biokinetic parameters of *F. trogii* ATCC 200800. A column test was conducted using a continuous-flow fluidized bed reactor with a recycling pump to approximate a completely-mixed flow reactor for model verification. The experimental results indicated that *F. trogii* biofilm bioregenerated the fly ash-chitosan beads after attached *F. trogii* has grown significantly. The removal efficiency of RB5 was about 95 % when RB5 concentration in the effluent was approximately 0.34 mg/L at a steady-state condition. The concentration of suspended *F. trogii* cells reached up to about 1.74 mg/L while the thickness of attached *F. trogii* cells was estimated to be 80 µm at a steady-state condition by

model prediction. The comparisons of experimental data and model prediction show that the model system for adsorption and biodegradation of RB5 can predict the experimental results well. The approaches of experiments and mathematical modeling in this study can be applied to design a full-scale fluidized bed process to treat reactive dye in textile wastewater.

Jianqiao Wang, Yotaro Yamamoto, Hirofumi Hirai, Hirokazu Kawagishi. (¹ Graduate School of Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka, 422-8529, Japan, ² Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka, 422-8529, Japan). **Dimerization of Bisphenol A by Hyper Lignin-Degrading Fungus *Phanerochaete sordida* YK-624 Under Ligninolytic Condition. Current Microbiology, Volume 66(6) (2013): 544-547**

Bisphenol A (BPA) was treated with hyper lignin-degrading fungus *Phanerochaete sordida* YK-624 under ligninolytic condition. After preculturing *P. sordida* YK-624 for 4 days, BPA (final concentration, 1 and 0.1 mM) was added to cultures. Both 1- and 0.1-mM BPA were effectively decreased within a 24-h treatment and two metabolites were detected. Two metabolites (5,5'-bis-[1-(4-hydroxy-phenyl)1-methyl-ethyl]-biphenyl-2,2'-diol and 4-(2-(4-hydroxy-phenyl) propan-2-yl)-2-(4-(2-(4-hydroxyphenyl) propan-2-yl) phenoxy)phenol) were identified by ESI-MS and NMR analysis. These results indicated that BPA was oxidized to BPA phenoxy radicals by ligninolytic enzymes and then dimerized at extracellular region.

Cervantes-González Elsa^{1*}, Rojas-Avelizapa Luz Irene², Cruz-Camarillo Ramón², Rojas-Avelizapa Norma Gabriela³ and Corona-Rivera Miguel Angel¹. (¹Departamento de Ingeniería Química, Coordinación Académica Región Altiplano, Universidad Autónoma de San Luis Potosí, Mexico, ²Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico., ³Departamento de Investigación, Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada, Instituto Politécnico Nacional, Mexico. *Corresponding author: E-mail: elsa.cervantes@uaslp.mx). **Microbial removal of weathered hydrocarbons by well adapted-bacteria. African Journal of Biotechnology Vol. 12(9)(2013):941-948**

The effectiveness of bioremediation processes may be limited by the physical and chemical properties of the pollutant, such as availability, recalcitrance, concentration and weathering, among others. The aim of this work was to evaluate the removal of recalcitrant oil fractions (aliphatic-aromatic and asphaltenic fractions) from a weathered soil, by two bacteria adapted to a high concentration of oil hydrocarbons, isolated from a soil with a concentration of 227,000 mg of total petroleum hydrocarbons per kg soil. Kinetics of hydrocarbons removal by *Bacillus coagulans* and/or *Serratia liquefaciens* was performed in liquid culture for 168 h; hydrocarbons from soil as sole carbon and energy source (600 mg/l) were added and each of the microorganisms was inoculated for evaluation independently or as a mixed culture. The aromatic fraction was removed by *B. coagulans* at 330 mg/l; by *S. liquefaciens* at 130 mg/l; and by both microorganisms at 360 mg/l. The asphaltenic fraction was removed by *B. coagulans* at 23 mg/l; by *S. liquefaciens* at 15 mg/l; and by both microorganisms at 34 mg/l. Chromatographic analysis of the aliphatic-aromatic fraction showed the presence of branched aliphatic C6 to C26, polyaromatic substituted compounds of two and three rings, and heteroaromatic compounds of

dibenzothiophene type. The compounds that were removed from the aliphatic-aromatic fraction were of all types in the range of C6 to C13.

Keywords: Asphaltenes, aliphatic-aromatic fraction, weathered, biodegradation.

Neha Singh, Tuhina Verma and Rajeeva Gaur*. (Department of Microbiology (Centre of Excellence), Dr. Ram Manohar Lohia Avadh University, Faizabad-224001, Uttar Pradesh, India. *Corresponding author. E-mail: rajeevagaur@gmail.com). **Detoxification of hexavalent chromium by an indigenous facultative anaerobic *Bacillus cereus* strain isolated from tannery effluent. African Journal of Biotechnology Vol. 12(10)(2013): 1091-1103**

A chromate resistant facultative anaerobic bacterial strain (FA-3) was isolated from the treated tannery effluent of Jajmau, Kanpur (India) and was identified as *Bacillus cereus*. FA-3 was tolerant to 1400 µg/ml of Cr (VI) and reduced a maximum of 72% Cr (VI) at 1000 µg/ml chromate concentration. The rate of growth of *B. cereus* decreased with the increase in Cr (VI) concentration of the medium and the chromate reduction was directly correlated to the growth of the strain. The strain FA-3 was capable of reducing Cr (VI) under a wide range of temperatures (25 to 40°C) and pH (6 to 10) with optimum at 37°C and initial pH 8.0. Glucose (0.5%) slightly increased the Cr (VI) reduction (78%). Heavy metal ions such as Zn²⁺, Ni²⁺ and Co²⁺ slightly affected the Cr (VI) reduction, while arsenic (As³⁺) significantly affected the Cr (VI) reduction. However, in the presence of Hg²⁺, the reduction of Cr (VI) was totally inhibited. Since, the strain has potential to survive and multiply in a wide range of environmental conditions and due to its high Cr (VI) reduction efficiency in the presence of other metal cations, it can be exploited for the bioremediation of chromate containing wastes.

Keywords: Treated tannery effluent, chromate resistant bacteria, minimal inhibitory concentration, heavy metal ions.

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A real-time PCR quantification method for indigenous hydrocarbon-degrading bacteria (HDB) carrying the *alkB* gene in the soil environment was developed to investigate their distribution in soil. The detection limit of indigenous HDB by the method was 1×10^6 cells/g-soil. The indigenous HDB were widely distributed throughout the soil environment and ranged from 3.7×10^7 to 5.0×10^8 cells/g-soil, and the ratio to total bacteria was 0.1–4.3 %. The dynamics of total bacteria, indigenous HDB, and *Rhodococcus erythropolis* NDKK6 (carrying *alkB* R2) during bioremediation were analyzed. During bioremediation with an inorganic nutrient treatment, the numbers of these bacteria were slightly increased. The numbers of HDB (both indigenous bacteria and strain NDKK6) were gradually decreased from the middle stage of bioremediation. Meanwhile, the numbers of these bacteria were highly increased and were maintained during bioremediation with an organic nutrient. The organic treatment led to activation of not only the soil bacteria but also the HDB, so an efficient bioremediation was carried out.

Young-Cheol Chang, Kazunori Takada, DuBok Choi, Tadashi Toyama, Ken Sawada, Shintaro Kikuchi. (¹ Division of Applied Sciences, College of Environmental Technology, Graduate School of Engineering, Muroran Institute of Technology, 27-1 Mizumoto, Muroran, 050-8585, Hokkaido, Japan, ² Biotechnology Lab, BK Company R&D Center, Jeonbuk, 579-879, Republic of Korea, ³ Department of Pharmacy, College of Pharmacy, Chungbuk National University, Cheongju, 361-763, Republic of Korea, ⁴ Department of Research, Interdisciplinary Graduate School of Medical and Engineering, University of Yamanashi, 4-3-11 Takeda, Kofu, 400-8511, Japan). **Isolation of Biphenyl and Polychlorinated Biphenyl-Degrading Bacteria and Their Degradation Pathway.** *Applied Biochemistry and Biotechnology*, Volume 170(2) (2013): 381-398

Four strains of biphenyl-degrading bacteria were isolated from a sewage and identified from the *Rhodococcus* genus (SK-1, SK-3, and SK-4) and *Aquamicrobium* genus (SK-2) by 16S rRNA sequence. Among these strains, strain SK-2 was most suitable for biphenyl degradation. When 0.65, 1.3, 2.6, or 3.9 mM of biphenyl was used, the biphenyl was completely degraded within 24 and 96 h of culture, respectively. However, in the case of 6.5 and 9.75 mM of biphenyl, the biphenyl degradation yields were about 80 % and 46.7 % after 120 h of culture, respectively. The isolated strains could degrade a broad spectrum of aromatic compounds including high-chlorinated polychlorinated biphenyl (PCB) congeners in the presence of biphenyl. In addition, strain SK-2 could utilize PCB congeners containing one to six chlorine substituents such as 2,2',4,4',5,5'-hexachlorobiphenyl. The PCB utilization rate by the strain SK-2 was increased compared to that of other PCB congener-utilizing bacteria. The four isolates metabolized 4-chlorobiphenyl to 4-chlorobenzoic acid and 2-hydroxy-6-oxo-6-(4'-chlorophenyl)-hexa-2,4-dienoic acid. These results suggest the isolated strains might be good candidates for the bioremediation of PCB-contaminated soil, especially high-saline soils.

U. D. Phalgune, P. R. Rajamohanam, B. G. Gaikwad, R. J. Varma, S. George. (¹ Central NMR Facility, National Chemical Laboratory, Pune, 411008, Maharashtra, India, ² Chemical Engineering Division, National Chemical Laboratory, Pune, 411008, Maharashtra, India, ³ Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram, Tamil Nadu, India). **Biodegradation of Phenol by the Yeast *Candida tropicalis*: An Investigation by NMR Spectroscopy.** *Applied Biochemistry and Biotechnology*, Volume 169(7) (2013): 2029-2037

The process of phenol biodegradation by the yeast *Candida tropicalis* NCIM 3556 in aqueous medium was studied by ¹H, ¹³C, and DOSY NMR techniques. Samples at regular intervals were centrifuged to separate the cells, and ¹H spectral data were collected at 400 MHz. Though a gradual decrease in the concentration of phenol was observed, after an incubation period of ~8 h, formation of any intermediate products could not be detected. Experiments carried out with uniformly ¹³C-labeled phenol also failed to detect formation of any carboxylic acid intermediates during degradation. The studies indicated that the phenol was completely degraded to carbon dioxide and water in approximately 20 h. Self-diffusion coefficient measurements showed that the lifetime of phenol in the bound form is too small to impart any change in its diffusion behavior and the intermediates formed are converted to carbon dioxide and water at a very fast rate.

V. S. Priya, Ligy Philip. (¹Environmental and Water Resources Engineering Division, Department of Civil Engineering, IIT Madras, Chennai, 600 036, India). **Biodegradation of Dichloromethane Along with Other VOCs from Pharmaceutical Wastewater.** *Applied Biochemistry and Biotechnology*, Volume 169(4) (2013):1197-1218

The present study dealt with the interaction of dichloromethane (DCM) with other non-chlorinated organic solvents such as methanol, acetone, toluene, and benzene, which are commonly present in the pharmaceutical wastewater, during biodegradation by mixed bacterial consortium. Non-chlorinated solvents were easily degradable even at an initial concentration of 1,000 mg/L, whereas only 20 mg/L of DCM was degraded when used as sole carbon source. The Monod Inhibition model appears to simulate the single pollutant biodegradation kinetics satisfactorily. In dual substrate systems, low concentrations (100 mg/L) of non-chlorinated solvents did not interfere with the DCM degradation. Non-interaction sum kinetics model was able to simulate the experimental results well in this case. However, high concentrations of non-chlorinated solvents (1,000 mg/L) affected the DCM degradation significantly. There was severe competition between the chlorinated and the non-chlorinated solvents. In this case, competitive inhibition model predicted the experimental results better compared to co-metabolism model. In multiple substrate system also, presence of DCM prolonged the degradation of the other non-chlorinated solvents. However, the presence of non-chlorinated compounds accelerated the degradation of DCM. The results of the present study may be helpful in optimal design of biological systems treating mixed pollutants.

Thanunchanok Chairin, Thitinard Nitheranont, Akira Watanabe, Yasuhiko Asada, Chartchai Khanongnuch, Saisamorn Lumyong. (¹Biotechnology Program, Graduate School, Chiang Mai University, Chiang Mai, 50200, Thailand, ²Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Kagawa, 761-0795, Japan, ³Division of Biotechnology, School of Agro-Industry, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, 50100, Thailand, ⁴Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand). **Biodegradation of Bisphenol A and Decolorization of Synthetic Dyes by Laccase from White-Rot Fungus, *Trametes polyzona*.** *Applied Biochemistry and Biotechnology*, Volume 169(2) (2013): 539-545

Purified laccase from *Trametes polyzona* WR710-1 was used as biocatalyst for bisphenol A biodegradation and decolorization of synthetic dyes. Degradation of bisphenol A by laccase with or without redox mediator, 1-hydroxybenzotriazole (HBT) was studied. The quantitative analysis by HPLC showed that bisphenol A rapidly oxidized by laccase with HBT. Bisphenol A was completely removed within 3 h and 4-isopropenylphenol was found as the oxidative degradation product from bisphenol A when identified by GC-MS. All synthetic dyes used in this experiment, Bromophenol Blue, Remazol Brilliant Blue R, Methyl Orange, Relative Black 5, Congo Red, and Acridine Orange were decolorized by *Trametes* laccase and the percentage of decolorization increased when 2 mM HBT was added in the reaction mixture. This is the first report showing that laccase from *T. polyzona* is an effective enzyme having high potential for environmental detoxification, bisphenol A degradation and synthetic dye decolorization.

Thanunchanok Chairin, Thitinard Nitheranont, Akira Watanabe, Yasuhiko Asada, Chartchai Khanongnuch, Saisamorn Lumyong. (¹Biotechnology Program, Graduate School, Chiang Mai University, Chiang Mai, 50200, Thailand, ²Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Kagawa, 761-0795, Japan, ³Division of Biotechnology, School of Agro-Industry, Faculty of

Agro-Industry, Chiang Mai University, Chiang Mai, 50100, Thailand, ⁴Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand). Biodegradation of Bisphenol A and Decolorization of Synthetic Dyes by Laccase from White-Rot Fungus, *Trametes polyzona*. *Applied Biochemistry and Biotechnology*, Volume 169(2) (2013): 539-545

Purified laccase from *Trametes polyzona* WR710-1 was used as biocatalyst for bisphenol A biodegradation and decolorization of synthetic dyes. Degradation of bisphenol A by laccase with or without redox mediator, 1-hydroxybenzotriazole (HBT) was studied. The quantitative analysis by HPLC showed that bisphenol A rapidly oxidized by laccase with HBT. Bisphenol A was completely removed within 3 h and 4-isopropenylphenol was found as the oxidative degradation product from bisphenol A when identified by GC-MS. All synthetic dyes used in this experiment, Bromophenol Blue, Remazol Brilliant Blue R, Methyl Orange, Relative Black 5, Congo Red, and Acridine Orange were decolorized by *Trametes* laccase and the percentage of decolorization increased when 2 mM HBT was added in the reaction mixture. This is the first report showing that laccase from *T. polyzonais* an affective enzyme having high potential for environmental detoxification, bisphenol A degradation and synthetic dye decolorization.

Shi-Ling Ding, Xi-Kui Wang, Wen-Qiang Jiang, Xia Meng, Ru-Song Zhao, Chen Wang, Xia Wang. (¹School of Chemical Engineering, Shandong Polytechnic University, Jinan, 250353, China, ²Shandong Key Laboratory of Cleaner Production and Reuse of Industrial Waste, Jinan, 250353, China, ³School of Light Chemistry and Environmental Engineering, Shandong Polytechnic University, Jinan, 250353, China, ⁴College of Environment and Safety Engineering, Qingdao University of Science and Technology, Qingdao, 266042, China, ⁵Analysis and Test Center, Shandong Academy of Sciences, Jinan, 250014, China). Photodegradation of the antimicrobial triclocarban in aqueous systems under ultraviolet radiation. *Environmental Science and Pollution Research*, Volume 20(5) (2013): 3195-3201

This work aimed to investigate the effectiveness of ultraviolet (UV) radiation on the degradation of the antimicrobial triclocarban (TCC). We investigated the effects of several operational parameters, including solution pH, initial TCC concentration, photocatalyst TiO₂ loading, presence of natural organic matter, and most common anions in surface waters (e.g., bicarbonate, nitrate, and sulfate). The results showed that UV radiation was very effective for TCC photodegradation and that the photolysis followed pseudo-first-order kinetics. The TCC photolysis rate was pH dependent and favored at high pH. A higher TCC photolysis rate was observed by direct photolysis than TiO₂ photocatalysis. The presence of the inorganic ions bicarbonate, nitrate, and sulfate hindered TCC photolysis. Negative effects on TCC photolysis were also observed by the addition of humic acid due to competitive UV absorbance. The main degradation products of TCC were tentatively identified by gas chromatograph with mass spectrometer, and a possible degradation pathway of TCC was also proposed.

Hong-Guang Guo, Nai-Yun Gao, Wen-Hai Chu, Lei Li, Yong-Ji Zhang, Jin-Shan Gu, Yu-Liang Gu. (¹State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai, 200092, China, ²National Engineering Research Center for Urban Water Resource, Shanghai, 200082, China). Photochemical degradation of ciprofloxacin in UV and UV/H₂O₂ process: kinetics,

parameters, and products. Environmental Science and Pollution Research, Volume 20(5) (2013): 3202-3213

Photochemical degradation of fluoroquinolone ciprofloxacin (CIP) in water by UV and UV/H₂O₂ were investigated. The degradation rate of CIP was affected by pH, H₂O₂ dosage, as well as the presence of other inorganic components. The optimized pH value and H₂O₂ concentration were 7.0 and 5 mM. Carbonate and nitrate both impeded CIP degradation. According to liquid chromatography–tandem mass spectrometry analysis, four and 16 products were identified in UV and UV/H₂O₂ system, respectively. Proposed degradation pathways suggest that reactions including the piperazinyl substituent, quinolone moiety, and cyclopropyl group lead to the photochemical degradation of CIP. Toxicity of products assessed by *Vibrio qinghaiensis* demonstrated that UV/H₂O₂ process was more capable on controlling the toxicity of intermediates in CIP degradation than UV process.

Puthiya Veetil Nidheesh, Rajan Gandhimathi, Srikrishnaperumal Thanga Ramesh. (¹Department of Civil Engineering, National Institute of Technology, Tiruchirappalli, Tamil Nadu, India). Degradation of dyes from aqueous solution by Fenton processes: a review. Environmental Science and Pollution Research, Volume 20(4) (2013): 2099-2132

Several industries are using dyes as coloring agents. The effluents from these industries are increasingly becoming an environmental problem. The removal of dyes from aqueous solution has a great potential in the field of environmental engineering. This paper reviews the classification, characteristics, and problems of dyes in detail. Advantages and disadvantages of different methods used for dye removal are also analyzed. Among these methods, Fenton process-based advanced oxidation processes are an emerging prospect in the field of dye removal. Fenton processes have been classified and represented as “Fenton circle”. This paper analyzes the recent studies on Fenton processes. The studies include analyzing different configurations of reactors used for dye removal, its efficiency, and the effects of various operating parameters such as pH, catalyst concentration, H₂O₂ concentration, initial dye concentration, and temperature of Fenton processes. From the present study, it can be concluded that Fenton processes are very effective and environmentally friendly methods for dye removal.

Yuan Zhang, Jian Xu, Zhenxing Zhong, Changsheng Guo, Lei Li, Yan He, Wenhong Fan, Yucheng Chen. (¹State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing, 100012, China, ²Laboratory of Riverine Ecological Conservation and Technology, Chinese Research Academy of Environmental Sciences, Beijing, 100012, China, ³College of Resources and Environment, Southwest University, Chongqing, 400716, China, ⁴Department of Environmental Science and Engineering, School of Chemistry and Environment, Beihang University, Beijing, 100191, China). Degradation of sulfonamides antibiotics in lake water and sediment. Environmental Science and Pollution Research, Volume 20(4) (2013): 2372-2380

Degradation of three sulfonamides (SAs), namely sulfamethoxazole (SMX), sulfamethazine (SMZ), and sulfadimethoxine (SDM) in surface water and sediments collected from Taihu Lake and Dianchi Lake, China was investigated in this study. The surface water (5–10 cm) was collected from the east region of Taihu Lake, China. Two sets of degradation experiments were conducted in 3-L glass bottles containing 2 L of fresh lake water and 100 µg/L of individual SAs

aerated by bubbling air at a rate of approximately 1.2 L/min, one of which was sterilized by the addition of NaN_3 (0.1 %). Sediment samples were taken from Taihu Lake and Dianchi Lake, China. For the sediment experiment, 5 g of sediment were weighed into a 50-mL glass tube, with 10 mg/kg of individual SAs. Different experimental conditions including the sediment types, sterilization, light exposure, and redox condition were also considered in the experiments. The three SAs degraded in lake water with half-lives ($t_{1/2}$) of 10.5–12.9 days, and the half-lives increased significantly to 31.9–49.8 days in the sterilized water. SMZ and SDM were degraded by abiotic processes in Taihu and Dianchi sediments, and the different experimental conditions and sediments characteristics had no significant effect on their declines. SMX, however, was mainly transformed by facultative anaerobes in Taihu and Dianchi sediments under anaerobic conditions, and the degradation rate of SMX in non-sterile sediment ($t_{1/2}$ of 9.6–16.7 days) were higher than in sterilized sediment ($t_{1/2}$ of 18.7–135.9 days). Under abiotic conditions, degradation of SMX in Dianchi sediment was faster than in Taihu sediment, probably due to the higher organic matter content and inorganic photosensitizers concentrations in Dianchi sediment. High initial SAs concentration inhibited the SAs degradation, which was likely related to the inhibition of microorganism activities by high SAs levels in sediments. Results from this study could provide information on the persistence of commonly used sulfanomides antibiotics in lake environment.

M. I. Vasquez, E. Hapeshi, D. Fatta-Kassinos, K. Kümmerer. (¹Department of Civil and Environmental Engineering, University of Cyprus, 75 Kallipoleos Street, 1678, Nicosia, Cyprus, ²Nireas International Water Research Center, University of Cyprus, Nicosia, Cyprus, ³Institute for Sustainable Chemistry and Resources, Leuphana University Lüneburg, Scharnhorststraße 1/C13, 21335, Lüneburg, Germany). **Biodegradation potential of ofloxacin and its resulting transformation products during photolytic and photocatalytic treatment. Environmental Science and Pollution Research, Volume 20(3) (2013):1302-1309**

The release of pharmaceuticals in the environment, as parent compounds, metabolites and transformation products, and the consequent risks posed to living organisms due to the unintended exposure of the latter to these chemicals are nowadays of increasing scientific concern. The development of advanced oxidation processes able to degrade these substances is in the core of the current research objectives, the main target being the removal of these compounds from wastewaters. Often the focus is on the removal of the parent compound only. However, these processes can form transformation products. Knowledge on the risk related to such transformation products is scarce. Among others, knowledge on their toxic effects and their biodegradability is of importance not only when they are present in the environment but also for the assessment of the advanced oxidation processes' efficiency applied for their degradation. Photolytic (UV irradiation) and photocatalytic treatment (UV irradiation in the presence of TiO_2) of the fluoroquinolone ofloxacin were applied, and the biodegradability of the formed products was investigated using the Closed Bottle test (OECD 301 D). Various transformation products, formed both during the photo(cata)lytic treatment and the Closed Bottle test, were identified using chromatographic analysis with an ultra high-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) system. The transformation products formed during the phototreatments were found to be non-readily biodegradable as the biodegradation percentages were close to zero. The persistence of the various photo(cata)lytic

transformation products during the Closed Bottle test may be attributed to the fluorine present in all the transformation products formed. The transformation products identified suggest that two transformation routes were present: decarboxylation and opening of the piperazine ring. Interestingly, it was observed that in the presence of a readily biodegradable carbon source (sodium acetate), the biodegradation percentage increased drastically for some of the photolytically treated samples. This was not the case for the photocatalytically treated samples, in which also mineralization of the parent compound was achieved faster. Further research is needed, however, in order to increase the understanding of the conditions that may lead to less potent and persistent substances during the application of such engineered or natural processes.

Prosun Tribedi, Alok K. Sil. (¹Department of Microbiology, University of Calcutta, 35 B. C. Road, Kolkata, 700019, India). **Bioaugmentation of polyethylenesuccinate-contaminated soil with *Pseudomonas* sp. AKS2 results in increased microbial activity and better polymer degradation.** *Environmental Science and Pollution Research*, Volume 20(3) (2013): 1318-1326

Pseudomonas sp. AKS2 isolated from soil degrades polyethylene succinate (PES) efficiently in the laboratory. However, this organism may not be able to degrade PES with similar efficiency in a natural habitat. Since in situ remediation is preferred for the effective removal of recalcitrant materials like plastic, in the current study, bioaugmentation potential of this organism was investigated. To investigate the potential of the AKS2 strain to bioaugment the PES-contaminated soil, a microcosm-based study was carried out wherein naturally attenuated, biostimulated, and AKS2-inoculated (bioaugmented) soil samples were examined for their ability to degrade PES. The results showed better degradation of PES by bioaugmented soil than other microcosms. Consistent with it, a higher number of PES-degrading organisms were found in the bioaugmented microcosm. The bioaugmented microcosm also exhibited a higher level of average well color development in BiOLOGECO plate assay than the other two. The corresponding Shannon–Weaver index and Gini coefficient revealed a higher soil microbial diversity of bioaugmented microcosm than the others. This was further supported by community-level physiological profile of three different microcosms wherein we have observed better utilization of different carbon sources by bioaugmented microcosms. Collectively, these results demonstrate that bioaugmentation of PES-contaminated soil with AKS2 not only enhances polymer degradation but also increases microbial diversity. Bioaugmentation of soil with AKS2 enhances PES degradation without causing damage to soil ecology. Thus, *Pseudomonas* sp. AKS2 has the potential to be implemented as a useful tool for in situ bioremediation of PES.

Xiao Xiao, Sheng-Peng Sun, Murray B. McBride, Ann T. Lemley. (¹Graduate Field of Environmental Toxicology, FSAD, HEB, Cornell University, Ithaca, NY, 14853-4401, USA, ²Department of Crop and Soil Sciences, Cornell University, Ithaca, NY, 14853, USA). **Degradation of ciprofloxacin by cryptomelane-type manganese(III/IV) oxides.** *Environmental Science and Pollution Research*, Volume 20(1) (2013): 10-21

The objective of this study is to investigate and understand the oxidizing properties of a manganese oxide, specifically synthetic cryptomelane ($\text{KMn}_8\text{O}_{16}$) and its derivatives, in aqueous solution. Ciprofloxacin (CIP), a commonly used fluoroquinolone antibiotic, was used as the probe. Synthetic cryptomelane, known as octahedral molecular sieves (OMS-2), was synthesized, and its derivatives were prepared by adding transition metal oxides, V_2O_5 or MoO_3 ,

as dopants during synthesis. The solids were characterized by x-ray powder diffraction (XRD), SEM–energy-dispersive spectrometry (SEM-EDX), x-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectra (FTIR), Raman spectra, and N₂-Brunauer-Emmett-Teller method. Degradation of CIP by different doped OMS-2 was carried out. Process conditions were optimized using response surface methodology (RSM). XRD patterns indicated the crystal phase of regular and doped OMS-2 as the cryptomelane type. Presence of the dopants in doped cryptomelane was confirmed by SEM-EDX and XPS. FTIR and Raman results suggested that the dopants were substituted into the framework in place of manganese. SEM images, XRD analysis, and surface area analysis of doped OMS-2 indicated decreased particle size, decreased crystallinity, and increased surface area compared to regular OMS-2. Higher oxidizing reactivity of doped OMS-2 was also observed with increased CIP removal rates from aqueous solution. The enhancement of reactivity may be due to the increase of surface areas. Nine percent Mo/OMS-2, the most effective oxidant of all synthesized derivatives, was selected for optimization study. Favorable treatment conditions were obtained using RSM at pH 3 with molar ratio [9 % Mo/OMS-2]/[CIP] \geq 50. Under such conditions, more than 90 % CIP can be removed in 30 min. The degradation kinetics was modeled by a modified first order rate with introduction of a retardation factor- α ($R^2 > 0.98$). Analysis of degradation products indicated that oxidation takes place mainly on the piperazine ring of CIP.

Shujuan Wang, Karen Poon, Zongwei Cai. (¹United International College, Beijing Normal University–Hong Kong Baptist University, Zhuhai, China, ²Department of Chemistry, Hong Kong Baptist University, 224 Waterloo Road, Kowloon Tong, Hong Kong SAR, China). **Biodegradation and removal of 3,4-dichloroaniline by *Chlorella pyrenoidosa* based on liquid chromatography-electrospray ionization-mass spectrometry. Environmental Science and Pollution Research, Volume 20(1) (2013): 552-557**

3,4-Dichloroaniline (3,4-DCA), widely used in the synthesis of dyes, textile and herbicides, is toxic to living organisms. The purpose of this study was to investigate the capability of green algae in degrading and removing 3,4-DCA in water. An environmentally ubiquitous green alga *Chlorella pyrenoidosa* was isolated from fresh aquatic environment. Then unicellular alga was incubated with 3,4-DCA at a concentration of 4.6 $\mu\text{g}/\text{mL}$ in water. The residual concentration of 3,4-DCA in the medium and the metabolites were analyzed. A removal percentage of 78.4 % was obtained over a 7-day period. Two major metabolites with less toxicity were identified as 3,4-dichloroformanilide and 3,4-dichloroacetanilide from the liquid chromatography-electrospray ionization-mass spectrometry analysis. The application of microalga *C. pyrenoidosa* may have potential for removing the environmental pollutant in aquatic environment.

Guang-can Zhou, Ying Wang, Shan Zhai, Feng Ge, Zhong-hua Liu, Yi-jun Dai, Sheng Yuan, Jun-yi Hou. (¹Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Research Center for Industrialization of Microbial Resources, College of Life Science, Nanjing Normal University, Nanjing, 210023, People's Republic of China, ²Nanjing Institute of Environmental Sciences, Ministry of Environmental Protection, Nanjing, 210042, People's Republic of China). **Biodegradation of the neonicotinoid insecticide thiamethoxam by the nitrogen-fixing and plant-growth-**

promoting rhizobacterium *Ensifer adhaerens* strain TMX-23. Applied Microbiology and Biotechnology, Volume 97(9) (2013): 4065-4074

Thiamethoxam (THIA), a second generation neonicotinoid insecticide in the thianicotinyl subclass, is used worldwide. Environmental studies revealed that microbial degradation is the major mode of removal of this pesticide from soil. However, microbial transformation of THIA is poorly understood. In the present study, we isolated a bacterium able to degrade THIA from rhizosphere soil. The bacterium was identified as *Ensifer adhaerens* by its morphology and 16S ribosomal DNA sequence analysis. High-performance liquid chromatography and mass spectrometry analysis suggested that the major metabolic pathway of THIA in *E. adhaerens* TMX-23 involves the transformation of its N-nitroimino group (=N-NO₂) to N-nitrosoimino (=N-NO) and urea (=O) metabolites. *E. adhaerens* TMX-23 is a nitrogen-fixing bacterium harboring two types of *nifH* genes in its genome, one of which is 98 % identical to the *nifH* gene in the cyanobacterium *Calothrix* sp. MCC-3A. *E. adhaerens* TMX-23 released various plant-growth-promoting substances including indole-3-acetic acid, exopolysaccharides, ammonia, HCN, and siderophores. Inoculation of *E. adhaerens* TMX-23 onto soybean seeds (*Glycine max* L.) with NaCl at 50, 100, or 154 mmol/L increased the seed germination rate by 14, 21, and 30 %, respectively. THIA at 10 mg/L had beneficial effects on *E. adhaerens* TMX-23, enhancing growth of the bacterium and its production of salicylic acid, an important plant phytohormone associated with plant defense responses against abiotic stress. The nitrogen-fixing and plant-growth-promoting rhizobacterium *E. adhaerens* TMX-23, which is able to degrade THIA, has the potential for bioaugmentation as well as to promote growth of field crops in THIA-contaminated soil.

Weiwei Zhang, Zongliang Niu, Chunyang Liao, Lingxin Chen. (¹Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS), Yantai, Shandong, 264003, People's Republic of China, ²Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, 17 Chunhui Road, Yantai, Shandong, 264003, China). **Isolation and characterization of *Pseudomonas* sp. strain capable of degrading diethylstilbestrol. Applied Microbiology and Biotechnology, Volume 97(9) (2013): 4095-4104**

Since diethylstilbestrol (DES) interrupts endocrine systems and generates reproductive abnormalities in both wildlife and human beings, methods to remove DES from the environments are urgently recommended. In this study, bacterial strain J51 was isolated and tested to effectively degrade DES. J51 was identified as *Pseudomonas* sp. based on its nucleotide sequence of 16S rRNA. The quinoprotein alcohol dehydrogenase and isocitrate lyase were identified to be involved in DES degradation by MALDI-TOF-TOF MS/MS analysis. In the presence of 40 mg/l DES, increase of the genes encoding quinoprotein alcohol dehydrogenase and isocitrate lyase in both RNA and protein levels was determined. The HPLC/MS analysis showed that DES was hydrolyzed to a major degrading metabolite DES-4-semiquinone. It was the first time to demonstrate the characteristics of DES degradation by specific bacterial strain and the higher degradation efficiency indicated the potential application of *Pseudomonas* sp. strain J51 in the treatment of DES-contaminated freshwater and seawater environments.

Ryota Kataoka, Kazuhiro Takagi. (¹Department of Environmental Sciences, Faculty of Life and Environmental Sciences, University of Yamanashi, 4-4-37 Takeda, Kofu, Yamanashi, 400-8510, Japan, ²Organochemicals Division, National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba, Ibaraki, 305-8604, Japan).

Biodegradability and biodegradation pathways of endosulfan and endosulfan sulfate. Applied Microbiology and Biotechnology, Volume 97(8) (2013): 3285-3292

Endosulfan and endosulfan sulfate are persistent organic pollutants that cause serious environmental problems. Although these compounds are already prohibited in many countries, residues can be detected in soils with a history of endosulfan application. Endosulfan is transformed in the environment into endosulfan sulfate, which is a toxic and persistent metabolite. However, some microorganisms can degrade endosulfan without producing endosulfan sulfate, and some can degrade endosulfan sulfate. Therefore, biodegradation has the potential to clean up soil contaminated with endosulfan. In this review, we provide an overview of aerobic endosulfan degradation by bacteria and fungi, and a summary of recent advances and prospects in this research field.

Lili Zhang, Jun Hu, Runye Zhu, Qingwei Zhou, Jianmeng Chen. (¹School of Biological and Environmental Engineering, Zhejiang University of Technology, No.6 District, Zhaohui, Hangzhou, 310032, China, ²State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China). **Degradation of paracetamol by pure bacterial cultures and their microbial consortium. Applied Microbiology and Biotechnology, Volume 97(8) (2013): 3687-3698**

Three bacterial strains utilizing paracetamol as the sole carbon, nitrogen, and energy source were isolated from a paracetamol-degrading aerobic aggregate, and assigned to species of the genera *Stenotrophomonas* and *Pseudomonas*. The *Stenotrophomonas* species have not included any known paracetamol degraders until now. In batch cultures, the organisms f1, f2, and fg-2 could perform complete degradation of paracetamol at concentrations of 400, 2,500, and 2,000 mg/L or below, respectively. A combination of three microbial strains resulted in significantly improved degradation and mineralization of paracetamol. The co-culture was able to use paracetamol up to concentrations of 4,000 mg/L, and mineralized 87.1 % of the added paracetamol at the initial of 2,000 mg/L. Two key metabolites of the biodegradation pathway of paracetamol, 4-aminophenol, and hydroquinone were detected. Paracetamol was degraded predominantly via 4-aminophenol to hydroquinone with subsequent ring fission, suggesting new pathways for paracetamol-degrading bacteria. The degradation of paracetamol could thus be performed by the single isolates, but is stimulated by a synergistic interaction of the three-member consortium, suggesting a possible complementary interaction among the various isolates. The exact roles of each of the strains in the consortium need to be further elucidated.

Jian Sun, Youming Li, Yongyou Hu, Bin Hou, Yaping Zhang, Sizhe Li. (¹Ministry of Education Key Laboratory of Pollution Control and Ecological Remediation for Industrial Agglomeration area, College of Environmental Science and Engineering, South China University of Technology, Guangzhou, 510006, China, ²State Key Lab of Pulp and Paper Engineering, College of Light Industry and Food Science, South China University of Technology, Guangzhou, 510640, China). **Understanding the degradation of Congo red and bacterial diversity in an air-cathode microbial fuel cell being evaluated for simultaneous azo dye removal from wastewater and bioelectricity generation. Applied Microbiology and Biotechnology, Volume 97(8) (2013): 3711-3719**

We investigated the mechanism of Congo red degradation and bacterial diversity in a single-chambered microbial fuel cell (MFC) incorporating a microfiltration membrane and air-cathode. The MFC was operated continuously for more than 4 months using a mixture of Congo red and glucose as fuel. We demonstrated that the Congo red azo bonds were reduced at the anode to form aromatic amines. This is consistent with the known mechanism of anaerobic biodegradation of azo dyes. The MFC developed a less dense biofilm at the anode in the presence of Congo red compared to its absence indicating that Congo red degradation negatively affected biofilm formation. Denaturing gradient gel electrophoresis and direct 16S ribosomal DNA gene nucleotide sequencing revealed that the microbial communities differed depending on whether Congo red was present in the MFC. *Geobacter*-like species known to generate electricity were detected in the presence or absence of Congo red. In contrast, *Azospirillum*, *Methylobacterium*, *Rhodobacter*, *Desulfovibrio*, *Trichococcus*, and *Bacteroides* species were only detected in its presence. These species were most likely responsible for degrading Congo red.

Yukiko Shinozaki, Tomotake Morita, Xiao-hong Cao, Shigenobu Yoshida, Motoo Koitabashi, Takashi Watanabe, Ken Suzuki, Yuka Sameshima-Yamashita, Toshiaki Nakajima-Kambe, Takeshi Fujii. (¹National Institute for Agro-Environmental Sciences (NIAES), 3-1-3 Kannondai, Tsukuba, Ibaraki, 305-8604, Japan, ²Research Institute for Innovation in Sustainable Chemistry, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 5-2, 1-1-1 Higashi, Tsukuba, Ibaraki, 305-8565, Japan, ³Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8572, Japan). **Biodegradable plastic-degrading enzyme from *Pseudozyma antarctica*: cloning, sequencing, and characterization.** *Applied Microbiology and Biotechnology*, Volume 97(7) (2013): 2951-2959

Pseudozyma antarctica JCM 10317 exhibits a strong degradation activity for biodegradable plastics (BPs) such as agricultural mulch films composed of poly(butylene succinate) (PBS) and poly(butylene succinate-co-adipate) (PBSA). An enzyme named PaE was isolated and the gene encoding PaE was cloned from the strain by functional complementation in *Saccharomyces cerevisiae*. The deduced amino acid sequence of PaE contains 198 amino acids with a predicted molecular weight of 20,362.41. High identity was observed between this sequence and that of cutinase-like enzymes (CLEs) (61–68 %); therefore, the gene encoding PaE was named *PaCLE1*. The specific activity of PaE against emulsified PBSA was 54.8 ± 6.3 U/mg. In addition to emulsified BPs, PaE degraded solid films of PBS, PBSA, poly(ϵ -caprolactone), and poly(lactic acid).

Guiqiu Chen, Song Guan, Guangming Zeng, Xiaodong Li, Anwei Chen, Cui Shang, Ying Zhou, Huanke Li, Jianmin He. (¹College of Environmental Science and Engineering, Hunan University, Changsha, 410082, People's Republic of China, ²Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha, 410082, People's Republic of China). **Cadmium removal and 2,4-dichlorophenol degradation by immobilized *Phanerochaete chrysosporium* loaded with nitrogen-doped TiO₂ nanoparticles.** *Applied Microbiology and Biotechnology*, Volume 97(7) (2013): 3149-3157

Phanerochaete chrysosporium has been identified as an effective bioremediation agent for its biosorption and degradation ability. However, the applications of *P. chrysosporium* are limited owing to its long degradation time and low resistance to pollutants. In

this research, nitrogen-doped TiO₂ nanoparticles were loaded on *P. chrysosporium* to improve the remediation capacity for pollutants. The removal efficiencies were maintained at a high level: 84.2 % for Cd(II) and 78.9 % for 2,4-dichlorophenol (2,4-DCP) in the wide pH range of 4.0 to 7.0 in 60 h. The removal capacity of immobilized *P. chrysosporium* loaded with nitrogen-doped TiO₂ nanoparticles (PTNs) was strongly affected by the initial Cd(II) and 2,4-DCP concentrations. The hyphae of PTNs became tight, and a large amount of crystals adhered to them after the reaction. Fourier transform infrared spectroscopy showed that carboxyl, amino, and hydroxyl groups on the surface of PTNs were responsible for the biosorption. In the degradation process, 2,4-DCP was broken down into *o*-chlorotoluene and 4-hexene-1-ol. These results showed that PTNs is promising for simultaneous removal of Cd(II) and 2,4-DCP from wastewater.

Ana R. Lopes, Anthony S. Danko, Célia M. Manaia, Olga C. Nunes. (¹LEPAE, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, 4200-465, Porto, Portugal, ²Centro de Investigação em Geo-Ambiente e Recursos, Dpto Engenharia de Minas, Faculdade de Engenharia, Universidade do Porto, 4200-465, Porto, Portugal, ³CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, 4200-072, Porto, Portugal). **Molinate biodegradation in soils: natural attenuation versus bioaugmentation.** *Applied Microbiology and Biotechnology*, Volume 97(6) (2013): 2691-2700

The aims of the present study were to assess the potential of natural attenuation or bioaugmentation to reduce soil molinate contamination in paddy field soils and the impact of these bioremediation strategies on the composition of soil indigenous microbiota. A molinate mineralizing culture (mixed culture DC) was used as inoculum in the bioaugmentation assays. Significantly higher removal of molinate was observed in bioaugmentation than in natural attenuation microcosms (63 and 39 %, respectively) after 42 days of incubation at 22 °C. In the bioaugmentation assays, the impact of *Gulosibacter molinativorax* ON4^T on molinate depletion was observed since the gene encoding the enzyme responsible for the initial molinate breakdown (harboured by that actinobacterium) was only detected in inoculated microcosms. Nevertheless, the exogenous mixed culture DC did not overgrow as the heterotrophic counts of the bioaugmentation microcosms were not significantly different from those of natural attenuation and controls. Moreover, the actinobacterial clone libraries generated from the bioaugmentation microcosms did not include any 16S rRNA gene sequences with significant similarity to that of *G. molinativorax* ON4^T. The multivariate analysis of the 16S rRNA DGGE patterns of the soil microcosm suggested that the activity of mixed culture DC did not affect the soil bacterial community structure since the DGGE patterns of the bioaugmentation microcosms clustered with those of natural attenuation and controls. Although both bioremediation approaches removed molinate without indigenous microbiota perturbation, the results suggested that bioaugmentation with mixed culture DC was more effective to treat soils contaminated with molinate.

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Albareda 1, 18008 Granada, Spain. E-mail: tino.krell@eez.csic.es). Tactic responses to pollutants and their potential to increase biodegradation efficiency. Journal of Applied Microbiology, Volume 114(4) (2013): 923–933

A significant number of bacterial strains are able to use toxic aromatic hydrocarbons as carbon and energy sources. In a number of cases, the evolution of the corresponding degradation pathway was accompanied by the evolution of tactic behaviours either towards or away from these toxic carbon sources. Reports are reviewed which show that a chemoattraction to heterogeneously distributed aromatic pollutants increases the bioavailability of these compounds and their biodegradation efficiency. An extreme form of chemoattraction towards aromatic pollutants, termed 'hyperchemotaxis', was described for *Pseudomonas putida* DOT-T1E, which is based on the action of the plasmid-encoded McpT chemoreceptor. Cells with this phenotype were found of being able to approach and of establishing contact with undiluted crude oil samples. Although close McpT homologues are found on other degradation plasmids, the sequence of their ligand-binding domains does not share significant similarity with that of NahY, the other characterized chemoreceptor for aromatic hydrocarbons. This may suggest the existence of at least two families of chemoreceptors for aromatic pollutants. The use of receptor chimeras comprising the ligand-binding region of McpT for biosensing purposes is discussed.

Keywords: biosensor; chemoreceptor; chemotaxis; pollutant; *Pseudomonas putida*

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To explore rhizospheric microbial communities from Arctic native plant species evaluating their bacterial hydrocarbon-degrading capacities.

Eriophorum scheuchzeri, *Potentilla* cf. *rubricaulis*, *Oxyria digyna*, *Salix arctica* and *Puccinellia angustata* plant species were collected at Eureka (Canadian high Arctic) along with their rhizospheric soil samples. Their bacterial community fingerprints (16S rRNA gene, DGGE) were distinctive encompassing members from the phyla: *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Proteobacteria*. Isolated diesel-degrading bacteria belonged to the phyla *Actinobacteria* and *Proteobacteria*. Strains of *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Intrasporangiaceae*, *Leifsoni* and *Arthrobacter* possessed *alkB* and *Pseudomonas* possessed both *ndoB* and *xylE* gene sequences. Two *Rhodococcus* strains mineralized [1-¹⁴C] hexadecane at 5 and -5°C. From the rhizosphere of *P. angustata*, larger numbers of hydrocarbon-degrading

bacteria were isolated than from other plant rhizosphere samples and all three genes *alkB* (from *Actinobacteria*), *ndoB* and *xylE* (from *Pseudomonas*) were detected by PCR.

(i) Arctic plants have unique rhizospheric bacterial communities. (ii) *P. angustata* has potential for phytoremediation research at high Arctic soils. (iii) Isolated bacteria mineralized hydrocarbons at ambient low temperatures.

To the best of our knowledge, this is the first rhizospheric exploration examining the phytoremediation potential of five Arctic plants and evaluating their microbial hydrocarbon-degrading capacities.

Keywords: *alkB* ; Arctic; bacteria; hydrocarbons; *ndoB* ; plants; rhizosphere; *xylE*

Biosensor

H. Harmens^a, L. Foan^{b, c}, V. Simon^{b, c}, G. Mills^a. (^aCentre for Ecology and Hydrology, Environment Centre Wales, Deiniol Road, Bangor LL57 2UW, UK, ^bUniversité de Toulouse, INPT, LCA (Laboratoire de Chimie Agro-Industrielle), ENSIACET, 4 Allée, Emile Monso, BP 44362, F-31030 Toulouse Cedex 4, France, ^cINRA, LCA (Laboratoire de Chimie Agro-Industrielle), F-31030 Toulouse, France). **Terrestrial mosses as biomonitors of atmospheric POPs pollution: A review. Environmental Pollution, Volume 173(2013) : 245–254**

Worldwide there is concern about the continuing release of persistent organic pollutants (POPs) into the environment. In this study we review the application of mosses as biomonitors of atmospheric deposition of POPs. Examples in the literature show that mosses are suitable organisms to monitor spatial patterns and temporal trends of atmospheric concentrations or deposition of POPs. These examples include polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), dioxins and furans (PCDD/Fs), and polybrominated diphenyl ethers (PBDEs). The majority of studies report on PAHs concentrations in mosses and relative few studies have been conducted on other POPs. So far, many studies have focused on spatial patterns around pollution sources or the concentration in mosses in remote areas such as the polar regions, as an indication of long-range transport of POPs. Very few studies have determined temporal trends or have directly related the concentrations in mosses with measured atmospheric concentrations and/or deposition fluxes.

Keywords: Biomonitoring; Bryophytes; Persistent organic pollutants; Polycyclic aromatic hydrocarbons; Atmospheric deposition

Marcos A.E. Chaparro^{a, b}, Juan M. Lavornia^c, Mauro A.E. Chaparro^d, Ana M. Sinito^a. (^aInstituto de Física Arroyo Seco (IFAS, UNCPBA)-CONICET, Pinto 399, 7000 Tandil, Argentina, ^bCentro de Geociencias, UNAM, Blvd. Juriquilla 3001, 76230 Juriquilla, Querétaro, Mexico, ^cCentro de Investigaciones y Estudios Ambientales (CINEA, UNCPBA)-CONICET, Pinto 399, 7000 Tandil, Argentina, ^dInstituto Multidisciplinario de Ecosistemas y Desarrollo Sustentable (UNCPBA)-CONICET, Pinto 399, B7000GHG Tandil, Argentina). **Biomonitoring of urban air pollution: Magnetic studies and SEM**

observations of corticolous foliose and microfoliose lichens and their suitability for magnetic monitoring. Environmental Pollution, Volume 172(2013): 61–69

This study explored the suitability of available lichen species as air pollution biomonitors and assessed their potential for magnetic monitoring in cities. Several lichens on tree bark were collected in urban and industrial sites from Tandil city, as well as control sites. The results showed that magnetite-like minerals were the main magnetic carriers in all sites and samples. However, the concentration varied between clean and polluted sites. In addition, magnetic-grain size-distribution showed clear differences between sites. Observations by scanning electron microscopy showed different particles in a variety of shapes and grain sizes; moreover, the presence of iron oxides and several toxic elements was detected by energy dispersive spectroscopy analysis. Although eleven lichen species were identified that appeared suitable for use as air-pollution monitors, three of them, *Parmotrema pilosum*, *Punctelia hipoleucites* and *Dirinaria picta*, occurred more frequently in the area, thus constituting appropriate species for future monitoring in the study area.

Keywords: Biomonitoring; Magnetic susceptibility; Lichen; Atmospheric pollution; Multivariate analysis

Snježana Zrnčić, Dražen Oraić, Marko Čaleta, Željko Mihaljević, Davor Zanella, Nina Bilandžić. (¹Croatian Veterinary Institute, Savska 143, Zagreb, Croatia, ²Faculty of Science, Department of Zoology, Rooseveltov trg 6, Zagreb, Croatia). **Biomonitoring of heavy metals in fish from the Danube River. Environmental Monitoring and Assessment, Volume 185(2) (2013): 1189-1198**

The Croatian part of the Danube River extends over 188 km and comprises 58 % of the country's overall area used for commercial freshwater fishing. To date, the heavy metal contamination of fish in the Croatian part of the Danube has not been studied. The main purpose of this study was to determine heavy metal levels in muscle tissue of sampled fish species and to analyze the measured values according to feeding habits of particular groups. Lead ranged from 0.015 μg^{-1} dry weight in planktivorous to 0.039 μg^{-1} dry weight in herbivorous fish, cadmium from 0.013 μg^{-1} dry weight in herbivorous to 0.018 μg^{-1} dry weight in piscivorous fish, mercury from 0.191 μg^{-1} dry weight in omnivorous to 0.441 μg^{-1} dry weight in planktivorous fish and arsenic from 0.018 μg^{-1} dry weight in planktivorous to 0.039 μg^{-1} dry weight in omnivorous fish. Among the analyzed metals in muscle tissue of sampled fish, only mercury exceeded the maximal level (0.5 mg kg^{-1}) permitted according to the national and EU regulations determining maximum levels for certain contaminants in foodstuffs, indicating a hazard for consumers of fish from the Danube River.

Samar Al Sayegh Petkovšek. (ERICo Velenje, Environmental Research & Industrial Cooperation, Koroška 58, 3320 Velenje, Slovenia). **Forest biomonitoring of the largest Slovene thermal power plant with respect to reduction of air pollution. Environmental Monitoring and Assessment, Volume 185(2)(2013): 1809-1823**

The condition of the forest ecosystem in the vicinity of the largest Slovene power plant [the Šoštanj Thermal Power Plant (ŠTPP)] was monitored during the period 1991–2008 by determining the total concentration of sulphur, ascorbic acid and chlorophyll in Norway spruce needles. After 1995, the introduction of cleaning devices at the ŠTPP dramatically reduced the former extremely high SO_2 and dust emissions. The most significant findings of this

comprehensive, long-duration survey are as follows: (1) the chosen parameters are suitable bioindicators of stress caused by air pollution in Norway spruce needles; they reflect both spatial and temporal variations in air pollution as well as the degree of efficiency of the cleaning devices; (2) observations show that the physiological condition of Norway spruce in northern Slovenia has significantly improved since 1995, when the first desulphurization device at ŠTPP was built, together with a reduction in the area influenced by pollution from ŠTPP; (3) metabolic processes in spruce needles react to air pollution according to the severity of the pollution and the length of exposure; exposure to high SO₂ ambient levels and/or spread over a long duration can damage the antioxidant defence mechanisms of spruce trees as well as diminishing the concentration of ascorbic acid; (4) a reduction in the exposure to air pollution improves the vitality of the trees (e.g. higher concentrations of total (a + b) chlorophyll), as well as restoring their defence capabilities as shown by higher concentrations of ascorbic acid; and (5) forest monitoring should be continued and focused on integrating the effects of multiple stressors, which can additionally affect a forest ecosystem.

Izabela Gierach^{1,2,†}, Kayle Shapero^{1,†}, Thomas W. Eyster¹, David W. Wood^{1,2,*}
(¹Department of Chemical Engineering, Princeton University, Princeton, New Jersey 08544, USA, ²Department of Chemical and Biomolecular Engineering, Ohio State University, Columbus, Ohio 43210, USA. [†]Email: David W. Wood, wood@chbmeng.ohio-state.edu. *Department of Chemical Engineering, Princeton University, Princeton, New Jersey 08544, USA [†]). **Bacterial biosensors for evaluating potential impacts of estrogenic endocrine disrupting compounds in multiple species. *Environmental Toxicology*, Volume 28(4) (2013): 179–189**

To study the effects and possible mechanisms of suspected endocrine disrupting compounds (EDCs), a wide variety of assays have been developed. In this work, we generated engineered *Escherichia coli* biosensor strains that incorporate the ligand-binding domains (LBDs) of the β -subtype estrogen receptors (ER β) from *Solea solea* (sole), and *Sus scrofa* (pig). These strains indicate the presence of ligands for these receptors by changes in growth phenotype, and can differentiate agonist from antagonist and give a rough indication of binding affinity via dose-response curves. The resulting strains were compared with our previously reported *Homo sapiens* ER β biosensor strain. In initial tests, all three of the strains correctly identified estrogenic test compounds with a high degree of certainty (Z' typically greater than 0.5), including the weakly binding test compound bisphenol A (BPA) ($Z' \approx 0.1$ – 0.3). The modular design of the sensing element in this strain allows quick development of new species-based biosensors by simple LBD swapping, suggesting its use in initial comparative analysis of EDC impacts across multiple species. Interestingly, the growth phenotypes of the biosensor strains indicate similar binding for highly estrogenic control compounds, but suggest differences in ligand binding for more weakly binding EDCs. © 2011 Wiley Periodicals, Inc. *Environ Toxicol*, 2013.

Keywords: endocrine disruptors; estrogen receptor; bacterial biosensor; *Solea solea*; *Sus scrofa*; *Homo sapiens*

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Drive, Aberdeen, AB24 3UU, UK, ²Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China, ³Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, 361021, China). **Application of Microbial Biosensors to Complement Geochemical Characterisation: a Case Study in Northern China. *Water, Air, & Soil Pollution*, 224(2013): 1409**

There are significant concerns about the impact of heavy metal contamination in soils as a consequence of urbanisation and industrialisation in developing countries. Routine chemical analysis of soils is used to measure the total concentration of metals from point source or diffuse activities, but this fails to put in context the bioavailability of the analyte or the potential toxicity of multiple contaminants. Bacterial biosensors provide a useful tool for assessing the toxicity of the bioavailable fraction of heavy metals in soils and for complementing chemical analysis. There are few examples of genuine environmental applications of biosensors for pollutant diagnosis. This study applied constitutively marked biosensors (which were comprehensively characterised) to soils collected from across Northern China (60,000 km²). The biosensors were responsive to soils impacted by As, Cd, Cr, Cu, Hg, Pb, and Zn when compared to 'uncontaminated controls'. The response of the biosensor correlated with individual (or groups of) metals related to their concentration and source. The geo-accumulation index (I_{geo}) assisted in explaining the biosensor response. The constitutively marked biosensors offered a focussed understanding of analyte bioavailability and placed in a relevant context the elemental analysis. When matrix-matched control samples can be collected, then such a biosensor procedure (as adopted here) is applicable to contrasting soils exposed to a wide range of contaminants. Biosensor applications complemented routine soil chemical analysis for this regional-scale study.

Anish Khan, Aftab Aslam Parwaz Khan, Abdullah M. Asiri, Malik Abdul Rub, Naved Azum, Mohammed M. Rahman, Sher Bahadar Khan, Sulaiman Ab Ghani. (¹Chemistry Department, King Abdulaziz University, Jeddah, 21589, Saudi Arabia. ²Center of Excellence for Advanced Materials Research (CEAMR), King Abdulaziz University, Jeddah, 21589, Saudi Arabia, ³Pusat Pengajian Sains Kimia, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia). A New Trend on Biosensor for Neurotransmitter Choline/Acetylcholine—an Overview. *Applied Biochemistry and Biotechnology*, Volume 169(6) (2013): 1927-1939

Technology always has been an indispensable part in the development of biosensors. The performance of biosensors is being tremendously improved using new materials as transducer as well as binding material in their construction. The use of new materials allowed innovation on transduction technology in biosensor preparations. Because of the submicron dimensions of these sensors, simple and rapid analyses in vitro as well as in vivo are now possible. Portable instruments capable of analysing multiple components are becoming available, too. Sensors that provide excellent temporal and spatial resolution for in vivo monitoring such as for measurement of neurotransmitters have become prominent. The interest to improve the stability, sensitivity and selectivity of the sensors is paramount. This study tries to give an overview of the present status of the material-based biosensor design and new generation of choline/acetylcholine neurotransmitter biosensors.

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Surface and Interface Chemistry and Engineering Technology, Nanjing University, Nanjing 210093, PR China, ^cNanjing Institute of Supervision and Testing on Product Quality, Nanjing 210028, PR China). Innovative biocompatible nanospheres as biomimetic platform for electrochemical glucose biosensor. *Biosensors and Bioelectronics*, Volume 44(2013): 1–5

In this work, the silica– phytic acid (SiO₂–PA) nanocomposites were synthesized by the method of reverse microemulsion and electrostatic binding. The newly designed materials were used to develop a novel glucose biosensor by immobilizing glucose oxidase (GOx) onto the SiO₂–PA nanocomposites film on the surface of glassy carbon electrode (GCE). The characteristics of SiO₂–PA nanocomposites and GOx were obtained by using transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy and circular dichroism (CD) technique. All the results indicated that silica nanoparticles were modified with phosphate radicals successfully and the biomimetic surface was built. The entrapped GOx could preserve its bioactivity and exhibited an excellent electrochemical behavior with a formal potential of –0.548 V in phosphate buffer solution (PBS) (pH=7). Response studies to glucose were carried out using differential pulse voltammetry (DPV). The results indicated that the modified electrode can be used to determine glucose without interference from l-ascorbic acid (AA) and uric acid (UA) with the low detection limit of 0.012 mM. The comparison tests of DPVs of different electrodes in the absence and presence of glucose were also studied. The biosensor can also be used for quantification of the concentration of glucose in real samples.

Keywords: Silica; Phytic acid; Biomimetic; Glucose oxidase; Biosensor

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The mechanically induced release of adenosine-5'-triphosphate (ATP) from osteoblastic cells (MC3T3-E1) was measured in real time. A stretching device integrated into scanning electrochemical microscopy was developed to apply controlled mechanical strain to MC3T3-E1 cells. For ATP secretion, a stepwise yet uniform mechanical stress was imposed onto MC3T3-E1 cells. The ATP biosensors were positioned at a distance of approximately 30–40 μm above the cell surface. Calibration functions were recorded prior to the cell measurements and revealed a linear response up to 40 μM with a sensitivity of 1–5 pA/μM ATP. Stretching MC3T3-E1 cells up to 21% resulted in a concentration of 30.57±4.82 μM of extracellular ATP (N=12) detected above the cell surface. As a control experiment, nifedipine, a L-type voltage sensitive calcium channel (L-VSCC) inhibitor was applied, which blocks Ca²⁺ entry from the outer medium into the cell. Inhibition resulted in a significantly smaller amount of released ATP, i.e., 7.08±1.93 μM ATP (N=10). Further control experiments with glucose microbiosensors did not yield significant changes of the baseline current (N=8).

Keywords: ATP microbiosensor; Cell stretching device; MC3T3-E1 osteoblastic cells; Bone cells; Localized ATP measurements; Scanning electrochemical microscopy

Wenjing Lian^a, Su Liu^b, Jinghua Yu^a, Jie Li^b, Min Cui^a, Wei Xu^a, Jiadong Huang^{a, c}. (^aKey Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, PR China, ^bCollege of Resources and Environment, University of Jinan, Jinan 250022, PR China, ^cCollege of Medicine and Life Sciences, University of Jinan, Jinan 250022, PR China). **Electrochemical sensor using neomycin-imprinted film as recognition element based on chitosan-silver nanoparticles/graphene-multiwalled carbon nanotubes composites modified electrode. Biosensors and Bioelectronics, Volume 44(2013): 70–76**

A novel imprinted electrochemical sensor for neomycin recognition was developed based on chitosan-silver nanoparticles (CS-SNP)/graphene-multiwalled carbon nanotubes (GR-MWCNTs) composites decorated gold electrode. Molecularly imprinted polymers (MIPs) were synthesized by electropolymerization using neomycin as the template, and pyrrole as the monomer. The mechanism of the fabrication process and a number of factors affecting the activity of the imprinted sensor have been discussed and optimized. The characterization of imprinted sensor has been carried out by scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR). The performance of the proposed imprinted sensor has been investigated using cyclic voltammetry (CV) and amperometry. Under the optimized conditions, the linear range of the sensor was from 9×10^{-9} mol/L to 7×10^{-6} mol/L, with the limit of detection (LOD) of 7.63×10^{-9} mol/L ($S/N=3$). The film exhibited high binding affinity and selectivity towards the template neomycin, as well as good reproducibility and stability. Furthermore, the proposed sensor was applied to determine the neomycin in milk and honey samples based on its good reproducibility and stability, and the acceptable recovery implied its feasibility for practical application.

Keywords: Electrochemical sensor; Molecularly imprinted polymers; Chitosan-silver nanoparticles composites; Graphene-multiwalled carbon nanotubes composites; Neomycin detection

Hamid SadAbadi^{a, b}, Simona Badilescu^a, Muthukumaran Packirisamy^a, Rolf Wüthrich^b. (^aOptical-Bio Microsystems Laboratory, Department of Mechanical Engineering, Concordia University, 1515 Saint Catherine Street West, EV13.235, Montreal, Quebec, Canada H3G 1M8, ^bElectrocatalytic Green Engineering Group, Department of Mechanical Engineering, Concordia University 1515 Saint Catherine Street West, EV14.205, Montreal, Quebec, Canada H3G 1M8). **Integration of gold nanoparticles in PDMS microfluidics for lab-on-a-chip plasmonic biosensing of growth hormones. Biosensors and Bioelectronics, Volume 44(2013): 77–84**

Gold nanoparticles were synthesized in a poly(dimethylsiloxane) (PDMS) microfluidic chip by using an *in-situ* method, on the basis of reductive properties of the cross-linking agent of PDMS. The proposed integrated device was further used as a sensitive and low-cost LSPR-based biosensor for the detection of polypeptides.

Synthesis of nanoparticles in the microfluidic environment resulted in improvement of size distribution with only 8% variation, compared with the macro-environment that yields about 67% variation in size. The chemical kinetics of the *in-situ* reaction in the microfluidic environment was studied in detail and compared with the reaction carried out at the macro-scale. The effect of temperature and gold precursor concentration on the kinetics of the reaction was investigated and the apparent activation energy was estimated to be $E_{a}^{\text{app}} = 30 \text{ kJ/mol}$. $E_{a} = 30 \text{ kJ/mol}$.

The sensitivity test revealed that the proposed sensor has a high sensitivity of 74 nm/RIU to the surrounding medium. The sensing of bovine growth hormone also known as bovine somatotropin (bST) shows that the proposed biosensor can reach a detection limit of as low as 3.7 ng/ml (185 pM). The results demonstrate the successful integration of microfluidics and nanoparticles which provides a potential alternative for protein detection in clinical diagnostics.

Keywords: Nano-microfluidic integrated biosensor; Gold-PDMS nanocomposite; *In-situ* reduction; Bovine somatotropin (bST); Localized surface plasmon resonance (LSPR); Chemical kinetics

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Silicon-on-insulator (SOI) wafer is one of the most appealing platforms for optical integrated circuit with the potential to realize high performance Ultra Large Scale Integration (ULSI) and device miniaturization. In this work, based on simulations to obtain appropriate optical properties of a porous silicon microcavity (PSM), we successfully fabricated a highly efficient PSM on SOI wafer by electrochemical etching for DNA detection at optical wavelength 1555.0 nm. The narrow resonance peak with a full width at half maximum about 26.0 nm in the reflectance spectrum gives a high Q factor which causes high sensitivity for sensing performance. The sensitivity of this sensor is investigated through 19-base pair DNA hybridization in the PSM by surface modification using a standard cross link chemistry method. The red shift of the reflectance spectra shows a good linear relationship with complementary DNA concentration, ranging from 0.625 to 12.500 μ M, and the detection limit is 43.9 nM. This optical PSM on SOI is highly sensitive, fast responsive, easy to fabricate and low-costly, that will broadly benefit to develop a new optical label-free biosensor on SOI wafer and has a great potential for biochips based on integrated optical devices.

Keywords: Silicon-on-insulator wafer; Porous silicon microcavity; DNA biosensor; High sensitivity

Ting Wei^{a, b}, Chao Zhang^{a, b}, Xin Xu^c, Michelle Hanna^d, Xiaohua Zhang^a, Yan Wang^a, Heping Dai^a, Wei Xiao^{c, d}. (^aInstitute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, PR China, ^bUniversity of Chinese Academy of Sciences, Beijing 100049, PR China, ^cCollege of Life Sciences, Capital Normal University, Beijing 100048, PR China, ^dDepartment of Microbiology and Immunology, University of Saskatchewan, Saskatoon SK S7N 5E5, Canada). **Construction and evaluation of two biosensors based on yeast transcriptional response to genotoxic chemicals. Biosensors and Bioelectronics, Volume 44(2013): 138–145**

It has been well established that essentially all microbial mutagens are rodent carcinogens, yet current mutagen detection systems are limited by their detection sensitivity. Here we report the construction of a pair of hypersensitive biosensors by optimizing both reporters and the host strain. The resulting *RNR3-yEGFP* and *HUG1-yEGFP* reporters and the septuple yeast mutant in combination with the automated protocol not only remarkably enhance the detection sensitivity, but also allow a high throughput screen of environmental genotoxins. This system is deemed much more sensitive than similar yeast and bacterium-based tests for all selected chemicals examined in this study.

Keywords: Genotoxin; Reporter; Biosensor; Yeast; Sensitivity

Prasana Sahoo^a, Sumathi Suresh^b, Sandip Dhara^a, Garima Saini^c, S. Rangarajan^b, A.K. Tyagi^a. (^aSurface and Nanoscience Division, Indira Gandhi Center for Atomic Research, Kalpakkam, India, ^bWSCD, BARC Facilities, Indira Gandhi Center for Atomic Research, Kalpakkam, India, ^cNational Institute Technology, Kurukshetra, India). **Direct label free ultrasensitive impedimetric DNA biosensor using dendrimer functionalized GaN nanowires. Biosensors and Bioelectronics, Volume 44(2013): 164–170**

We demonstrate a very simple and generic protocol for ultrasensitive in-situ label-free detection of DNA hybridization using third generation poly(amidoamine)dendrimer (G3-PAMAM) functionalized GaN nanowires (NWs). PAMAM modified GaN NWs provides large density of docking site to immobilize significant number of probe (p-) DNA covalently. These p-DNA/PAMAM/GaN NWs sensor probes are employed to achieve an ultra-high detection limit down to attomolar level concentration of complementary target (t-) DNA. Comparative in-situ studies on single/triple base-pair mismatched, γ -irradiated and complementary t-DNA in the hybridization process reveal selectivity and specificity of the p-DNA/PAMAM/GAN NWs sensor probe over a wide range, 10^{-8} to 10^{-19} M, of analyte concentration. During the hybridization process, there is a substantial change in t-DNA concentration dependent interfacial polarization resistance during electrochemical impedance measurement, which forms the basis of the present DNA biosensor. This novel methodology for specific DNA sequence detection, as compared with the existing methods, is found to be very robust, highly sensitive, and reproducible.

Keywords: Gallium nitride nanowire; DNA; Biosensor; SWINE flu (H1N1); In-situ detection; Dendrimer

Wei Shen, Huimin Deng, Yuqian Ren, Zhiqiang Gao. (Department of Chemistry, National University of Singapore, Singapore 117543, Singapore). **A label-free microRNA biosensor based on DNAzyme-catalyzed and microRNA-guided formation of a thin insulating polymer film. Biosensors and Bioelectronics, Volume 44(2013): 171–176**

Herein we report a label-free microRNA (miRNA) biosensor in which the formation of a thin insulating film is used to amplify the analytical signal. Briefly, the biosensor is made of an oligonucleotide-coated gold electrode. After hybridizing with a target miRNA, free capture probe (CP) strands on the biosensor are removed by a nuclease digestion. A second hybridization with an oligonucleotide-tailed DNAzyme is performed to introduce the DNAzyme to the biosensor. The DNAzyme triggers the polymerization of 3,3'-dimethoxybenzidine (DB) in the presence of H_2O_2 and the hybridized miRNA-CP duplexes serve as templates to guide the deposition of poly (3,3'-dimethoxybenzidine) (PDB). The formation of the insulating PDB film

alters the impedance of the biosensor, rendering it readily distinguishable by electrochemical impedance measurements. The accumulative nature of the PDB deposition drastically improves the detectability of the biosensor. A proof-of-concept study is conducted on the detection of miRNAs in total RNA extracted from cultured cells.

Keywords: MicroRNA; DNase; Nuclease I; 3,3'-Dimethoxybenzidine; Electrochemical impedance

Yan Li^a, Cancan Huang^a, Jianbin Zheng^a, Honglan Qi^b, Wei Cao^a, Yinmao Wei^a. (^aShaanxi Provincial Key Laboratory of Electroanalytical Chemistry, Institute of Analytical Science, Northwest University, Xi'an, Shaanxi 710069, China, ^bKey Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an, Shaanxi 710062, China). **Label-free electrogenerated chemiluminescence biosensing method for trace bleomycin detection based on a Ru(phen)₃²⁺-hairpin DNA composite film electrode. *Biosensors and Bioelectronics*, Volume 44(2013): 177–182**

A novel label-free electrogenerated chemiluminescence (ECL) DNA-based biosensing method for the determination of trace bleomycin (BLM) was developed on basis of Fe(II)-BLM-mediated DNA strand scission and Ru(phen)₃²⁺ as an ECL probe. A thiolated ss-DNA, as substrate for BLMs, was self-assembled onto surface of a gold electrode to form a hairpin structure. Ru(phen)₃²⁺ was intercalated into the hairpin DNA structure. In the presence of Fe(II)-BLM, the hairpin DNA sequence undergoes the irreversible cleavage event under the oxidative effect of BLM with Fe(II) as a cofactor and the intercalated Ru(phen)₃²⁺ released from the gold electrode, which can be transduced into a significant decrease in ECL intensity. The ECL intensity versus the concentration of BLMs was linear in the range from 0.1 pM to 50 pM. The detection limit was 0.03 pM. This work demonstrates that using the sequence selectivity of DNA cleavage strategy for the fabrication of the label-free ECL biosensing method is a promising approach for the determination of antitumor drugs.

Keywords: Biosensing; DNA; Electrogenerated chemiluminescence; Bleomycin; Ru(phen)₃²⁺

Jing Chen^a, Gaowu Qin^a, Jiansheng Wang^a, Jiangyu Yu^b, Bo Shen^a, Song Li^a, Yuping Ren^a, Liang Zuo^a, Wen Shen^c, Biswajit Das^c. (^aKey Laboratory for Anisotropy and Texture of Materials (Ministry of Education), Northeastern University, Shenyang 110819, China, ^bCollege of Materials and Metallurgy, Northeastern University, Shenyang 110819, China, ^cNevada Nanotechnology Center, Howard R. Hughes College of Engineering, University of Nevada, Las Vegas, NV 89154-4026, USA). **One-step fabrication of sub-10-nm plasmonic nanogaps for reliable SERS sensing of microorganisms. *Biosensors and Bioelectronics*, Volume 44(2013): 191–197**

Nanoscale gaps in noble metal films can produce intense electromagnetic enhancement. When Raman-active molecules are positioned in these regions, their surface-enhanced Raman scattering (SERS) signals can be dramatically enhanced. However, the lack of convenient and reliable fabrication methods with ultrasmall nanogaps (<10 nm) severely block the application of SERS. Here, we propose a cost-effective and reproducible technique to fabricate the large-area Ag SERS-active substrates which are full of the high-density, sub-10-nm nanogaps by high

pressure sputtering, and the enhancement factor (EF) is testified to improve by 10^3 times compared to the continuous Ag film with a smooth surface (the roughness is 0.5 nm) and without nanogaps. Since there are no chemicals used during fabrication, this substrate has a clean surface, which is crucial for acquiring reliable SERS spectra. This SERS-active substrate has then been applied to identify a series of microorganisms, and excellent, reproducible SERS spectra were obtained. Finally, a set of piecewise-linear equations is provided according to the correlation between SERS intensity and rhodamine 6G (R6G) concentration, and the detection limit is calculated to be 0.2×10^{-8} M. These results suggest that the high pressure sputtering is an excellent, reliable technique for fabricating sub-10-nm plasmonic nanogaps, and the SERS-based methodology is very promising for being used in biological sensing field.

Keywords: Biosensor; High pressure sputtering; Microorganisms; Sub-10-nm nanogaps; Surface enhanced Raman scattering

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We describe within this paper the construction of a label-free immunosensor for the protein psoriasis (S100A7), which is associated with a number of clinical conditions such as skin diseases or cancer. Antibodies to psoriasis were immobilised onto screen-printed carbon electrodes that had been pre-modified with the conductive polymer polyaniline. We compared and contrasted a number of different methods of assembly to optimise the construction and properties of the immunosensor. Immunosensors were fabricated using both manual liquid handling (pipette) and an automated liquid dispensing platform, the BioDot AD3200TM. Two immobilisation methods were also utilised; simple electrostatic binding of the antibody to polyaniline as well as a more complex procedure using a biotin–neutravidin bridge. The optimum results in terms of sensitivity and reproducibility were obtained utilising the automated system and the biotin–avidin assembly procedure. The resultant immunosensors could be interrogated using AC impedance without the need for any labelling and demonstrated quantification of psoriasis from 250 pg ml^{-1} to 10 ng ml^{-1} —a concentration range suitable for determining physiological levels of psoriasis.

Keywords: Psoriasis; S100A7; Immunosensor; Impedance; Label-free

Xiulan Sun^a, Jian Ji^a, Donglei Jiang^a, Xiaowei Li^c, Yinzhi Zhang^a, Zaijun Li^b, Yongning Wu^c. (^aState Key Laboratory of Food Science and Technology, School of Food Science of Jiangnan University, Wuxi, Jiangsu 214122, China, ^bSchool of Chemical and Material Engineering of Jiangnan University, Wuxi, Jiangsu 214122, China, ^cChina National Center for Food Safety Risk Assessment, Beijing, Panjiayuan 100017, China). **Development of a novel electrochemical sensor using pheochromocytoma cells and its assessment of acrylamide cytotoxicity. Biosensors and Bioelectronics, Volume 44(2013): 122–126**

We report on a sensitive, simple, label-free cell-based electrochemical sensor to monitor the toxic effect of acrylamide on the Pheochromocytoma cells. The surface of the electrode was modified with gold nanoparticles and electrochemically reduced graphene oxide. Cyclic

voltammetry, impedance spectroscopy and differential pulse voltammetry were applied to characterize the modified electrode. Reduced graphene oxide was proved to increase electron-transfer rate between the cell and the surface of electrode, while gold nanoparticle retain cell bioactivity. The sensor exhibited good correlation to the logarithmic value of cell numbers ranging from 1.6×10^4 to 1.6×10^7 cells mL^{-1} , with R.S.D value of 1.68%. The value of differential pulse voltammetry (cell adsorption concentration of 1.6×10^7 cells mL^{-1}) decreased with the concentration of acrylamide in range of 0.1–5 mM with the detection limit as 0.04 mM. Scanning electron microscope-based morphological and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide analysis confirmed the results of the electrochemical study. This sensor was proved to be a useful tool for probing the toxicity of cells, and assisted in the development of a labeling-free, simple, rapid and immediate detection method.

Keywords: Electrochemical sensor; PC-12 cell; Acrylamide; Gold nanoparticle; Graphene oxide

Fengxing Jiang^{a, b}, Ruirui Yue^{a, b}, Yukou Du^a, Jingkun Xu^b, Ping Yang^a. (^aCollege of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, PR China, ^bJiangxi Key Laboratory of Organic Chemistry, Jiangxi Science and Technology Normal University, Nanchang 330013, PR China. **A one-pot ‘green’ synthesis of Pd-decorated PEDOT nanospheres for nonenzymatic hydrogen peroxide sensing. *Biosensors and Bioelectronics*, Volume 44(2013): 127–131**

We developed a novel nonenzymatic biosensor based on palladium/poly(3,4-ethylenedioxythiophene) (Pd/PEDOT) nanocomposite modified glassy carbon electrode (GCE) for the detection of hydrogen peroxide (H_2O_2). Pd/PEDOT has been successfully fabricated by a facile one-pot ‘green’ method using H_2PdCl_4 as an oxidant and a source of metal nanoparticles without any surfactants and templates. The as-synthesized PEDOT nanospheres are quite uniform in size (~ 60 nm) without aggregation and provide a good platform for anchoring the Pd nanoparticles (NPs). Pd NPs (~ 4.5 nm) are homogeneously dispersed on surface of PEDOT nanospheres. The Pd/PEDOT nanospheres on GCE exhibit a good electrocatalytic activity towards the H_2O_2 reduction. The electrochemical response of Pd/PEDOT to H_2O_2 exhibits a low detection limit of $2.84 \mu\text{M}$ in the range of 2.5×10^{-3} –1.0 mM with a high sensitivity, good repeatability, acceptable reproducibility and good long-term stability. The good recoveries achieved in spiked human urine samples demonstrated the potential application of Pd/PEDOT for H_2O_2 detection.

Keywords: Poly(3,4-ethylenedioxythiophene); Nanosphere; Palladium; Hydrogen peroxide; Nonenzymatic biosensor

Kobra Omidfar, Fahimeh Khorsand, Maedeh Darziani Azizi. (Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, P.O. Box 14395/1179, Tehran, Islamic Republic of Iran). **New analytical applications of gold nanoparticles as label in antibody based sensors. *Biosensors and Bioelectronics*, Volume 43(2013): 336–347**

Gold nanoparticles (AuNPs) with optical and electrochemical distinctiveness as well as biocompatibility characteristics have proven to be powerful tools in nanomedicinal application. This review article discusses recent advances in the application of AuNPs as label in

bioanalytical devices, especially electrochemical immunosensors, rapid and point-of-care (PoC) tests. A crucial assessment regarding implementation of different formats of antibodies allowing rapid and sensitive analysis of a range of analytes is also provided in this study. In addition to this, different approaches to minimize antibodies into Fab, scFv or even single-domain antibody fragments like VHHs will be reviewed. Given the high level of target specificity and affinity, such biomolecules are considered to be excellent elements for on-site or PoC analysis.

Keywords: Gold nanoparticles; Label; Immunosensor; Antibody

Lei Hong^{a, b}, Ai-Lin Liu^{a, b}, Guang-Wen Li^b, Wei Chen^{a, b}, Xin-Hua Lin^{a, b}. (^aDepartment of Pharmaceutical Analysis, School of Pharmacy, Fujian Medical University, Fuzhou 350004, China, ^bNano Medical Technology Research Institute, Fujian Medical University, Fuzhou 350004, China). **Chemiluminescent cholesterol sensor based on peroxidase-like activity of cupric oxide nanoparticles. Biosensors and Bioelectronics, Volume 43(2013): 1–5**

A chemiluminescent cholesterol sensor with good selectivity and enhanced sensitivity was constructed based upon the peroxidase-like activity of cupric oxide nanoparticles. Cupric oxide nanoparticles can catalyze the oxidation of luminol by H₂O₂, which was produced by the reaction of cholesterol and oxygen that was catalyzed by cholesterol oxidase. Therefore, the oxidation of cholesterol could be transduced into the chemiluminescence of luminol by combining these two reactions. Under the optimum conditions, the CL intensity was proportional to the concentration of cholesterol over the range of 0.625–12.5 μM and a detection limit was 0.17 μM. The applicability of proposed method has been validated by determination of cholesterol in milk powder and human serum samples with satisfactory results.

Keywords: Cholesterol; Cupric oxide nanoparticles; Enzyme mimic; Chemiluminescence; Luminol

Bo Liang^a, Lu Fang^a, Guang Yang^a, Yichuan Hu^a, Xishan Guo^b, Xuesong Ye^{a, c}. (^aBiosensor National Special Laboratory, Key Laboratory of Biomedical Engineering of Ministry of Education, College of Biomedical Engineering and Instrument Science, Zhejiang University, Hangzhou 310027, PR China, ^b College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, PR China, ^c Cyrus Tang Center for Sensor Materials and Applications, Zhejiang University, Hangzhou 310058, PR China). **Direct electron transfer glucose biosensor based on glucose oxidase self-assembled on electrochemically reduced carboxyl grapheme. Biosensors and Bioelectronics, Volume 43(2013): 131–136**

A glucose biosensor based on direct electron transfer of glucose oxidase (GOD) self-assembled on the surface of the electrochemically reduced carboxyl graphene (ERCGr) modified glassy carbon electrode has been reported. X-ray photoelectron spectroscopy (XPS) analyses of ERCGr indicate most of the oxygen-containing groups such as epoxy/ether groups and hydroxyl groups in the carboxyl graphene were eliminated, while carboxylic acid groups remained. GOD was immobilized on the ERCGr modified glassy carbon electrode via self-assembly. The cyclic voltammetric result of the electrode shows a pair of well-defined and quasi-reversible redox peaks with a formal potential of –0.467 V and a peak to peak separation of 49 mV, revealing that the direct electron transfer between GOD and the electrode has been achieved. The proposed biosensor exhibits a linear response to glucose concentrations ranging from 2 to 18 mM with a detection limit of 0.02 mM. Moreover, this facile, fast, environment-friendly and economical

preparation strategy of ERCGr may be extended for the preparation of other graphene based enzyme electrode biosensors.

Keywords: Glucose sensor; Carboxyl graphene; Electrochemical reduction; Direct electrochemistry

Bioengineering

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Hydrogen gas exhibits potential as a sustainable fuel for the future. Therefore, many attempts have been made with the aim of producing high yields of hydrogen gas through renewable biological routes. Engineering of strains to enhance the production of hydrogen gas has been an active area of research for the past 2 decades. This includes overexpression of hydrogen-producing genes (native and heterologous), knockout of competitive pathways, creation of a new productive pathway, and creation of dual systems. Interestingly, genetic mutations in 2 different strains of the same species may not yield similar results. Similarly, 2 different studies on hydrogen productivities may differ largely for the same mutation and on the same species. Consequently, here we analyzed the effect of various genetic modifications on several species, considering a wide range of published data on hydrogen biosynthesis. This article includes a comprehensive metabolic engineering analysis of hydrogen-producing organisms, namely *Escherichia coli*, *Clostridium*, and *Enterobacter* species, and in addition, a short discussion on thermophilic and halophilic organisms. Also, apart from single-culture utilization, dual systems of various organisms and associated developments have been discussed, which are considered potential future targets for economical hydrogen production. Additionally, an indirect contribution towards hydrogen production has been reviewed for associated species.

Keywords: hydrogen, metabolic engineering, *Escherichia coli*, *Clostridium*, *Enterobacter*, dual systems

Pollen Biotechnology

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Shimoga, India, ⁴Department of Dermatology, University Hospital Gloriastrasse, Zurich, Switzerland. * Correspondence Richard E. Goodman, Department of Food Science & Technology, 143 Food Industry Complex, Lincoln, NE 68583-0955, USA. Tel.: +1 (402) 472 0452, Fax: +1 (402) 472 1693, E-mail: rgoodman2@unl.edu). Challenges in testing genetically modified crops for potential increases in endogenous allergen expression for safety Allergy, Volume 68(2) (2013): 142–151

Premarket, genetically modified (GM) plants are assessed for potential risks of food allergy. The major risk would be transfer of a gene encoding an allergen or protein nearly identical to an allergen into a different food source, which can be assessed by specific serum testing. The potential that a newly expressed protein might become an allergen is evaluated based on resistance to digestion in pepsin and abundance in food fractions. If the modified plant is a common allergenic source (e.g. soybean), regulatory guidelines suggest testing for increases in the expression of endogenous allergens. Some regulators request evaluating endogenous allergens for rarely allergenic plants (e.g. maize and rice). Since allergic individuals must avoid foods containing their allergen (e.g. peanut, soybean, maize, or rice), the relevance of the tests is unclear. Furthermore, no acceptance criteria are established and little is known about the natural variation in allergen concentrations in these crops. Our results demonstrate a 15-fold difference in the major maize allergen, lipid transfer protein between nine varieties, and complex variation in IgE binding to various soybean varieties. We question the value of evaluating endogenous allergens in GM plants unless the intent of the modification was production of a hypoallergenic crop.

Keywords: endogenous allergen; genetically modified; IgE; maize; rice; soybean

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Airway epithelial cells are the first to encounter aeroallergens and therefore have recently become an interesting target of many studies investigating their involvement in the modulation of allergic inflammatory responses. Disruption of a passive structural barrier composed of epithelial cells by intrinsic proteolytic activity of allergens may facilitate allergen penetration into local tissues and additionally affect chronic and ongoing inflammatory processes in respiratory tissues. Furthermore, the ability of rhinoviruses to disrupt and interfere with epithelial tight junctions may alter the barrier integrity and enable a passive passage of inhaled allergens through the airway epithelium. On the other hand, epithelial cells are no longer considered to act only as a physical barrier toward inhaled allergens, but also to actively

contribute to airway inflammation by detecting and responding to environmental factors. Epithelial cells can produce mediators, which may affect the recruitment and activation of more specialized immune cells to the local tissue and also create a microenvironment in which these activated immune cells may function and propagate the inflammatory processes. This review presents the dual role of epithelium acting as a passive and active barrier when encountering an inhaled allergen and how this double role contributes to the start of local immune responses.

Keywords: allergens; dendritic cells; epithelium; immunology; innate immunity

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The immune system is regulated to protect the host from exaggerated stimulatory signals establishing a state of tolerance in healthy individuals. The disequilibrium in immune regulatory vs effector mechanisms results in allergic or autoimmune disorders in genetically predisposed subjects under certain environmental conditions. As demonstrated in allergen-specific immunotherapy and in the healthy immune response to high-dose allergen exposure models in humans, T regulatory cells are essential in the suppression of Th2-mediated inflammation, maintenance of immune tolerance, induction of the two suppressive cytokines interleukin-10 and transforming growth factor- β , inhibition of allergen-specific IgE, and enhancement of IgG4 and IgA. Also, suppression of dendritic cells, mast cells, and eosinophils contributes to the construction of peripheral tolerance to allergens. This review focuses on mechanisms of peripheral tolerance to allergens with special emphasis on recent developments in the area of immune regulation.

Keywords: allergy; dendritic cells; peripheral tolerance; T helper cells; T regulatory cells

Saeed Irian, Ahamad Majd, Aboozar Hoseinizadeh, Parisa Jonubi. (¹Department of Biology, Tarbiat Moallem University, Tehran, Iran). **A study on the allergenicity and ontogeny of *Acacia farnesiana* pollen grains in guinea pigs. Aerobiologia, Volume 29 (1) (2013): 21-29**

Pollen grains as the angiosperm male gametophytes transfer male genetic material during sexual reproduction. Pollen grains are among the important plant allergens, such that almost 80–90 % of plant allergens are of pollen origin. *Acacia farnesiana* is a plant with economical values, and due to its resistance to dry climates, it has had a widespread distribution in Southern Iran. This study was aimed at investigating the allergenicity and the ontogeny of

pollen grains of *A. farnesiana*. Pollen grains were collected from the blossoms and flowers of *A. farnesiana* in the suburbs of Ahvaz-Iran. Pollen extracts (15 %) were prepared in PBS (pH 7.2). 4–6-week-old male guinea pigs (*Hartly*) were used for allergenicity tests. Skin tests showed a significant increase in flare diameter. Clinical tests also showed a significant change in the levels of eosinophils, neutrophils, and IgE. Histotechnical analysis was performed on male gametophytes, and photographs were taken using a camera-equipped light microscope. Pollen characteristics were identified using both light and electron microscopy. Sporoderm ultrastructure and pollen morphology were studied using Scanning Electron Microscopy. PAGE analysis of the total protein content of mature pollen grains showed seven clear bands of 10–83 kDa.

Myszkowska Dorota. (¹Department of Clinical and Environmental Allergology, Jagiellonian University Medical College, 31-531, Cracow, Sniadeckich 10, Poland). **Prediction of the birch pollen season characteristics in Cracow, Poland using an 18-year data series.** *Aerobiologia*, Volume 29 (1) (2013):31-44,

The aim of the study was to construct the model forecasting the birch pollen season characteristics in Cracow on the basis of an 18-year data series. The study was performed using the volumetric method (Lanzoni/Burkard trap). The 98/95 % method was used to calculate the pollen season. The Spearman's correlation test was applied to find the relationship between the meteorological parameters and pollen season characteristics. To construct the predictive model, the backward stepwise multiple regression analysis was used including the multi-collinearity of variables. The predictive models best fitted the pollen season start and end, especially models containing two independent variables. The peak concentration value was predicted with the higher prediction error. Also the accuracy of the models predicting the pollen season characteristics in 2009 was higher in comparison with 2010. Both, the multi-variable model and one-variable model for the beginning of the pollen season included air temperature during the last 10 days of February, while the multi-variable model also included humidity at the beginning of April. The models forecasting the end of the pollen season were based on temperature in March–April, while the peak day was predicted using the temperature during the last 10 days of March.

Tamara Voskresensky Baričić, Slavica Dodig. (¹Department for Pulmonology, Allergology and Clinical Immunology, Pediatric Clinic Klaićeva, Klaićeva 16, 10000, Zagreb, Croatia, ²Department of Clinical Laboratory Diagnosis, Srebrnjak Children's Hospital, 10000, Zagreb, Croatia). **Birch pollen-associated peanut allergies in children.** *Aerobiologia*, Volume 29 (1) (2013): 85-93

Panallergens show structural similarities, and they are responsible for many cross-reactions between pollen and plant food sources. The aim of the present study was to investigate IgE reactivity to peanut allergen components in children with birch pollen allergy. Patients experienced symptoms of allergic asthma, allergic rhinitis, and urticaria, and they underwent a complete diagnostic evaluation, including skin prick test (SPT), specific IgE (sIgE) to birch pollen allergen (t3), peanut allergen (f13). In addition, measurement of sIgE to the major birch allergen components, *Betula verrucosa* (Bet v1, Bet v2), and to peanut allergen components, *Arachis hypogaea* (genuine components: Ara h1, Ara h2, Ara h3, and cross-reactive Ara h8) was performed, by using a microarray technique (component resolved diagnosis, CRD). SPT to birch extract was positive in all children, and SPT to peanut extract was positive in 51 % of them. sIgE to both allergens was increased in 39 % of children, 55 % of them had increased

sIgE (t3), and one child had increased sIgE (f13). CRD results confirmed that some children were sensitized to Bet v1 only, and some children to genuine Ara h only. Bet v1/Ara h8 cross-reactivity was found in 16 % of children. Results of the present study reveal that SPT, sIgE, and CRD may detect sensitization and co-sensitization with birch and peanut allergens/allergen components, and CRD may help to differentiate sensitization to genuine peanut components from sensitization to peanut cross-reactive component in birch-sensitive children. Diagnostic approach has to be individualized for each patient.

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The objective of this investigation was to identify the overall pollen types and, more particularly, the allergenic pollen content in the investigated area and then to explore their seasonal variations. The measurement point was located in the Timișoara city, Romania. A Lanzoni volumetric trap was used for sample collection. Duration of the pollen season of allergenic plants and respective variation in airborne pollen concentration are presented in the pollen calendar for the year 2009. Among the identified pollen of 23 types, 20 were allergenic: Taxaceae/Cupressaceae, *Alnus* sp., *Fraxinus* sp., *Betula* sp., *Corylus* sp., *Carpinus* sp., *Salix* sp., *Populus* sp., *Ulmus* sp., *Juglans* sp., *Quercus* sp., Pinaceae, *Tilia*, Poaceae, Urticaceae, Chenopodiaceae/Amaranthaceae, *Rumex* sp., *Plantago* sp., *Artemisia* sp., *Ambrosia* sp. These species prevail throughout almost the entire pollen season, from February–October, accounting for 87.03 % of the total pollen count. The greatest diversity of pollen types is detected in the months of spring. The summer months were characterized mostly by non-arboreal pollen types. In late summer and early autumn, *Ambrosia* airpollen was the most abundant in the atmosphere. The relationships between pollen concentrations and nine meteorological parameters are presented too. To analyze the correlation between pollen data and variables, the Spearman rank correlation coefficient was used. The correlation analysis of daily pollen counts and meteorological parameters showed that arboreal pollen and non-arboreal pollen counts were significantly correlated with temperature. The prevalence of pollen sensitization resulted to be very high in our patients with respiratory symptoms.

M. J. Velasco-Jiménez, P. Alcázar, E. Domínguez-Vilches, C. Galán. (University of Cordoba, Córdoba, Spain). **Comparative study of airborne pollen counts located in different areas of the city of Córdoba (south-western Spain). *Aerobiologia*, Volume 29(1) (2013):113-120**

Airborne pollen counts are mainly determined using a volumetric suction sampler based on the impact principle, that is, a Hirst-type spore trap. As a consequence of their volumetric nature, samplers detect pollen from a wide area, and therefore, a single sampler is frequently used to

acquire information on airborne pollen counts for the whole city. The main goal of the present study was to compare airborne pollen counts at two sites located at opposite ends (south-west vs. north-east) of the southern Spanish city of Córdoba, to assess the advantages and disadvantages of using more than one sampler in the city. Also, a comparative study was carried out using two samplers at the same site, in order to confirm the efficiency of the samplers. Results revealed that data from one volumetric sampler—located within a city of medium size with uniform topography and vegetation conditions—are sufficient to establish monitoring of the main airborne pollen types, the pollen seasons involved and the timing of peak counts. For clinical studies, however, data on pollen counts in specific areas of the city may be of value, since pollen intensity may vary from one district to another, mainly in the case of ornamental plants with a local distribution inside the city. Comparison of data obtained by the two samplers running at the same site indicated that potential inter-site differences could not be attributed to differences in sampler efficiency.

Biotechnology Policy Issue

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(† Department of Agricultural Economics, Purdue University, West Lafayette, Indiana, United States, ‡ Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, Indiana, United States. **Environmental and Economic Trade-Offs in a Watershed When Using Corn Stover for Bioenergy. *Environ. Sci. Technol.*, Volume 47 (4)(2013) : 1784–1791.**

There is an abundant supply of corn stover in the United States that remains after grain is harvested which could be used to produce cellulosic biofuels mandated by the current Renewable Fuel Standard (RFS). This research integrates the Soil Water Assessment Tool (SWAT) watershed model and the DayCent biogeochemical model to investigate water quality and soil greenhouse gas flux that results when corn stover is collected at two different rates from corn–soybean and continuous corn crop rotations with and without tillage. Multiobjective watershed-scale optimizations are performed for individual pollutant-cost minimization criteria based on the economic cost of each cropping practice and (individually) the effect on nitrate, total phosphorus, sediment, or global warming potential. We compare these results with a purely economic optimization that maximizes stover production at the lowest cost without taking environmental impacts into account. We illustrate trade-offs between cost and different environmental performance criteria, assuming that nutrients contained in any stover collected must be replaced. The key finding is that stover collection using the practices modeled results in increased contributions to atmospheric greenhouse gases while reducing nitrate and total phosphorus loading to the watershed relative to the status quo without stover collection. Stover collection increases sediment loading to waterways relative to when no stover is removed for each crop rotation–tillage practice combination considered; no-till in combination with stover collection reduced sediment loading below baseline conditions without stover collection. Our results suggest that additional information is needed about (i) the level of nutrient replacement required to maintain grain yields and (ii) cost-effective management practices capable of reducing soil erosion when crop residues are removed in order to avoid contributions to climate change and water quality impairments as a result of using corn stover to satisfy the RFS.

Stefan Merz †, **Georg Steinhauser** *‡†, and **Nobuyuki Hamada** §. († Vienna University of Technology, Atominstitut, Stadionallee 2, 1020 Wien, Austria, ‡ Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, Colorado 80523, United States, § Radiation Safety Research Center, Nuclear Technology Research Laboratory, Central Research Institute of Electric Power Industry (CRIEPI), 2-11-1 Iwado-kita, Komae, Tokyo 201-8511, Japan). **Anthropogenic Radionuclides in Japanese Food: Environmental and Legal Implications.** *Environ. Sci. Technol.*, Volume 47 (3)(2013):1248–1256

The Japanese government ordered the analysis of thousands of foods after the Fukushima nuclear accident to ascertain compliance with regulatory limits for anthropogenic radionuclides in food. Four hundred and forty-five samples obtained until 31 December 2011 from 11 prefectures exceeded the regulatory limits that were in force until 31 March 2012. The possibility of these 445 samples representing localized areas of high radiocesium concentration was investigated. The objective of this study was to determine the radiocesium activity ratio ($^{134}\text{Cs}/^{137}\text{Cs}$) in foods from each geographic area to possibly identify the radioactive signature of the four different reactors (i.e., four independent sources) in the distinct regions. The average $^{134}\text{Cs}/^{137}\text{Cs}$ activity ratio was 0.98 ± 0.01 for all samples. However, no statistically significant deviations from this value could be confirmed in the various regions. Therefore, we conclude that the releases from reactor No. 4 (carrying a significantly smaller activity ratio) are assumed to be small when compared with the other three reactor releases. The individual radioisotopic signatures of reactors No. 1, 2, and 3 could not be identified in various Japanese regions using the food samples, indicating integral radiocesium contamination from these sources. Subsequent releases of fission products from the reactors (e.g., after possible criticalities reported in October 2011) proved to have no impact on the radiocesium activity ratio. A discussion of the development of the regulatory limits in Japan and Europe with regard to the current limits and radiological food safety are also included.

Agricultural Biotechnology

Jose Cuesta^a, **Svetlana Edmeades**^b, **Lucia Madrigal**^c. (^a World Bank, Poverty Reduction and Equity Unit, 1818 H Street NW, Washington, DC 20477, USA, ^b World Bank, Latin America and Caribbean Agriculture and Rural Development, 1818 H Street NW, Washington, DC 20477, USA, ^c International Food Policy Research Institute, 2033 K Street NW, Washington, DC 20006, USA). **Food security and public agricultural spending in Bolivia: Putting money where your mouth is?** *Food Policy*, Volume 40 (2013): 1–13

This paper explores the reduction of food insecurity in Bolivia, adopting a supply-side approach that analyzes the role of agricultural spending on vulnerability to food insecurity. Vulnerability to food insecurity is captured by a municipal-level composite indicator for all 327 municipalities in 2003, 2006, and 2007. Econometric analysis indicates that levels of public agricultural spending are positively associated with high or very high vulnerability—especially investments in infrastructure and research and extension. The authors interpret this to indicate that agricultural spending allocation is driven by high or very high vulnerability levels, but has small effects on reducing high vulnerability.

Keywords: Food security; Vulnerability; Agricultural spending; Municipalities; Latin America; Bolivia

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The biofuels industry in both the UK and the EU as a whole has expanded significantly in recent years in response to various EU biofuel policy initiatives. Further expansion of biofuel demand will increase the impact of the biofuels sector on agricultural markets. This paper examines the impact that increasing levels of first generation biofuel demand to 10% of total transport fuel use in the UK and other EU Member States would have on agricultural markets within the EU and specifically the UK using a partial equilibrium modelling system. Increasing overall biodiesel demand raises demand for vegetable oil and oilseed and in turn their prices. The increased grain demand in response to the increased bioethanol demand is mostly sourced from changes in EU net trade.

Keywords: Biofuels; EU Renewable Energy Directive; UK Renewable Transport Fuel Obligation Order; Partial equilibrium model; Agricultural market; Land use change

Bioenergy

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We tested the hypothesis that the biodegradation of volatile petroleum hydrocarbons (VPHs) in aerobic sandy soil is affected by the blending with 10 percent ethanol (E10) or 20 percent biodiesel (B20). When inorganic nutrients were scarce, competition between biofuel and VPH degraders temporarily slowed monoaromatic hydrocarbon degradation. Ethanol had a bigger impact than biodiesel, reflecting the relative ease of ethanol compared to methyl ester biodegradation. Denaturing gradient gel electrophoresis (DGGE) of bacterial 16S rRNA genes revealed that each fuel mixture selected for a distinct bacterial community, each dominated by *Pseudomonas* spp. Despite lasting impacts on soil bacterial ecology, the overall effects on VPH biodegradation were minor, and average biomass yields were comparable between fuel types, ranging from 0.40 ± 0.16 to 0.51 ± 0.22 g of biomass carbon per gram of fuel carbon degraded. Inorganic nutrient availability had a greater impact on petroleum hydrocarbon biodegradation than fuel composition.

Keywords: Biofuels; Petroleum; Fuel biodegradability; Soil pollution; Microbial ecology

Rebecca A. Efroymsen, Virginia H. Dale, Keith L. Kline, Allen C. McBride, Jeffrey M. Bielicki, Raymond L. Smith, Esther S. Parish, Peter E. Schweizer, Denice M. Shaw. (¹Center for BioEnergy Sustainability, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, USA, Email: efroymsonra@ornl.gov ² Center for BioEnergy Sustainability, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, USA ³ Hubert H. Humphrey School of Public Affairs, University of Minnesota, 301 19th Avenue South, Minneapolis, MN, 55455, USA ⁴ National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH, 45268, USA ⁵ National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC, 20460, USA). **Environmental Indicators of Biofuel Sustainability: What About Context? Environmental Management, Volume 51(2) (2013): 291-306**

Indicators of the environmental sustainability of biofuel production, distribution, and use should be selected, measured, and interpreted with respect to the context in which they are used. The context of a sustainability assessment includes the purpose, the particular biofuel production and distribution system, policy conditions, stakeholder values, location, temporal influences, spatial scale, baselines, and reference scenarios. We recommend that biofuel sustainability questions be formulated with respect to the context, that appropriate indicators of environmental sustainability be developed or selected from more generic suites, and that decision makers consider context in ascribing meaning to indicators. In addition, considerations such as technical objectives, varying values and perspectives of stakeholder groups, indicator cost, and availability and reliability of data need to be understood and considered. Sustainability indicators for biofuels are most useful if adequate historical data are available, information can be collected at appropriate spatial and temporal scales, organizations are committed to use indicator information in the decision-making process, and indicators can effectively guide behavior toward more sustainable practices.

Timothy Lawrence Johnson, Jeffrey M. Bielicki, Rebecca S. Dodder, Michael R. Hilliard, P. Ozge Kaplan, C. Andrew Miller. (¹Nicholas School of the Environment, Duke University, 90328, Durham, NC, 27708, USA, Email: timothy.l.johnson@duke.edu ² Hubert H. Humphrey Institute of Public Affairs, University of Minnesota, Minneapolis, MN, USA ³ Office of Research and Development, 109 TW Alexander Drive, MD E305-02, Research Triangle Park, Durham, NC, 27711, USA ⁴ Oak Ridge National Laboratory, Oak Ridge, TN, USA). **Advancing Sustainable Bioenergy: Evolving Stakeholder Interests and the Relevance of Research. Environmental Management, Volume 51(2) (2013): 339-353**

The sustainability of future bioenergy production rests on more than continual improvements in its environmental, economic, and social impacts. The emergence of new biomass feedstocks, an expanding array of conversion pathways, and expected increases in overall bioenergy production are connecting diverse technical, social, and policy communities. These stakeholder groups have different—and potentially conflicting—values and cultures, and therefore different goals and decision making processes. Our aim is to discuss the implications of this diversity for bioenergy researchers. The paper begins with a discussion of bioenergy stakeholder groups and their varied interests, and illustrates how this diversity complicates efforts to define and promote “sustainable” bioenergy production. We then discuss what this diversity means for research

practice. Researchers, we note, should be aware of stakeholder values, information needs, and the factors affecting stakeholder decision making if the knowledge they generate is to reach its widest potential use. We point out how stakeholder participation in research can increase the relevance of its products, and argue that stakeholder values should inform research questions and the choice of analytical assumptions. Finally, we make the case that additional natural science and technical research alone will not advance sustainable bioenergy production, and that important research gaps relate to understanding stakeholder decision making and the need, from a broader social science perspective, to develop processes to identify and accommodate different value systems. While sustainability requires more than improved scientific and technical understanding, the need to understand stakeholder values and manage diversity presents important research opportunities.

Sunil Kumar Narwal, Reena Gupta. (Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla, 171005, India. Reena Gupta Email: reenagupta_2001@yahoo.com). Biodiesel production by transesterification using immobilized lipase. *Biotechnology Letters*, Volume 35(4) (2013): 479-490

Biodiesel can be produced by transesterification of vegetable or waste oil catalysed by lipases. Biodiesel is an alternative energy source to conventional fuel. It combines environmental friendliness with biodegradability, low toxicity and renewability. Biodiesel transesterification reactions can be broadly classified into two categories: chemical and enzymatic. The production of biodiesel using the enzymatic route eliminates the reactions catalysed under acid or alkali conditions by yielding product of very high purity. The modification of lipases can improve their stability, activity and tolerance to alcohol. The cost of lipases and the relatively slower reaction rate remain the major obstacles for enzymatic production of biodiesel. However, this problem can be solved by immobilizing the enzyme on a suitable matrix or support, which increases the chances of re-usability. The main factors affecting biodiesel production are composition of fatty acids, catalyst, solvents, molar ratio of alcohol and oil, temperature, water content, type of alcohol and reactor configuration. Optimization of these parameters is necessary to reduce the cost of biodiesel production.

Wei Zhang^a, Elaine A. Yu^b, Scott Rozelle^c, Jun Yang^d, Siwa Msangi^a. (^aEnvironment and Production Technology Division, International Food Policy Research Institute, Washington, DC, USA, ^b Division of Nutritional Sciences, Cornell University, NY, USA, ^cShorenstein Asia Pacific Research Center, Stanford University, Stanford, CA, USA, ^dCenter for Chinese Agricultural Policy, Chinese Academy of Sciences, Beijing, China). The impact of biofuel growth on agriculture: Why is the range of estimates so wide? *Food Policy*, Volume 38 (2013): 227–239

The rapid expansion of biofuel production has generated considerable interest within the body of empirical economic literature that has sought to understand the impact of biofuel growth on the global food economy. While the consensus within the literature is that biofuel emergence is likely to have some effect on future world agricultural market, there is a considerable range in the estimated size of the impact. Despite the importance of this topic to policy makers, there has been no study that has tried to reconcile the differences among various outlook studies. This paper undertakes an in-depth review of some key outlook studies which quantify the impacts of biofuels on agricultural commodities, and which are based on either general-equilibrium (GE) or partial-equilibrium (PE) modeling approaches. We attempt to reconcile the systematic differences in the estimated impacts of biofuel production growth on the prospective prices and

production of three major feedstock commodities, maize, sugar cane, and oilseeds across these studies. Despite the fact that all models predict positive impacts on prices and production, there are large differences among the studies. Our findings point to a number of key assumptions and structural differences that seem to jointly drive the variations we observe, across these studies. The differences among the PE models are mainly due to differences in the design of scenarios, the presence or absence of biofuel trade, and the structural way in which agricultural and energy market linkages are modeled. The differences among the GE models are likely to be driven by model assumptions on agricultural land supply, the inclusion of the byproducts, and assumptions on crude oil prices and the elasticity of substitution between petroleum and biofuels.

Keywords: Biofuels; Agricultural commodity markets; Economic modeling; Energy

Frank R. Bengelsdorf^{1,*}, Ulrike Gerischer², Susanne Langer¹, Manuel Zak³, Marian Kazda³. (¹Institute for Microbiology and Biotechnology, University of Ulm, Ulm, Germany, ²Theoretical and Computational Biophysics Department, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, ³Institute for Systematic Botany and Ecology, University of Ulm, Ulm, Germany. *Correspondence: Frank Bengelsdorf, Institut für Mikrobiologie und Biotechnologie, Albert-Einstein-Allee 11, D-89081 Ulm, Universität Ulm, Germany. Tel.: +49 731 50 22713; fax: +49 731 50 22719; e-mail: frank.bengelsdorf@uni-ulm.de). **Stability of a biogas-producing bacterial, archaeal and fungal community degrading food residues. FEMS Microbiology Ecology, Volume 84(1) (2013): 201–212**

The resident microbiota was analyzed in a mesophilic, continuously operating biogas plant predominantly utilizing food residues, stale bread, and other waste cosubstrates together with pig manure and maize silage. The dominating bacterial, archaeal, and eukaryotic community members were characterized by two different 16S/18S rRNA gene culture-independent approaches. Prokaryotic 16S rRNA gene and eukaryotic 18S rRNA gene clone libraries were constructed and further analyzed by restriction fragment length polymorphism (RFLP), 16S/18S rRNA gene sequencing, and phylogenetic tree reconstruction. The most dominant bacteria belonged to the phyla *Bacteroidetes*, *Chloroflexus*, and *Firmicutes*. On the family level, the bacterial composition confirmed high differences among biogas plants studied so far. In contrast, the methanogenic archaeal community was similar to that of other studied biogas plants. Furthermore, it was possible to identify fungi at the genus level, namely *Saccharomyces* and *Mucor*. Both genera, which are important for microbial degradation of complex compounds, were up to now not found in biogas plants. The results revealed their long-term presence as indicated by denaturing gradient gel electrophoresis (DGGE). The DGGE method confirmed that the main members of the microbial community were constantly present over more than one-year period.

Keywords: biowaste; biogas plant; 16S/18S rRNA gene; *Saccharomyces*; denaturing gradient gel electrophoresis

Anil Kuruvilla Mathew, Mitch Crook, Keith Chaney, Andrea Claire Humphries. (Crops Department, Harper Adams University College, Edgmond, Newport, Shropshire TF10 8NB, UK). **Comparison of entrapment and biofilm mode of immobilisation for bioethanol**

production from oilseed rape straw using *Saccharomyces cerevisiae* cells. Biomass and Bioenergy, Volume 52 (2013): 1–7

Cell immobilisation provides the opportunity to reduce the cost of producing bioethanol from lignocellulosic biomass such as oilseed rape (OSR) straw, in addition to enhancing operational stability. Bioethanol fermentation of OSR straw hydrolysate by free and immobilised *Saccharomyces cerevisiae* was studied. Cells were either entrapped in alginate beads or Lentikat[®] discs or immobilised as a biofilm on spent grains, Leca, or reticulated foam. The overall aims of the research were to compare bioethanol yields produced from free and immobilised systems, and to identify the most suitable immobilisation technique in terms of bioethanol yield and longevity of the immobilised cell system. Cell entrapment in alginate beads and Lentikat[®] discs resulted in significantly higher bioethanol yields compared to when cells were free in suspension or immobilised as a biofilm on a support material. The maximum amount of bioethanol produced by cells immobilised in alginate beads and Lentikat[®] discs were 169.26 ± 0.24 and 165.13 ± 0.67 g bioethanol kg⁻¹ OSR straw after 3 h and 7 h of fermentation, respectively. Due to the high mechanical stability and bioethanol yield, immobilisation of *S. cerevisiae* in Lentikat[®] discs was considered the most appropriate immobilisation technique for bioethanol production.

Keywords: Alkaline pre-treatment; Immobilisation; Oilseed rape straw; Glucose; Bioethanol.

Pramila Tamunaidu^a, Naohiro Matsui^b, Yasuyuki Okimori^b, Shiro Saka^a. (^aDepartment of Socio-Environmental Energy Science, Graduate School of Energy Science, Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto 606-8501, Japan, ^b The General Environmental Technos Co., Ltd., 1-3-5 Azuchimachi, Chuo-ku, Osaka 541-0052, Japan). **Nipa (*Nypa fruticans*) sap as a potential feedstock for ethanol production. Biomass and Bioenergy, Volume 52 (2013): 96–102**

The current study was initiated to evaluate the potential of sugar saps from nipa (*Nypa fruticans*) palm as sustainable feedstock for ethanol production. Nipa palms managed as plantations on four sites was chosen for this study with palms within 8–100 years of age. All palms studied were found to have the potential to produce sugar saps from 0.4 to 1.2 L d⁻¹ per palm. Further chemical characterization of its saps gave a total composition of 159–214 g kg⁻¹ mainly composed of sucrose, glucose and fructose. In addition, the elemental analysis gave 5 g kg⁻¹ of inorganics with Na, K and Cl being its main inorganic elements. Preliminary batch fermentative assays using *Saccharomyces cerevisiae* showed that nipa saps can be converted to ethanol within 30–48 h in conditions with and without nutrient supplementation. Furthermore, the fermentation trends were similar to sugarcane sap with high ethanol conversions up to 96.9% and 95.5% achieved for both nutrient conditions. Further analysis on inorganic elements before and after fermentation showed that specific elements of Mg, Ca, P and S were significantly reduced and could have assisted the fermentation. Based on the results obtained from sap collection, chemical characterization and fermentation, the ethanol potential from nipa planted at a density of 1000 ha⁻¹ would range from 4550–9100 L ha⁻¹ y⁻¹. Conclusively, nipa sap showed some interesting characteristics which makes it a potential feedstock for ethanol production.

Keywords: Nipa sap; Chemical composition; Fermentation; *Saccharomyces cerevisiae*; Ethanol

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Building Society, Level 4, 17 Albert Street, Auckland, New Zealand). Extractable liquid, its energy and hydrocarbon content in the green alga *Botryococcus braunii*. Biomass and Bioenergy, Volume 52(2013): 103–112

Due to sparse sampling across races, studies on various strains of *Botryococcus braunii* have effectively been indiscriminate, and so the target strains for energy production have not come clearly into focus. This study compares extractable liquid biofuel content, bioenergy content and hydrocarbon content across 16 strains *B. braunii* (A, B and L races) by direct combustion of algal biomass using thermogravimetric analysis (TGA), pressure differential scanning calorimetry (PDSC) and gas chromatography/mass spectrometry (GC/MS). All *B. braunii* strains were cultured in the same environmental conditions in 250 ml flasks, and were harvested for analysis when algae reached the exponential growth phase. Significant differences were detected within and between races A, B and L. The ranges of variation in extractable liquid, biofuel energy and hydrocarbon contents in algal dry biomass were 10–40%, 10–60% and 4–25%, respectively. The race B strains (Ayame 1, Kossou 4, Overjuyo 3 and Paquemar) had more than 21% of dry weight comprising C₃₁-C₃₆ hydrocarbons, which are suitable for biofuel and bioenergy production. The Overjuyo 7 and CCAP 807/2 strains in race A and the Madras 3 and Yamoussoukro 4 strains in race L also showed high biofuel production with extractable liquid biofuel accounting for >30% of dry weight. This study identified particular *B. braunii* strains that are suitable for biofuel production. The application of TGA and PDSC provides a useful analytical approach for assessing the biodiesel production potential of microalgae.

Keywords: *Botryococcus braunii*; Extractable liquid; Biofuel energy; Hydrocarbon production; Algae

Camelia Ciubota-Rosie^a, Matei Macoveanu^a, Carmen María Fernández^b, María Jesús Ramos^b, Angel Pérez^b, Andrés Moreno^c. (^aTechnical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, Department of Environmental Engineering and Management, 71 Mangeron Blvd., 700050 Iasi, Romania, ^b Chemical Engineering Department, Institute of Chemical and Environmental Technology, University of Castilla-La Mancha, Avd. Camilo José Cela s/n, 13071 Ciudad Real, Spain, ^c Department of Organic Chemistry, Faculty of Chemistry, University of Castilla-La Mancha, Avd. Camilo José Cela s/n, 13071 Ciudad Real, Spain). *Sinapis alba* seed as a prospective biodiesel source. Biomass and Bioenergy, Volume 51(2013): 83–90

Seed processing and oil pre-treatment were optimised to improve the yield and quality of biodiesel obtained from *Sinapis alba*. By using solvent extraction or mechanical pressing followed by solvent extraction, an oil extraction yield greater than 41 wt.% and a de-oiled cake with less than 2 wt.% of oil could be obtained. The oil characterisation showed that erucic acid was the most predominant fatty acid in the oil composition (>50 wt.%). A high phosphorous content (>36 mg/kg) and acid value (>1.5 mg KOH/g) were obtained. To obtain a biodiesel with a high methyl ester content, it was necessary to carry out an oil refining process (acid degumming and chemical de-acidification) followed by a standard transesterification with methanol and sodium methoxide as a catalyst. Finally, the quality of the purified biodiesel was tested according to the EN 14214 and ASTM D6751 standards. The results of this work revealed the possibility of using the oil from *Sinapis alba* seeds as a suitable source for biodiesel.

Keywords: Mustard seeds; Oil extraction; Refining; Biodiesel; Biopesticide

Anders Michael Nielsen^{a, b}, Knud Villy Christensen^b, Henrik Bjarne Møller^a. (^aAarhus University, Department of Engineering, Postbox 50, Blichers Allé 20, DK-8830 Tjele, Denmark, ^b University of Southern Denmark, Faculty of Engineering, Niels Bohrs Allé 1, DK-5230 Odense, Denmark). **Inline NH₃ removal from biogas digesters. Biomass and Bioenergy, Volume 50(2013): 10–18**

During biogas production from various types of substrates such as animal manure, fats and proteins, bacterial growth and biogas production can be inhibited by excessive ammonia (NH₃) concentrations. If NH₃ is removed from the biogas digester without damaging the digestion process, inhibition of the methane (CH₄) producing bacteria will diminish. This study shows that it is possible to remove a significant quantity of NH₃ from the biogas digester headspace and liquid phase by a simple gas circulation method where gas bubbles free of NH₃ is forced through the upper 30 cm of the liquid phase in the biogas digester, into the headspace and out of the digester. The suggested method improves conditions for anaerobic bacteria exposed to high concentrations of NH₃ by simply removing NH₃ from the digester.

In full-scale biogas production the system presented in this study can be improved by circulating headspace gas through an ammonia absorber and returning the NH₃ depleted biogas into the biogas digester. This method can also replace the need for mixing in biogas digesters.

Keywords: Ammonia; Biogas; Inhibition; K_{La} ; Removal

Vít Kermes, Petr Bělohradský. (Faculty of Mechanical Engineering, Brno University of Technology, Technická 2, 616 69 Brno, Czech Republic). **Biodiesel (EN 14213) heating oil substitution potential for petroleum based light heating oil in a 1 MW stationary combustion facility. Biomass and Bioenergy, Volume 49(2013): 10–21**

The aim of the present work was to experimentally investigate the combustion properties of methyl-ester of rapeseed oil (RME) that was preheated for three distinct temperatures ((30, 70, 110) °C) in the study. The experiments were carried out in a water-cooled horizontal combustion chamber with a nominal thermal load of 1500 kW. The experimental results were compared to the results obtained from the combustion tests with extra light heating oil (ELHO) that was used as a comparative fuel. The fuel was atomized by means of the twin-fluid effervescent atomizer with a maximal output of 100 kg h⁻¹ of oil at gas to liquid mass flow rate ratio (GLR) equals to 20%.

Primarily the experiments were focused on the investigation of the flame characteristics and the quality of combustion at distinct heat outputs ((475, 700, 900) kW) and distinct GLR ratios ((10, 15, 20) %). Second, experiments focused on the evaluation and comparison of local wall heat fluxes along the flame length and were performed only for one setting of operating conditions, namely for the heat output of 900 kW and the GLR 15%.

The results revealed that substitution of ELHO requires preheating of RME to the temperature of about 70 °C, if pneumatic atomization is to be used. The preheating of RME to higher temperature did not show any significant improvement in terms of quality of combustion. Moreover, it causes the increase in nitrogen oxides formation up to 60% in comparison with the values measured during ELHO combustion.

Keywords: Combustion; Extra light heating oil; Methyl-ester of rapeseed oil; Pollutant emissions; Burning stability; Heat transfer

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This paper presents the life cycle inventory (LCI) of hydrogen production by *Clostridium butyricum* fermentation of *Scenedesmus obliquus* hydrolysate. The main purpose of this work was to evaluate the potential of H₂ production from microalgal biomass and the respective energy consumption and CO₂ emissions in the bioconversion process considering the microalga production, acid hydrolysis of *S. obliquus* biomass, preparation of the inoculum and culture media, and fermentation. The scale-up to industrial production was not envisaged.

The hydrogen yield obtained in this work was 2.9 ± 0.3 mol H₂/mol sugars in *S. obliquus* hydrolysate. Results show that this process of biological production of hydrogen can achieve 7270 MJ/MJ_{H₂}/MJ/MJ_{H₂} of energy consumption and 670 kg CO₂/MJ_{H₂} kgCO₂/MJ_{H₂}. The microalgal culture is the stage responsible for 98% of these total final values due to the use of artificial lighting. All stages and processes with the highest values of energy consumption and CO₂ emissions were identified for future energetic and environmental optimisation.

Keywords: *Scenedesmus obliquus*; Dark fermentation; *Clostridium butyricum*; Biohydrogen; Life cycle inventory

Sarah L. Hemstock. (School of Animal, Rural & Environmental Sciences, Nottingham Trent University, Brackenhurst Campus, Burton Street, Nottingham NG1 4BU, UK). The potential of coconut toddy for use as a feedstock for bioethanol production in Tuvalu. *Biomass and Bioenergy*, Volume 49(2013): 323–332

In Tuvalu the sap from the coconut palm (*Cocos nucifera*) is known as “toddy”. This paper examines toddy's current use as a foodstuff, the contribution of the sale of toddy products to household incomes and the potential use of sour toddy as a sustainable feedstock for bioethanol production for use as a petroleum substitute in Tuvalu.

The productivity and current uses of coconut woodlands are also assessed. At current levels of production and use, less than 1% of coconut palms are used for toddy production by 1133 producers. Over 5 dm³ of fresh toddy are produced annually and average toddy production per tree ranges from 1.7 to 4.3 dm³ d⁻¹.

The sale of toddy products provides 22–24% of annual household income for producers.

The production of bioethanol from toddy has been demonstrated by the NGO Alofa Tuvalu. However, it is not currently cost competitive with petroleum, although in terms of productivity,

the current toddy harvest could produce enough bioethanol to replace 31% of the nation's petroleum.

It is evident that there is a large untapped biomass energy potential in Tuvalu which should be utilised if the Government of Tuvalu is going to fulfil their commitment to be carbon neutral by 2020.

Keywords: Biomass; Bioethanol; Coconut; Land use; Toddy; Tuvalu

Luis Blanco-Cocom^{a, b}, Andrés Guerrero-Álvarez^a, Jorge Domínguez-Maldonado^a, Eric Ávila-Vales^b, Liliana Alzate-Gaviria^a. (^aUnidad de Energía Renovable, Centro de Investigación Científica de Yucatán A.C (CICY), Calle 43 No. 130 Col. Chuburná de Hidalgo, C.P. 97200 Mérida, Yucatán, México, ^bFacultad de Matemáticas, Universidad Autónoma de Yucatán, Anillo Periférico Norte, Tablaje Cat. 13615, Col. Chuburná de Hidalgo Inn, Mérida, Yucatán, México). **Mathematical model for a continuous hydrogen production system: Stirred fermenter connected to a biocatalyzed electrolysis cell. Biomass and Bioenergy, Volume 48(2013): 90–99**

This paper presents a mathematical model applied to a continuous hydrogen production system, composed of a stirred fermenter connected to a biocatalyzed electrolysis cell (BEC). The model contemplates two differential equation systems which describe the adaptation (start-up) and continuous phases between the fermenter and the BEC. The proposed model describes the dynamics of hydrogen and volatile fatty acid (VFA) production and substrate consumption (glucose for the stirred fermenter and acetate in the BEC), based on a Tessier-type bacterial kinetic which simulates the lag phase in the bacteria. A hybrid evolutionary algorithm and least squares method were used to estimate the parameters. Model validation and simulation were achieved by obtaining the volumes of hydrogen and VFAs produced and the statistical bacterial density via the most probable number (MPN) method.

Keywords: Hydrogen; Stirred fermenter; Biocatalyzed electrolysis cell; Genetic algorithm; Tessier

Bajrang Singh^a, Kripal Singh^a, G. Rejeshwar Rao^b, J. Chikara^c, Dinesh Kumar^d, D.K. Mishra^e, S.P. Saikia^f, U.V. Pathre^a, Nidhi Raghuvanshi^a, T.S. Rahi^a, Rakesh Tuli^g. (^aNational Botanical Research Institute, Lucknow 226 001, Uttar Pradesh, India, ^b Central Research Institute in Dryland Agriculture, Hyderabad 500 059, Andhra Pradesh, India, ^cCentral Salt and Marine Chemical Research Institute, Bhavnagar 364 002, Gujarat, India, ^d Forest Research Institute, Dehradun 248 195, Uttarakhand, India, ^e Arid Forest Research Institute, Jodhpur 342 005, Rajasthan, India, ^f North-East Institute of Science and Technology, Jorhat 785 006, Assam, India, ^g National Agri-Food Biotechnology Institute, Mohali 160 071, Chandigarh, India). **Agro-technology of *Jatropha curcas* for diverse environmental conditions in India. Biomass and Bioenergy, Volume 48(2013): 191–202**

Jatropha curcas has been widely planted without knowing its standard package of practice for optimizing the yield. Therefore, a standardized agro-technology of *Jatropha* is required. With this purpose, in this study an elite accession of *Jatropha* was planted at seven sites in India, covering a range of edapho-climatic conditions. Three experimental trials (spacing, pruning and irrigation and fertilizer) were carried out wherein its growth and yield performance were assessed for five years (2007–2012) at all the sites. The growth characters like plant height and number of branches showed significant variations among the sites and the effects of treatments

were not uniform across the sites, indicating that site-specific package of practices should be followed instead of adopting the general recommendations. The seed yield was disappointing to recommend it randomly for large scale plantations on degraded lands in India. But results from the multi-location trials have shown good prospects at semi-arid (Bhavnagar) and drylands (Hyderabad); where plants in close spacing (2 × 2 m) produced significantly higher seed yield per unit area upto 1.4 t ha⁻¹ (Bhavnagar) in comparison to wide spacing. Pruning showed a negative effect on seed yield during initial five years. We found that the following prescription was sufficient to optimize the yield on India's degraded soils: irrigation at a 30 day interval, and for each planting hole 2 kg of Farm Yard Manure (organic manure) and nitrogen, phosphorous and potassium at 10 g, 20 g, and 10 g, respectively.

Keywords: Biodiesel; *Jatropha curcas*; Seed yield; Soil properties; Climate effect

Prabodh Illukpitiya^a, John F. Yanagida^b, Richard Ogoshi^c, Goro Uehara^c. (^aDepartment of Agricultural and Environmental Sciences, Tennessee State University, 3500 John A. Merritt Blvd, Nashville, TN 37209, USA, ^b Department of Natural Resources and Environmental Management University of Hawaii at Manoa, USA, ^c Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, USA). **Sugar-ethanol-electricity co-generation in Hawai'i: An application of linear programming (LP) for optimizing strategies. Biomass and Bioenergy, Volume 48(2013): 203–212**

This research develops a linear programming (LP) model to assess various options for sugar and biofuel production from sugarcane and other feedstock in Hawaii. More specifically, the study focuses on finding optimal sugar and biomass feedstock that would maximize producer profits in the production of sugar, ethanol and electricity. Feedstock included in the model were sugarcane, banagrass, energy cane and sweet sorghum. Given available land resources for growing energy crops on the island of Maui, four land resource scenarios were considered. If available land resources were used in the production of sugarcane and energy crops with added utilization of non-prime lands, Hawaii's ethanol goal for year 2020 could be achieved while maintaining two-thirds of Hawaii's current sugar production. Crop yields and unit production costs are key factors in determining optimal quantities of feedstock in the optimization model tested in this study.

Keywords: Biofuel; Energy crops; Ethanol; Hawaii; Linear program; Non-prime land

Rumana Islam^a, Serpil Özmihçi^{a, c}, Nazim Cicek^a, Richard Sparling^b, David B. Levin^a. (^aDepartment of Biosystems Engineering, University of Manitoba, Winnipeg, Manitoba R3T 5V6, Canada, ^b Department of Microbiology, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada, ^c Department of Environmental Engineering, Dokuz Eylul University, 35160 Buca, Izmir, Turkey). **Enhanced cellulose fermentation and end-product synthesis by *Clostridium thermocellum* with varied nutrient compositions under carbon-excess conditions. Biomass and Bioenergy, Volume 48(2013): 213–223**

Sixteen combinations of seven growth nutrients, namely α -cellulose, yeast extract (YE), urea, CaCl₂·2H₂O, MgCl₂·6H₂O, FeSO₄·6H₂O and vitamins, were studied to improve direct cellulose fermentation by *Clostridium thermocellum* DSM 1237 under carbon-excess conditions. Varied nutrient compositions improved cellulose fermentation conditions for *C. thermocellum* and displayed two major types of effects: a general growth enhancement effect and a carbon-flux

shifting effect. Different concentrations of the four most influential nutrients (YE, α -cellulose, CaCl_2 and MgCl_2) resulted in enhanced yields of ethanol or H_2 . High ethanol yields, high ethanol to acetate (E/A) ratios, and low acetate and H_2 yields were obtained when YE, α -cellulose and CaCl_2 were present at high concentrations, in combination with low concentrations of magnesium. Vitamins were identified as a relatively less influential nutrient, but high concentrations of vitamins supported enhanced yields of acetate and H_2 . High urea and YE in combination with low MgCl_2 concentrations enhanced cellulose utilization per unit mass of cells and cell-specific yields of both ethanol and H_2 . Volumetric yields for ethanol and H_2 were improved by 2.3-fold (76.5 mM) and 2.04-fold (71.22 mmol per liter), respectively, compared with the basic combination. The highest hydrogen yield (1.64 mol/mol glucose) was obtained in the combination with the lowest ethanol yields while the lowest hydrogen yielding combination had the highest ethanol yield of 1.36 mol/mole glucose, representing 68% of the theoretical maximum for ethanol. The culture conditions determined by this study can be optimized further for enhanced production of either ethanol or H_2 by direct cellulose fermentation.

Keywords: *Clostridium thermocellum*; Cellulose fermentation; Growth nutrients; Medium composition; Resolution-IV design

E. Baranitharan, Maksudur R. Khan, D. M. R. Prasad, Jailani Bin Salihon. (¹ Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia). **Bioelectricity Generation from Palm Oil Mill Effluent in Microbial Fuel Cell Using Polyacrylonitrile Carbon Felt as Electrode.** *Water, Air, & Soil Pollution*, 224(2013): 1533

Palm oil mill effluent (POME) is an organic waste material produced at the oil palm mills. In its raw form, POME is highly polluting due to its high content of biological and chemical oxygen demand. In the present paper, POME was treated using double chamber microbial fuel cell with simultaneous generation of electricity. Polyacrylonitrile carbon felt (PACF), a new electrode material was used as electrode throughout the MFC experiments. Various dilutions of raw POME were used to analyze the effect of initial chemical oxygen demand (COD) on MFC power generation, COD removal efficiency and coulombic efficiency. Anaerobic sludge was used as inoculum for all the MFC experiments. Since this inoculum originated from POME, it showed higher potential to generate bioenergy from complex POME. Anaerobic sludge enhanced the power production due to better utilization of substrates by various types of microorganisms present in it. Among the raw POME and different concentrations of POME used, the PACF with raw POME showed the maximum power density and volumetric power density of about 45 mW/m² and 304 mW/m³, respectively, but it showed low coulombic efficiency and low COD removal efficiency of about 0.8 % and 45 %, respectively. The MFC PACF with 1:50 dilution showed higher COD removal efficiency and coulombic efficiency of about 70 % and 24 % but showed low power density and low volumetric power density of about 22 mW/m² and 149 mW/m³, respectively. The formation of biofilm onto the electrode surface has been confirmed from scanning electron microscopy (SEM) experiments. The results confirm that MFC possesses great potential for the simultaneous treatment of POME and power generation using PACF as electrode and also shows that initial COD has great influence on coulombic efficiency, COD removal efficiency and power generation.

Seonghun Kim, Jang Min Park, Chul Ho Kim. (¹ Jeonbuk Branch Institute, Korea Research Institute of Bioscience and Biotechnology, 181 Ipsin-gil, Jeongeup, 580-185, South Korea). **Ethanol Production Using Whole Plant Biomass of Jerusalem Artichoke**

by *Kluyveromyces marxianus* CBS1555. **Applied Biochemistry and Biotechnology, Volume 169(5) (2013): 1531-1545**

Jerusalem artichoke is a low-requirement sugar crop containing cellulose and hemicellulose in the stalk and a high content of inulin in the tuber. However, the lignocellulosic component in Jerusalem artichoke stalk reduces the fermentability of the whole plant for efficient bioethanol production. In this study, Jerusalem artichoke stalk was pretreated sequentially with dilute acid and alkali, and then hydrolyzed enzymatically. During enzymatic hydrolysis, approximately 88 % of the glucan and xylan were converted to glucose and xylose, respectively. Batch and fed-batch simultaneous saccharification and fermentation of both pretreated stalk and tuber by *Kluyveromyces marxianus* CBS1555 were effectively performed, yielding 29.1 and 70.2 g/L ethanol, respectively. In fed-batch fermentation, ethanol productivity was 0.255 g ethanol per gram of dry Jerusalem artichoke biomass, or 0.361 g ethanol per gram of glucose, with a 0.924 g/L/h ethanol productivity. These results show that combining the tuber and the stalk hydrolysate is a useful strategy for whole biomass utilization in effective bioethanol fermentation from Jerusalem artichoke.

David G. Wernick, James C. Liao. (¹Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, 5531 Boelter Hall, 420 Westwood Plaza, Los Angeles, CA, 90095, USA, ² Department of Chemistry and Biochemistry, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, CA, 90095, USA, ³ Institute for Genomics and Proteomics, University of California, Los Angeles, 201 Boyer Hall, 611 Charles E. Young Drive East, Los Angeles, CA, 90095, USA). **Protein-based biorefining: metabolic engineering for production of chemicals and fuel with regeneration of nitrogen fertilizers. Applied Microbiology and Biotechnology, Volume 97 (4) (2013): 1397-1406**

Threats to stable oil supplies and concerns over environmental emissions have pushed for renewable biofuel developments to minimize dependence on fossil resources. Recent biofuel progress has moved towards fossil resource-independent carbon cycles, but environmental issues regarding use of nitrogen fertilizers have not been addressed on a global scale. The recently demonstrated conversion of waste protein biomass into advanced biofuels and renewable chemicals, while recycling nitrogen fertilizers, offers a glimpse of the efforts needed to balance the nitrogen cycle at scale. In general, the catabolism of protein into biofuels is challenging because of physiological regulation and thermodynamic limitations. This conversion became possible with metabolic engineering around ammonia assimilation, intracellular nitrogen flux, and quorum sensing. This review highlights the metabolic engineering solutions in transforming those cellular processes into driving forces for the high yield of chemical products from protein.

Ioannis Dogaris, Diomi Mamma, Dimitris Kekos. (¹Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 9 Iroon Polytechniou Str., Zografou Campus, 15780, Athens, Greece). **Biotechnological production of ethanol from renewable resources by *Neurospora crassa*: an alternative to conventional yeast fermentations? Applied Microbiology and Biotechnology, Volume 97 (4) (2013): 1457-1473**

Microbial production of ethanol might be a potential route to replace oil and chemical feedstocks. Bioethanol is by far the most common biofuel in use worldwide. Lignocellulosic

biomass is the most promising renewable resource for fuel bioethanol production. Bioconversion of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation, and product separation/distillation. Conventional bioethanol processes for lignocellulosics apply commercial fungal cellulase enzymes for biomass hydrolysis, followed by yeast fermentation of resulting glucose to ethanol. The fungus *Neurospora crassa* has been used extensively for genetic, biochemical, and molecular studies as a model organism. However, the strain's potential in biotechnological applications has not been widely investigated and discussed. The fungus *N. crassa* has the ability to synthesize and secrete all three enzyme types involved in cellulose hydrolysis as well as various enzymes for hemicellulose degradation. In addition, *N. crassa* has been reported to convert to ethanol hexose and pentose sugars, cellulose polymers, and agro-industrial residues. The combination of these characteristics makes *N. crassa* a promising alternative candidate for biotechnological production of ethanol from renewable resources. This review consists of an overview of the ethanol process from lignocellulosic biomass, followed by cellulases and hemicellulases production, ethanol fermentations of sugars and lignocellulosics, and industrial application potential of *N. crassa*.

Lakshmi Prasanna, Vincent G. H. Eijnsink, Richard Meadow, Sigrid Gåseidnes. (¹ Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, 1432, Ås, Norway, ² Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432, Ås, Norway). **A novel strain of *Brevibacillus laterosporus* produces chitinases that contribute to its biocontrol potential. *Applied Microbiology and Biotechnology*, Volume 97 (4) (2013): 1601-1611**

A novel strain exhibiting entomopathogenic and chitinolytic activity was isolated from mangrove marsh soil in India. The isolate was identified as *Brevibacillus laterosporus* by phenotypic characterization and 16S rRNA sequencing and designated Lak1210. When grown in the presence of colloidal chitin as the sole carbon source, the isolate produced extracellular chitinases. Chitinase activity was inhibited by allosamidin indicating that the enzymes belong to the family 18 chitinases. The chitinases were purified by ammonium sulfate precipitation followed by chitin affinity chromatography yielding chitinases and chitinase fragments with 90, 75, 70, 55, 45, and 25 kDa masses. Mass spectrometric analyses of tryptic fragments showed that these fragments belong to two distinct chitinases that are almost identical to two putative chitinases, a 89.6-kDa four-domain chitinodextrinase and a 69.4-kDa two-domain enzyme called ChiA1, that are encoded on the recently sequenced genome of *B. laterosporus* LMG15441. The chitinase mixture showed two pH optima, at 6.0 and 8.0, and an optimum temperature of 70 °C. The enzymes exhibited antifungal activity against the phytopathogenic fungus *Fusarium equiseti*. Insect toxicity bioassays with larvae of diamondback moths (*Plutella xylostella*), showed that addition of chitinases reduced the time to reach 50 % mortality upon infection with non-induced *B. laterosporus* from 3.3 to 2.1 days. This study provides evidence for the presence of inducible, extracellular chitinolytic enzymes in *B. laterosporus* that contribute to the strain's antifungal activity and insecticidal activity.

Juan Daniel Rivaldi, Marta Luís C. Sousa Silva, Luis C. Duarte, António E. N. Ferreira, Carlos Cordeiro, Maria das Graças de Almeida Felipe, Ana de Ponces Freire, Ismael Maciel de Mancilha. (¹ Universidade de São Paulo, Escola de Engenharia de Lorena, Lorena, SP, Brazil, ² Universidad Nacional de Asunción, Facultad de Ciencias Químicas, San Lorenzo, Paraguay, ³ Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Lisboa,

Portugal, ⁴Unidade de Bioenergia, LNEG—Laboratório Nacional de Energia e Geologia, Lisboa, Portugal, ⁵Universidade Federal de Viçosa, Departamento de Tecnologia de Alimentos, Viçosa, MG, Brazil). **Metabolism of biodiesel-derived glycerol in probiotic *Lactobacillus* strains. *Applied Microbiology and Biotechnology*, Volume 97 (4) (2013): 1735-1743**

Three probiotic *Lactobacillus* strains, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus delbrueckii*, were tested for their ability to assimilate and metabolize glycerol. Biodiesel-derived glycerol was used as the main carbon and energy source in batch microaerobic growth. Here, we show that the tested strains were able to assimilate glycerol, consuming between 38 and 48 % in approximately 24 h. *L. acidophilus* and *L. delbrueckii* showed a similar growth, higher than *L. plantarum*. The highest biomass reached was 2.11 g L⁻¹ for *L. acidophilus*, with a cell mass yield ($Y_{X/S}$) of 0.37 g g⁻¹. *L. delbrueckii* and *L. plantarum* reached a biomass of 2.06 and 1.36 g L⁻¹. All strains catabolize glycerol mainly through glycerol kinase (EC 2.7.1.30). For these *Lactobacillus* species, kinetic parameters for glycerol kinase showed Michaelis–Menten constant (K_m) ranging from 1.2 to 3.8 mM. The specific activities for glycerol kinase in these strains were in the range of 0.18 to 0.58 U mg protein⁻¹, with *L. acidophilus* ATCC 4356 showing the maximum specific activity after 24 h of cultivation. Glycerol dehydrogenase activity was also detected in all strains studied but only for the reduction of glyceraldehyde with NADPH (K_m for DL-glyceraldehyde ranging from 12.8 to 32.3 mM). This enzyme shows a very low oxidative activity with glycerol and NADP⁺ and, most likely, under physiological conditions, the oxidative reaction does not occur, supporting the assumption that the main metabolic flux concerning glycerol metabolism is through the glycerol kinase pathway.

Sudharshan Sekar, Surianarayanan Mahadevan, Ranganathan Vijayaraghavan, Asit Baran Mandal, D. R. MacFarlane. (¹Thermochemical Lab, Chemical Engineering Department, Central Leather Research Institute (CLRI), Adyar, Chennai, 600020, Tamil Nadu, India, ²School of Chemistry, Monash University, Clayton Campus, Melbourne, VIC, 3800, Australia). **Bioenergetics for the growth of *Staphylococcus lentus* in biocompatible choline salts. *Applied Microbiology and Biotechnology*, Volume 97 (4) (2013): 1767-1774**

Choline-based biocompatible salts were used as “nutrients” for the growth of *Staphylococcus lentus* bacteria. Increase in the growth rate of bacteria was observed, compared to conventional carbon sources. In the case of the ionic liquid, choline lactate, the increase was pronounced. Bacterial growth was correlated with power–time curve in an investigation monitored online by reaction calorimetry. From the power–time curve, three phases of the growth can be distinctly seen. Heat yield coefficients estimated for the growth of *S. lentus* were found to match well with those reported hitherto. A comparative study of heat yields (catabolic) between glucose and choline lactate revealed significant information; the heat yield due to choline lactate ($Y_{Q/S}$) consumption and oxygen ($Y_{Q/O}$) were 23.4 kJ/g and 435 kJ/mol and whereas that for glucose with oxygen were 9.6 kJ/g and 427 kJ/mol, respectively, showing clearly the preferential affinity of choline lactate by the bacteria rather than glucose. This study also established that the use of ionic liquids as nutrients can be monitored using bioreaction calorimetry.

Stefano Mocali, Carlo Galeffi, Elena Perrin, Alessandro Florio, Melania Migliore, Francesco Canganella, Giovanna Bianconi, Elena Di Mattia, Maria Teresa Dell'Abate, Renato Fani. (¹CRA—Agrobiologia and Pedology Research Centre, Piazza D'Azeglio, 30, 50121, Firenze, Italy, ²CRA—Research Centre for the Soil-Plant System, via della Navicella 2, 00184, Roma, Italy, ³Evolutionary Biology Department, University of Florence, via Romana 17-19, 50125, Firenze, Italy, ⁴Department for Innovation in Biological, Agrofood and Forest systems, University of Tuscia, via C. de Lellis, 01100, Viterbo, Italy, ⁵Department of Sciences and Technologies for Agriculture, Forest, Nature and Energy, University of Tuscia, via C. de Lellis, 01100, Viterbo, Italy). **Alteration of bacterial communities and organic matter in microbial fuel cells (MFCs) supplied with soil and organic fertilizer. Applied Microbiology and Biotechnology, Volume 97 (3) (2013): 1299-1315**

The alteration of the organic matter (OM) and the composition of bacterial community in microbial fuel cells (MFCs) supplied with soil (S) and a composted organic fertilizer (A) was examined at the beginning and at the end of 3 weeks of incubation under current-producing as well as no-current-producing conditions. Denaturing gradient gel electrophoresis revealed a significant alteration of the microbial community structure in MFCs generating electricity as compared with no-current-producing MFCs. The genetic diversity of cultivable bacterial communities was assessed by random amplified polymorphic DNA (RAPD) analysis of 106 bacterial isolates obtained by using both generic and elective media. Sequencing of the 16S rRNA genes of the more representative RAPD groups indicated that over 50.4% of the isolates from MFCs fed with S were *Proteobacteria*, 25.1% Firmicutes, and 24.5% *Actinobacteria*, whereas in MFCs supplied with A 100% of the dominant species belonged to γ -*Proteobacteria*. The chemical analysis performed by fractionating the OM and using thermal analysis showed that the amount of total organic carbon contained in the soluble phase of the electrochemically active chambers significantly decreased as compared to the no-current-producing systems, whereas the OM of the solid phase became more humified and aromatic along with electricity generation, suggesting a significant stimulation of a humification process of the OM. These findings demonstrated that electroactive bacteria are commonly present in aerobic organic substrates such as soil or a fertilizer and that MFCs could represent a powerful tool for exploring the mineralization and humification processes of the soil OM.

Nano Biotechnology

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The environmental effects and bioavailability of nanoparticulate iron (Fe) to plants are currently unknown. Here, plant bioavailability of synthesized hematite Fe nanoparticles was evaluated

using *Arabidopsis thaliana* (*A. thaliana*) as a model. Over 56-days of growing wild-type *A. thaliana*, the nanoparticle-Fe and no-Fe treatments had lower plant biomass, lower chlorophyll concentrations, and lower internal Fe concentrations than the Fe-treatment. Results for the no-Fe and nanoparticle-Fe treatments were consistently similar throughout the experiment. These results suggest that nanoparticles (mean diameter 40.9 nm, range 22.3–67.0 nm) were not taken up and therefore not bioavailable to *A. thaliana*. Over 14-days growing wild-type and transgenic (Type I/II proton pump overexpression) *A. thaliana*, the Type I plant grew more than the wild-type in the nanoparticle-Fe treatment, suggesting Type I plants cope better with Fe limitation; however, the nanoparticle-Fe and no-Fe treatments had similar growth for all plant types.

Keywords: *Arabidopsis thaliana*; Bioavailability; Iron limitation; Nanomaterials; Nanoparticles

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This report presents an exhaustive literature review on the toxicity of manufactured ZnO nanoparticles (NPs) to ecological receptors across different taxa: bacteria, algae and plants, aquatic and terrestrial invertebrates and vertebrates. Ecotoxicity studies on ZnO NPs are most abundant in bacteria, and are relatively lacking in other species. These studies suggest relative high acute toxicity of ZnO NPs (in the low mg/l levels) to environmental species, although this toxicity is highly dependent on test species, physico-chemical properties of the material, and test methods. Particle dissolution to ionic zinc and particle-induced generation of reactive oxygen species (ROS) represent the primary modes of action for ZnO NP toxicity across all species tested, and photo-induced toxicity associated with its photocatalytic property may be another important mechanism of toxicity under environmentally relevant UV radiation. Finally, current knowledge gaps within this area are briefly discussed and recommendations for future research are made.

Keywords: ZnO nanoparticles; Ecotoxicity; Particle dissolution; Reactive oxygen species; Photo-induced toxicity

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Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. This biogenic reduction of metal ion to base metal is quite rapid, readily conducted at room temperature and pressure, and easily scaled up. Synthesis mediated by plant extracts is environmentally benign. The reducing agents involved include the

various water soluble plant metabolites (e.g. alkaloids, phenolic compounds, terpenoids) and co-enzymes. Silver (Ag) and gold (Au) nanoparticles have been the particular focus of plant-based syntheses. Extracts of a diverse range of plant species have been successfully used in making nanoparticles. In addition to plant extracts, live plants can be used for the synthesis. Here we review the methods of making nanoparticles using plant extracts. Methods of particle characterization are reviewed and potential applications of the particles in medicine are discussed.

Keywords: Metal nanoparticles; Nanogold; Nanosilver; Plant extracts; Drug delivery; Nanoparticle biosynthesis; Green synthesis

Haiying Li, Andrew Turner, Murray T. Brown. (¹School of Geography, Earth and Environmental Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA, UK, ²School of Marine Science and Engineering, University of Plymouth, Drake Circus, Plymouth, PL4 8AA, UK). **Accumulation of Aqueous and Nanoparticulate Silver by the Marine Gastropod *Littorina littorea*. Water, Air, & Soil Pollution, Volume 224 (2012): 1354**

The accumulation of Ag by the marine herbivorous gastropod, *Littorina littorea*, has been studied in a series of exposures in which the metal was added in aqueous form and as nanoparticles, both in the presence and absence of contaminated algal food (*Ulva lactuca*). Significant accumulation occurred in the gill, kidney, stomach and visceral mass when the snail was exposed to aqueous Ag in the absence of food. Despite the consumption of *U. lactuca* that had been previously contaminated by Ag, no accumulation was observed from the dietary route. When added as nanoparticles, accumulation of Ag was only measured in the head and gill and only in the absence of contaminated food. These observations suggest that Ag is most bioavailable to *L. littorina* when in true solution and that Ag measured in external tissues of the snail following exposure to nanoparticles arises from some physical association that does not result in significant transfer of the metal to internal organs.

Prem Lal Kashyap, Sudheer Kumar, Alok Kumar Srivastava, Arun Kumar Sharma. (¹National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, 275101, Uttar Pradesh, India). **Myconanotechnology in agriculture: a perspective. World Journal of Microbiology and Biotechnology, Volume 29(2) (2013): 191-207**

Myconanotechnology is an emerging field, where fungi can be harnessed for the synthesis of nanomaterials or nanostructures with desirable shape and size. Though myconanotechnology is in its infancy, potential applications provide exciting waves of transformation in agriculture and fascinate microbiologists and other researchers to contribute in providing incremental solutions through green chemistry approaches for advancing food security. In this article, we provide a brief overview of the research efforts on the mycogenic synthesis of nanoparticles with particular emphasis on mechanisms and potential applications in agriculture and allied sectors.

J. C. Tarafdar¹, Shikha Sharma² and Ramesh Raliya^{1*} (¹Central Arid Zone Research Institute, Jodhpur-342003, India, ²Panjab Agricultural University, Ludhiana-141001, India. *Corresponding author. E-mail: rameshraliya@gmail.com). **Nanotechnology: Interdisciplinary science of applications. African Journal of Biotechnology, Vol. 12(3) (2013): 219-226**

Nanotechnology is the study of particle sizes between 1 and 100 nanometers at least at one dimension. Particle size reduced to nanometer length scale exhibit more surface area to volume size ratio and showing unusual properties makes them enable for systematic applications in engineering, biomedical, agricultural and allied sectors. Nanomaterial can create from bottom up or top down approaches using physical, chemical and biological mode of synthesis.

Keywords: Nanotechnology, nanomaterial, nanobiotechnology, nanotech-applications.

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Nanoparticles offer the potential to improve environmental treatment technologies due to their unique properties. Adsorption of metal ions (Pb(II), Cd(II), Cu(II), Zn(II)) to nanohematite was examined as a function of sorbent concentration, pH, temperature, and exhaustion. Adsorption experiments were conducted with 0.05, 0.1, and 0.5 g/L nanoparticles in a pH 8 solution and in spiked San Antonio tap water. The adsorption data showed the ability of nanohematite to remove Pb, Cd, Cu, and Zn species from solution with adsorption increasing as the nanoparticle concentration increased. At 0.5 g/L nanohematite, 100 % Pb species adsorbed, 94 % Cd species adsorbed, 89 % Cu species adsorbed and 100 % Zn species adsorbed. Adsorption kinetics for all metals tested was described by a pseudo second-order rate equation with lead having the fastest rate of adsorption. The effect of temperature on adsorption showed that Pb(II), Cu(II), and Cd(II) underwent an endothermic reaction, while Zn(II) underwent an exothermic reaction. The nanoparticles were able to simultaneously remove multiple metals species (Zn, Cd, Pb, and Cu) from both a pH 8 solution and spiked San Antonio tap water. Exhaustion experiments showed that at pH 8, exhaustion did not occur for the nanoparticles but adsorption does decrease for Cd, Cu, and Zn species but not Pb species. The strong adsorption coupled with the ability to simultaneously remove multiple metal ions offers a potential remediation method for the removal of metals from water.

Alexandre G. Rodrigues, Liu Yu Ping, Priscyla D. Marcato, Oswaldo L. Alves, Maria C. P. Silva, Rita C. Ruiz, Itamar S. Melo, Ljubica Tasic, Ana O. De Souza. (¹Laboratório de Bioquímica e Biofísica, Instituto Butantan, Av. Vital Brasil, 1500,05503-900, São Paulo, São Paulo, Brazil, ²IQ, Universidade Estadual de Campinas, São Paulo, Brazil, ³ICB, Universidade de São Paulo, São Paulo, Brazil, ⁴Laboratório de Bacteriologia, Instituto Butantan, São Paulo, Brazil, ⁵Embrapa Meio Ambiente, São Paulo, Brazil). **Biogenic antimicrobial silver nanoparticles produced by fungi. Applied Microbiology and Biotechnology, Volume 97(2) (2013): 775-782**

Aspergillus tubingensis and *Bionectria ochroleuca* showed excellent extracellular ability to synthesize silver nanoparticles (Ag NP), spherical in shape and 35 ± 10 nm in size. Ag NP were characterized by transmission electron microscopy, X-ray diffraction analysis, and photon correlation spectroscopy for particle size and zeta potential. Proteins present in the fungal filtrate

and in Ag NP dispersion were analyzed by electrophoresis (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Ag NP showed pronounced antifungal activity against *Candida* sp, frequently occurring in hospital infections, with minimal inhibitory concentration in the range of 0.11–1.75 µg/mL. Regarding antibacterial activity, nanoparticles produced by *A. tubingenensis* were more effective compared to the other fungus, inhibiting 98.0 % of *Pseudomonas aeruginosa* growth at 0.28 µg/mL. *A. tubingenensis* synthesized Ag NP with surprisingly high and positive surface potential, differing greatly from all known fungi. These data open the possibility of obtaining biogenic Ag NP with positive surface potential and new applications.

Madan Lal Verma, Colin J. Barrow, Munish Puri. (¹Centre for Chemistry and Biotechnology, Deakin University, Victoria, 3217, Australia, ²Centre for Biotechnology and Interdisciplinary Sciences (BioDeakin), Geelong Technology Precinct, Waurn Ponds, Deakin University, Victoria, 3217, Australia, ³Centre for Biotechnology and Interdisciplinary Sciences BioDeakin, Deakin University, Victoria, Australia). **Nanobiotechnology as a novel paradigm for enzyme immobilisation and stabilisation with potential applications in biodiesel production. Applied Microbiology and Biotechnology, Volume 97(1) (2013): 23-39**

Nanobiotechnology is emerging as a new frontier of biotechnology. The potential applications of nanobiotechnology in bioenergy and biosensors have encouraged researchers in recent years to investigate new novel nanoscaffolds to build robust nanobiocatalytic systems. Enzymes, mainly hydrolytic class of enzyme, have been extensively immobilised on nanoscaffold support for long-term stabilisation by enhancing thermal, operational and storage catalytic potential. In the present report, novel nanoscaffold variants employed in the recent past for enzyme immobilisation, namely nanoparticles, nanofibres, nanotubes, nanopores, nanosheets and nanocomposites, are discussed in the context of lipase-mediated nanobiocatalysis. These nanocarriers have an inherently large surface area that leads to high enzyme loading and consequently high volumetric enzyme activity. Due to their high tensile strengths, nanoscale materials are often robust and resistant to breakage through mechanical shear in the running reactor making them suitable for multiple reuses. The optimisation of various nanosupports process parameters, such as the enzyme type and selection of suitable immobilisation method may help lead to the development of an efficient enzyme reactor. This might in turn offer a potential platform for exploring other enzymes for the development of stable nanobiocatalytic systems, which could help to address global environmental issues by facilitating the production of green energy. The successful validation of the feasibility of nanobiocatalysis for biodiesel production represents the beginning of a new field of research. The economic hurdles inherent in viably scaling nanobiocatalysts from a lab-scale to industrial biodiesel production are also discussed.

S. Roy Choudhury*, **A. Goswami.** (¹Biological Sciences Division, Indian Statistical Institute, Kolkata, India. *Correspondence :Samrat Roy Choudhury, Biological Sciences Division, Indian Statistical Institute, 203, B. T Road, Kolkata-700108, India. E-mail: samratroychoudhury@gmail.com). **Supramolecular reactive sulphur nanoparticles: a novel and efficient antimicrobial agent. Journal of Applied Microbiology, Volume 114(1): 1–10**

Antimicrobial resistance continues to be an inexorable threat for the biomedical and biochemical researchers. Despite the novel discoveries in drug designing and delivery, high-

throughput screening and surveillance data render the prospects for new antimicrobial agents as bleak as ever. The advent of nanotechnology, however, strengthens pharmacology by offering effective therapeutics to treat this aforementioned problem. Several nanoparticles of the known elements have already been reported for their antimicrobial efficacy. Nanosized fabrication of elemental sulphur with suitable surface modifications offers to retrieve the use of sulphur (man's oldest known ecofriendly microbicide) as a potential antimicrobial agent. Sulphur nanoparticles (SNPs) are effective against both conventionally sulphur-resistant and sulphur-susceptible microbes (fungi and bacteria). Moreover, biocompatible polymers present on the surface of SNPs minimize toxicity during application. Here, we focus on various aspects of physicochemical features of SNPs and their biochemical interactions with microbes. The present review also illustrates the effects of SNPs on plants and animals in terms of cytotoxicity and biocompatibility.

Keywords: antibacterial; antifungal; biocompatibility; cost-effectiveness; sulphur nanoparticles

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Engineered metal nanoparticles are increasingly used in consumer products, in part as additives that exhibit advantageous antimicrobial properties. Conventional nanoparticle susceptibility testing is based largely on determination of nontemporal growth profiles such as measurements of inhibition zones in common agar diffusion tests, counting of colony-forming units, or endpoint or regular-interval growth determination via optical density measurements. For better evaluation of the dynamic effects from exposure to nanoparticles, a cultivation-based assay was established in a 96-well format and adapted for time-resolved testing of the effects of nanoparticles on micro-organisms.

The modified assay allowed simultaneous cultivation and on-line analysis of microbial growth inhibition. The automated high-throughput assay combined continuous monitoring of microbial growth with the analysis of many replicates and was applied to *Cupriavidus necator* H16 test organisms to study the antimicrobial effects of spherical silver [Ag(0)] nanoparticles (primary particle size distribution $D_{90} < 15$ nm). Ag(0) concentrations above $80 \mu\text{g ml}^{-1}$ resulted in complete and irreversible inhibition of microbial growth, whereas extended lag phases and partial growth inhibition were observed at Ag(0) concentrations between 20 and $80 \mu\text{g ml}^{-1}$. Addition of Ag(0) nanoparticles at different growth stages led to either complete inhibition (addition of $40 \mu\text{g ml}^{-1}$ Ag(0) from 0 h to 6 h) or resulted in full recovery ($40 \mu\text{g ml}^{-1}$ Ag(0) addition ≥ 9 h).

Contrary to the expected results, our data indicate growth stimulation of *C. necator* at certain Ag(0) nanoparticle concentrations, as well as varying susceptibility to nanoparticles at different growth stages.

These results underscore the need for time-resolved analyses of microbial growth inhibition by Ag(0) nanoparticles. Due to the versatility of the technique, the assay will likely complement existing microbiological methods for cultivation and diagnostics of microbes, in addition to tests of other antimicrobial nanoparticles.

Keywords: Ag; antifouling; antimicrobial nanoparticles; biocidal nanoparticles; engineered metal nanoparticles; growth inhibition; growth kinetics; silver; surface

Huafeng Chen, Zhuangqiang Gao, Yuling Cui, Guonan Chen, Dianping Tang. (Key Laboratory of Analysis and Detection for Food Safety (Ministry of Education and Fujian Province), Department of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350108, PR China). Nanogold-enhanced graphene nanosheets as multienzyme assembly for sensitive detection of low-abundance proteins. Biosensors and Bioelectronics, Volume 44(2013): 108–114

A new electrochemical immunosensing protocol was designed for detection of carcinoembryonic antigen (CEA, as a model protein) by using graphene-carried poly(*o*-phenylenediamine)/gold hybrid nanosheets (GNPGs) as signal tags on the hierarchical dendritic gold microstructures (HDGMs)-modified glassy carbon electrode. To prepare the signal tags, poly(*o*-phenylenediamine) molecules were initially immobilized on the surface of graphene nanosheets via the π -stacking interaction. Then gold nanoparticles were assembled onto the poly(*o*-phenylenediamine)-modified graphene nanosheets, which were used for the labeling of anti-CEA detection antibodies and horseradish peroxidase (HRP). The as-prepared GNPGs were characterized by using atomic force microscopy (AFM), transmission electron microscopy (TEM) and UV–vis absorption spectroscopy. The assay was carried out with a sandwich-type immunoassay format in pH 5.5 acetic acid-buffered saline solutions containing $2.5 \text{ mmol L}^{-1} \text{ H}_2\text{O}_2$. Under optimal conditions, the electrochemical immunoassay exhibited a wide dynamic range of $0.005\text{--}80 \text{ ng mL}^{-1}$ toward CEA standards with a low detection limit of 5.0 pg mL^{-1} . Intra- and inter-assay coefficients of variation were less than 11.5%. No significant difference at the 0.05 significance level was encountered in the analysis of 6 clinical serum specimens and 6 spiked blank new born cattle serum specimens between the developed immunoassay and commercially available electrochemiluminescent (ECL) method for the detection of CEA.

Keywords: Electrochemical immunosensor; Multifunctional graphene nanosheets; Redox-active nanolabels; Multienzyme assembly; Carcinoembryonic antigen; Low-abundance proteins

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biosensor for ascorbic acid based on carbon-supported PdNinanoparticles. Biosensors and Bioelectronics, Volume 44(2013):183–190

Carbon-supported PdNi nanoparticles (PdNi/C) were synthesized using a novel synthetic route, and characterized by transmission electron microscopy (TEM) and X-ray diffractometry (XRD). The overall metallic content (Pd+Ni) was 10% (w/w) and uniformly distributed in the carbon black (90%) matrix. The electrocatalytic performance of the PdNi/C modified glassy carbon electrode (GCE) was investigated for ascorbic acid (AA) oxidation, and showed better catalytic activity than an equal amount of commercially available palladium carbon catalyst. The oxidation potential of AA was negatively shifted to -0.05 V. The biosensor tolerated a wide linear concentration range for AA, from 1.0×10^{-5} M to 1.8×10^{-3} M ($R=0.9973$), with a detection limit of $0.5 \mu\text{M}$ ($S/N=3$). Our results demonstrate that PdNi/C nanomaterials have excellent AA sensing capability, including a fast response time, high reproducibility and stability, with great promise in the quantification of AA in real samples. These qualities make the Pd-based bimetallic catalysts promising candidates for amperometric sensing.

Keywords: Pd; Ni; Ascorbic acid; Electrochemical sensor

Liyan Bi^a, Yanying Rao^a, Qin Tao^a, Jian Dong^a, Ting Su^{a, b}, Fangjing Liu^a, Weiping Qian^a. (^a State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, PR China, ^b School of Chemistry and Chemical Engineering, Southeast University, Nanjing 210096, PR China). **Fabrication of large-scale gold nanoplate films as highly active SERS substrates for label-free DNA detection. Biosensors and Bioelectronics, Volume 43 (2012):193–199**

We introduce a simple but robust label-free method to detect DNA based on large-scale gold nanoplate (GNP) films with tunable localized surface plasmon resonance (LSPR) and highly surface-enhanced Raman scattering (SERS) activity. The common probe molecule, Nile Blue A sulfate (NBA) is used for testing the SERS activity of the GNP films at very low concentrations. It is found that the SERS properties are highly dependent on the edge lengths of gold nanoplate and gold nanoplate density in the films. Multiple-layer GNP films which are constructed by gold nanoplate with an edge length of 134 ± 6 nm have the density of 916 ± 40 GNPs/GNPs/spot. It shows the highest signal intensity with SERS enhancement factor (EF) as high as 5.4×10^7 and also has excellent stability, reproducibility and repeatability. The optimized SERS-active substrate with the largest enhancement ability could be used to detect double-strand DNA without a dye label, and the detection limit is down to 10^{-6} mg/mL.

Keywords: GNP films; Localized surface plasmon resonance (LSPR); Surface enhanced Raman scattering (SERS); Label-free DNA detection

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Vilnius, Lithuania). Magnetic gold nanoparticles in SERS-based sandwich immunoassay for antigen detection by well oriented antibodies. Biosensors and Bioelectronics, Volume 43 (2013): 281–288

The aim of the study was to develop an indirect, robust and simple in application method for the detection of bovine leukemia virus antigen *gp51*. Surface-enhanced Raman scattering (SERS) was applied as detection method. Magnetic gold nanoparticles (MNP-Au) modified by antibodies in oriented or random manner were used for the binding of *gp51*. The high performance liquid chromatography analysis indicated that the best antibody immobilization and antigen capturing efficiency was achieved using fragmented antibodies obtained after reduction of intact antibodies with dithiothreitol. In order to increase efficiency and sensitivity of immunoassay Raman labels consisting of gold nanorods coated by 5-thio-nitrobenzoic acid layer with covalently bounded antibodies have been constructed. The LOD and LOQ of the proposed immunoassay for antigen *gp51* detection were found to be $0.95 \mu\text{g mL}^{-1}$ and $3.14 \mu\text{g mL}^{-1}$, respectively. This immunoassay was successfully applied for the detection of *gp51* in milk samples in a rapid, reliable and selective manner. We believe that the proposed SERS-based immunoassay format can be applied for the detection of other proteins.

Keywords: SERS; Immunoassay; Magnetic gold nanoparticles; Oriented immobilization; BLV

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
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