



ENVIS CENTER



on

ENVIRONMENTAL BIOTECHNOLOGY

Abstract Vol. XXIII

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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 sixteen 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 23rd publication of Abstract Volume of this ENVIS Centre. This contains the abstracts of research papers collected from the various areas of Environmental Biotechnology from different journals published in last six months upto December, 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 16 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 disciple of nanotechnology. This technical approach to biology allows scientists to imagine and create systems that can be used for biological research

Biomimicry: Biomimicry is an applied science that derives inspiration for solutions to human problems through the study of natural designs, systems and processes. Biomimicry on the other hand, which is not a science, is a more subtle way which we can benefit from nature. It is the modern, often high tech, equivalent of the historical practices of emulating nature. . The science of biomimicry is a newly developing field but the application of biomimicry has been around since the beginning of man. The biomimetic technologies (flight controls, bio-robotics, ventilation systems, etc.) and potential technologies (fin geometry, nacre materials, etc.) improve performance. The use of biomimicry as an approach to sustainable engineering, specifically the environmental components.

ABBREVIATIONS USED IN ADDRESSES AND CITED JOURNALS

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

John Chételat^{1, 2}, Louise Cloutier¹ and Marc Amyot¹. (¹Groupe de recherche interuniversitaire en limnologie, Département de sciences biologiques, Université de Montréal, Montreal, QC, H3C 3J7, Canada, ²Present address: Environment Canada, National Wildlife Research Centre, Carleton University, Ottawa, ON, K1A 0H3, Canada. Email: john.chetelat@ec.gc.ca). An investigation of enhanced mercury bioaccumulation in fish from offshore feeding. *Ecotoxicology*, Volume 22(6) (2013): 1020-1032

We investigated the dietary pathways of mercury transfer in the food web of Morency Lake (Canada) to determine the influence of carbon source and habitat use on mercury bioaccumulation in fish. Whole-body concentrations of methylmercury (MeHg) were significantly different in four fish species (white sucker, brown bullhead, pumpkinseed and smallmouth bass) and increased with both trophic position and greater feeding on offshore (versus littoral) carbon. An examination of fish gut contents and the depth distribution of invertebrates in Morency Lake showed that smallmouth bass and brown bullhead were supplementing their littoral diet with the consumption of either opossum shrimp (*Mysis diluviana*) or profundal amphipods in offshore waters. The zooplanktivore *Mysis* had significantly higher MeHg concentrations than zooplankton and benthic invertebrates, and it was an elevated source of MeHg to smallmouth bass. In contrast, profundal amphipods consumed by brown bullhead did not have higher MeHg concentrations than littoral amphipods. Instead, partitioning of benthic invertebrate resources likely explains the greater MeHg bioaccumulation in brown bullhead, associated with offshore feeding of amphipods. White sucker and brown bullhead had a similar trophic position but white sucker consumed more chironomids, which had one-third the MeHg concentration of amphipods. Our findings suggest that offshore feeding in a lake can affect fish MeHg bioaccumulation via two different processes: (1) the consumption of MeHg-enriched pelagic prey, or (2) resource partitioning of benthic primary consumers with different MeHg concentrations. These observations on the mechanisms of habitat-specific bioaccumulation highlight the complexity of MeHg transfer through lake food webs.

Keywords: Mercury Resource partitioning Pelagic Benthic *Mysis*

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Nine-banded armadillos (*Dasypus novemcinctus*) are widespread and abundant New World mammals with a lifestyle that entails prolonged, intimate contact with soils. Thus, armadillos would seem a promising candidate as a sentinel species to monitor chemical contamination in terrestrial ecosystems. Surprisingly, there have been virtually no toxicology studies on armadillos. Here, we provide the first analysis of metal contaminants for wild armadillos. Liver tissues were obtained from 302 armadillos collected at 6 sites in Georgia and Florida, USA that varied in their extent of human disturbance, from rural pine plantations to highly modified military/space installations. Data were stratified by age (juvenile and adult), sex, and site.

Temporal (yearly) variation was examined at two of the sites that were sampled over three consecutive years. Concentrations of aluminum, cadmium, copper, nickel, lead, and zinc were measured in liver samples from each site. Although reference levels are not available for armadillos, accumulated metal concentrations were comparable to those reported for other mammals. We found no evidence of sex or age differences in the concentrations of any metal, except for Cd (age) and Pb (sex and age). However, concentrations of most metals varied substantially across sites and over time. Finally, concentrations of many metals were positively correlated with one another, suggesting that they likely co-occurred in some areas. Collectively, this study indicates the utility of armadillos as a sentinel species for studies of metal contamination in terrestrial systems, and highlights the need for further studies of other toxicants in these animals.

Keywords: Aluminum Armadillo Cadmium Copper *Dasyus novemcinctus* Nickel Lead Zinc

M. D'Ambrosio¹, S. C. Marques², U. M. Azeiteiro³, M. A. Pardal², E. Pereira⁴, A. C. Duarte⁴ and P. G. Cardoso¹. (¹Institute of Marine Research (IMAR), Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal, ²Centre for Functional Ecology (CFE), Department of Life Sciences, University of Coimbra, PO Box 3046, 3001-401 Coimbra, Portugal, ³Centre for Functional Ecology (CFE), Department of Sciences and Technology, Universidade Aberta, 4200-055 Oporto, Portugal, ⁴Centre for Environmental and Marine Studies (CESAM), Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal. Email: gcardoso@ci.uc.pt). Mercury bioaccumulation and the population dynamics of *Mesopodopsis slabberi* (Crustacea: Mysidacea) along a mercury contamination gradient. *Ecotoxicology*, Volume 22(8) (2013): 1278-1288

The mercury bioaccumulation and population dynamics of the mysid *Mesopodopsis slabberi* was assessed along a mercury gradient in Ria de Aveiro (Portugal). *M. slabberi* is one of the most important mysid species in European temperate coastal shallow waters playing a key ecological role. Nevertheless, no references were found concerning the possible consequences of the Hg on the trophodynamics of these coastal ecosystems. *M. slabberi* showed a clear bioaccumulation along the Hg gradient and through life, with mature females reaching the highest concentrations. In terms of population structure, higher densities and biomasses of *M. slabberi* were assessed in the most contaminated areas contrarily to the least polluted areas. Despite the mercury accumulation in its tissues no strong negative effects on the structure and population dynamics of the species were observed. However, mysids might be important in the transfer of metals from the sediments and zooplankton to higher trophic levels such as fishes, most of them with commercial interest.

Keywords: Mercury contamination Mysids *Mesopodopsis slabberi* Bioaccumulation Biomagnification Population dynamics Life span

Aleksandra Nadgórska-Socha¹, Bartłomiej Ptasieński¹ and Andrzej Kita². (¹Department of Ecology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, ²Analytical Spectroscopy Research Group, Institute of Chemistry, University of Silesia, Szkolna 7, 40-007 Katowice, Poland. Email: aleksandra.nadgorska-socha@us.edu.pl). Heavy metal bioaccumulation and antioxidative responses in *Cardaminopsis arenosa* and *Plantago lanceolata* leaves from metalliferous and non-metalliferous sites: a field study. *Ecotoxicology*, Volume 22(9) (2013): 1422-1434

The purpose of this study was to determine the concentrations of heavy metals (cadmium, lead, zinc, copper, iron and manganese) in soil, their bioavailability and bioaccumulation in plants leaves. This study also examined their influences on the antioxidant response of the plants *Cardaminopsis arenosa* and *Plantago lanceolata* grown in metal-contaminated and non-contaminated soils. The activities of guaiacol peroxidase and superoxide dismutase and the levels of antioxidants such as glutathione, proline and non-protein thiols were measured. Concentrations of the examined metals were several to thousands of times lower in the potentially bioavailable fraction than in the acid-extracted fraction of the soil. Similar mode of antioxidant responses in plant leaves of metalliferous populations indicates the tolerance of plants towards heavy metals. However POD and GSht had a particularly strong role in defense reactions, as their increase was the most common reaction to heavy metal contamination. The levels of Zn, Cd and Pb in the leaves of *C. arenosa* better reflected metal concentrations in the metalliferous and non-metalliferous soil than the determined metal concentrations in *P. lanceolata*. Bioaccumulated Zn, Cd and Pb concentrations were above or in the ranges mentioned as toxic for plant tissues and therefore the studied plants have potential for use in phytostabilization.

Keywords: Heavy metals Antioxidants Antioxidant enzymes *Cardaminopsis arenosa* *Plantago lanceolata*

Nicola Stromsoe^a, J. Nikolaus Callow^b, Hamish A. McGowan^a, Samuel K. Marx^{c,d}. (^a Climate Research Group, School of Geography, Planning and Environmental Management, The University of Queensland, St Lucia, QLD 4072, Australia, ^b School of Earth and Environment, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia, ^c GeoQuEST, School of Earth and Environment Sciences, The University of Wollongong, Wollongong, NSW 2522, Australia, ^d Wollongong Isotope Geochronology Laboratory, School of Earth & Environmental Sciences, University of Wollongong, Wollongong, NSW 2522, Australia). Attribution of sources to metal accumulation in an alpine tarn, the Snowy Mountains, Australia. *Environmental Pollution*, Volume 181(2013) : 133–143

This study analyses 1800 years of heavy metal accumulation in a remote alpine lake experiencing long-range atmospheric contamination and additional inputs of Ag from cloud seeding. In comparison to previous work undertaken on peats, lake sediments show limited post-industrial metal enrichment with enrichment factors of Ag: 1.3, Pb: 1.3, Zn: 1.1, Cu: 1.2 compared to Ag: 2.2, Pb: 3.3, Zn: 2.1, Cu: 4.1 for peat. We show this to be the result of substantial fluvial lithogenic flux of metals (92–97% of total metal flux) to the lake. Total annual metal flux to the lake ranges from: Ag: 4–12 ng/cm²/yr to Zn: 3 383–11 313 ng/cm²/yr. As a result, any contribution of cloud seeding to additional enrichment of Ag in lake sediments is considered negligible. Results show that metal enrichment is not necessarily ubiquitous through a landscape. This has implications for predicting the impacts of atmospheric metal pollution to complex environmental systems.

Keywords: Lakes; Metals; Atmospheric pollution; Cloud seeding

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Meknes, Morocco. Correspondence: Naima EL Ghachtouli, Microbial Biotechnology Laboratory, Sidi Mohammed Ben Abdellah University, Faculty of Sciences and Techniques, Route Immouzer, P.O. Box 2202, 30000 Fez, Morocco. E-mail: naima.elghachtouli@usmba.ac.ma). **Bioaugmentation of chromium-polluted soil microcosms with *Candida tropicalis* diminishes phytoavailable chromium. Journal of Applied Microbiology, Volume 115(3) (2013): 727–734**

Localization of Cr(VI) removal activity in *Candida tropicalis* strain and the study of its Cr(VI) removal capacity in soil.

Candida tropicalis strain HE650140 showed a remarkable capacity to completely reduce 50 mg l⁻¹ of Cr(VI) in 48 h under aerobic conditions; however, a small change in total content of chromium in the culture liquid was detected, which can be explained by the formation of Cr(III). Fractionation of the cells showed that chromium removal activity was present in both the cytoplasm and membrane. The bioaugmentation of Cr(VI)-contaminated soil microcosms by live and dead biomass showed that yeast inoculation diminishes phytoavailable chromium from soils, improving different growth parameters of clover.

The Cr(VI) removal activity was found in both cytoplasmic and membrane fractions. Both live and dead biomass of *C. tropicalis* were capable to reduce Cr(VI) in the soil and limit the toxicity of this metal to clover seedlings.

This study is one of the few documents that present the ability of dead yeast to limit phytoavailability of Cr(VI) from soil. This is of great significance in bioremediation of Cr(VI)-contaminated soil.

Keywords: bioaugmentation; bioremediation; *Candida tropicalis* ; cell fractionation; Cr(VI) removal; soil

Bioremediation

M. Petriccione¹, D. Di Patre¹, P. Ferrante², S. Papa³, G. Bartoli³, A. Fioretto³ and M. Scortichini¹. (¹Consiglio per la Ricerca e la Sperimentazione in Agricoltura — Fruit Trees Unit Research, Via Torrino 3, 81100 Caserta, Italy, ²Consiglio per la Ricerca e la Sperimentazione in Agricoltura — Fruit Crops Research Centre, Via di Fioranello 52, 00134 Roma, Italy, ³Life Science Department, Second University of Naples, Via Vivaldi 43, 81100 Caserta, Italy. Email: milena.petriccione@entecra.it). **Effects of *Pseudomonas fluorescens* Seed Bioinoculation on Heavy Metal Accumulation for *Mirabilis jalapa* Phytoextraction in Smelter-Contaminated Soil. Water, Air, & Soil Pollution, 224(2013): 1645**

Some *Pseudomonas fluorescens* strains, consistently isolated from the rhizosphere of wild plants grown in a soil that was highly polluted with illegal waste of smelter residues, were utilised for *Mirabilis jalapa* seed bioinoculation to verify their effects on seed germination and on promoting a higher heavy metal accumulation in the plant rhizosphere and/or uptake in the leaves. The high content of heavy metals in the soil induced a decrease in either the leaf dry weight or photosynthetic pigment concentration during all vegetative phase of *M. jalapa*.

Bioinoculation with *P. fluorescens* strains significantly increased the germination of seeds and the root length in the contaminated soil. In some bacterial strain/seed combination, bioinoculation significantly increased the accumulation of heavy metals in *M. jalapa* rhizosphere. For Cd, the concentration of this metal in the rhizospheres of bioinoculated plants ranged from 270 to 910 $\mu\text{g g}^{-1}$ of dry weight compared with 200 $\mu\text{g g}^{-1}$ of dry weight for the non-coated plants. Two *P. fluorescens* strains, AA27 and MO49, which were isolated from *Artemisia annua* and *Melilotus officinalis*, respectively, induced a significantly higher rhizosphere availability also for Cr, Cu, Ni and Zn. However, despite the relevant accumulation of the heavy metals in the plant rhizosphere, generally the metal uptake into the leaves was rather low. Both analysis of variance and principal component analysis confirmed this finding. However, one *P. fluorescens* strain, CD1, which was isolated from the multi-metal accumulator *Cynodon dactylon*, significantly promoted the *M. jalapa* leaf uptake for Cr, Cu and Zn. The plant metal uptake assessment, confirmed the per se capability of *M. jalapa* to effectively uptake Cd (30 %) and Cu (12.72 %) from the rhizosphere to the leaves, whereas the uptake for the other metals was low: Ni (2.66 %), Zn (2.46 %), Cr (1.75 %), Pb (0.73 %).

Keywords: Bacteria-assisted phytoremediation, Soil pollution, PGPR Fluorescent pseudomonads, Hyperaccumulator plants

Olushola S. Ayanda¹, Olalekan S. Fatoki¹, Folahan A. Adekola² and Bhekumusa J. Ximba¹. (¹Department of Chemistry, Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 1906, Bellville, South Africa, ²Department of Chemistry, University of Ilorin, P.M.B 1515, Ilorin, Nigeria. Email: osayanda@gmail.com). **Remediation of Tributyltin Contaminated Seawater by Adsorption Using nFe₃O₄, Activated Carbon and nFe₃O₄/Activated Carbon Composite Material. Water, Air, & Soil Pollution, 224(2013): 1684**

The remediation of tributyltin (TBT) by adsorption onto nFe₃O₄, activated carbon and nFe₃O₄/activated carbon composite material as a function of adsorbent dose, contact time, pH, stirring speed, initial TBT concentration and temperature was studied. The effect of temperature on kinetics and equilibrium of TBT sorption on the precursors and the composite was thoroughly examined. The adsorption kinetics is well fitted using a pseudo-second-order kinetic model, and the adsorption isotherm data of nFe₃O₄, activated carbon could be described by the Freundlich isotherm model whereas nFe₃O₄/activated carbon composite could be described by the Freundlich and Dubinin–Radushkevich isotherm models. Thermodynamic parameters (i.e. change in the free energy (ΔG°), the enthalpy (ΔH°) and the entropy (ΔS°)) were also evaluated. The overall adsorption process was endothermic and spontaneous in nature. The results obtained also showed that 99.9, 99.7 and 80.1 % TBT were removed from contaminated natural seawater by nFe₃O₄/activated carbon composite, activated carbon and nFe₃O₄, respectively.

Keywords: Tributyltin Adsorption, Nano-iron (III) oxide, Activated carbon Composite GC–FPD

M. Wilschut¹, P.A.W. Theuws², I. Duchhart. (Department of Landscape Architecture, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands). **Phytoremediative urban design: Transforming a derelict and polluted harbour area into a green and productive neighbourhood. Environmental Pollution, Volume 183(2013) : 81–88**

Many urban areas are polluted by industrial activities and waste disposal in landfills. Since conventional soil remediation techniques are costly and unsustainable, phytoremediation might offer an alternative. In this article, we explore how phytoremediation can be integrated into the transformation of urban post-industrial areas, while improving public space. Buiksloterham, a polluted and deprived industrial area in Amsterdam, serves as case study. Buiksloterham is polluted with heavy metals, with Zinc (Zn) concentrations being the highest. A regression-model for Alpine Pennycress (*Thlaspi caerulescens*) is used to estimate the time needed to remediate the site. This reveals a conflict in time between remediation and urban development. A research by design experiment shows how to overcome this conflict by dealing with polluted soil innovatively while emphasizing spatial and aesthetic qualities of the phytoremediation plant species. The resulting landscape framework integrates phytoremediation with biomass production and gives new ecological, economic and social value to Buiksloterham.

Keywords: Phytoremediation; Post-industrial; Research by design; Urban transformation

Vishal Verma^a, Roberto Rico-Martinez^b, Neel Kotra^a, Corey Rennolds^c, Jiumeng Liu^a, Terry W. Snell^c, Rodney J. Weber^a. (^a School of Earth and Atmospheric Sciences, Georgia Institute of Technology, 311 Ferst Drive, Atlanta, GA 30332, USA, ^b Universidad Autónoma de Aguascalientes, Centro de Ciencias Básicas, Departamento de Química, Avenida Universidad 940, Aguascalientes, Ags. C.P. 20131, Mexico, ^c School of Biology, Georgia Institute of Technology, Atlanta, GA 30332, USA). **Estimating the toxicity of ambient fine aerosols using freshwater rotifer *Brachionus calyciflorus* (Rotifera: Monogononta). *Environmental Pollution*, Volume 182(2013): 379–384**

The toxicity of atmospheric fine particulate matter (PM_{2.5}) in Atlanta is assessed using freshwater rotifers (*Brachionus calyciflorus*). The PM-laden quartz filters were extracted in both water and methanol. Aerosol extracts were passed through a C-18 column to separate the PM components into hydrophobic and hydrophilic fractions. Toxicity data reported in the units of LC₅₀ (concentration that kills 50% of the test population in 24 h) shows that ambient particles are toxic to the rotifers with LC₅₀ values ranging from 5 to 400 µg of PM. The methanol extract of the aerosols was substantially more toxic (8 ± 6 times) to the rotifers compared to the water extracts. A sizeable fraction (>70%) of toxicity was found to be associated with the hydrophobic fraction of PM. However, none of the bulk aerosol species was strongly correlated with the LC₅₀ values suggesting a complicated mechanism of toxicity probably involving synergistic interactions of various PM components.

Keywords: Freshwater rotifers; Aerosol toxicity; Hydrophobic compounds; Water-insoluble PM components

Débora M. Bayer¹, Alessandra C. O. Chagas-Spinelli², Sávia Gavazza³, Lourdinha Florencio¹ and Mario T. Kato¹. (¹Departamento de Engenharia Civil, Universidade Federal de Pernambuco (UFPE), Av. Acadêmico Hélio Ramos, s/n. CEP, 50740-530 Recife, PE, Brazil, ²Universidade Federal do Semi-Árido, Angicos, RN, Brazil, ³Centro Acadêmico do Agreste, Universidade Federal de Pernambuco, Caruaru, PE, Brazil). **Natural Attenuation and Biosurfactant-Stimulated Bioremediation of Estuarine Sediments Contaminated with Diesel Oil. *Applied Biochemistry and Biotechnology*, Volume 171(1) (2013): pp 173-188**

We evaluated the bioremediation, by natural attenuation (NA) and by natural attenuation stimulated (SNA) using a rhamnolipid biosurfactant, of estuarine sediments contaminated with

diesel oil. Sediment samples (30 cm) were put into 35 cm glass columns, and the concentrations of the 16 polycyclic aromatic hydrocarbons (PAHs) prioritized by the US Environmental Protection Agency were monitored for 111 days. Naphthalene percolated through the columns more than the other PAHs, and, in general, the concentrations of the lower molecular weight PAHs, consisting of two and three aromatic rings, changed during the first 45 days of treatment, whereas the concentrations of the higher molecular weight PAHs, consisting of four, five, and six rings, were more stable. The higher molecular weight PAHs became more available after 45 days, in the deeper parts of the columns (20–30 cm). Evidence of degradation was observed only for some compounds, such as pyrene, with a total removal efficiency of 82 and 78 % in the NA and SNA treatments, respectively, but without significant difference. In the case of total PAH removal, the efficiencies were significantly different of 82 and 67 %, respectively.

Keywords: Oil derivatives, Hydrocarbons, Naphthalene, Pyrene, Percolation, Rhamnolipid, Biodegradation

Izabela Michalak¹, Katarzyna Chojnacka¹ and Anna Witek-Krowiak². (¹Department of Chemistry, Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology, Smoluchowskiego 25, 50-372 Wrocław, Poland, ²Division of Chemical Engineering, Department of Chemistry, Wrocław University of Technology, Norwida 4/6, 50-373 Wrocław, Poland. Email: izabela.michalak@pwr.wroc.pl). **State of the Art for the Biosorption Process—a Review. Applied Biochemistry and Biotechnology, Volume 170(6) (2013): 1389-1416**

In recent years, biosorption process has become an economic and eco-friendly alternative treatment technology in the water and wastewater industry. In this light, a number of biosorbents were developed and are successfully employed for treating various pollutants including metals, dyes, phenols, fluoride, and pharmaceuticals in solutions (aqueous/oil). However, still there are few technical barriers in the biosorption process that impede its commercialization and thus to overcome these problems there has been a steadily growing interest in this research field. This resulted in large numbers of publications and patents each year. This review reports the state of the art in biosorption research. In this review, we provide a compendium of know-how in laboratory methodology, mathematical modeling of equilibrium and kinetics, identification of the biosorption mechanism. Various mathematical models of biosorption were discussed: the process in packed-bed column arrangement, as well as by suspended biomass. Particular attention was paid to patents in biosorption and pilot-scale systems. In addition, we provided future aspects in biosorption research.

Keywords: Biosorption, Research methodology, Kinetics Equilibrium Process, solutions Application in practice

Suzana Cláudia Silveira Martins^{1*}, Claudia Miranda Martins¹, Larissa Maria Cidrão Guedes Fiúza¹ and Sandra Tédde Santaella². (¹Laboratory of Environmental Microbiology, Department of Biology, Sciences Center, Federal University of Ceará, Fortaleza, CE, Brazil, ²Sea Sciences Institute, Federal University of Ceará, Fortaleza, CE, Brazil. Email: suzana220@gmail.com Tel: +55 85-3366 9815. Fax: +55 85 3366 9806). **Immobilization of microbial cells: A promising tool for treatment of toxic pollutants in industrial wastewater. African Journal of Biotechnology Volume 12(28)(2013): 4412-4418**

The review articles on cell immobilization have been published since 1980 and reflect the general interest in this topic. Immobilized microbial cells create opportunities in a wide range of sectors including environmental pollution control. Compared with suspended microorganism technology, cell immobilization shows many advantages, such as resistance to toxic chemicals. This review presents the potential of immobilized microbial cells for treatment of toxic pollutants in industrial wastewater, the fundamentals, history and advantages of immobilized cells compared with suspended cells, characteristics of support materials and the principal methods of immobilization, with special emphasis for natural immobilization by cell adsorption.

Keywords: Cell immobilization, microorganisms, adsorption, toxic pollutants, wastewater.

Onwu, F. K.^{1*}, Ogah, S. P. I.² and Ngele, S. O.² (¹Department of Chemistry, Michael Okpara University of Agriculture Umudike, P.M.B 7267, Umuahia, Abia State, Nigeria, ²Department of Industrial Chemistry, Ebonyi State University, P.M.B 053, Abakaliki, Ebonyi State, Nigeria. Email: frank4kalu2007@yahoo.com, frank4kalu2013@gmail.com). **Biosorption of cadmium (ii) ion from aqueous solution by Afzelia Africana. African Journal of Biotechnology, Volume 12(32)(2013): 5060-5068**

The batch adsorption of cadmium (II) ion from aqueous solution using low-cost adsorbent of biological origin, *Afzelia africana* shell under different experimental conditions was investigated in this study. The influences of initial Cd (II) ion concentration, initial pH, contact times and temperature were reported. Adsorption of Cd (II) was found to be pH dependent and the results indicate that the optimum pH for its removal from aqueous solution was 5. No marginal effect on the biosorption of cadmium was detected for temperatures between 298 and 313K as observed from their Langmuir isotherm constants (q_{max}) at different temperatures. q_{max} values obtained at the different temperatures of 298, 303 and 313K were 13.59, 14.04 and 14.27 mg g⁻¹, respectively. The adsorption equilibrium shows that the process followed both Freundlich and Langmuir models with Freundlich giving a better fit for the adsorption data in comparison to the Langmuir model. The fit of the adsorption data into Freundlich model shows that the adsorption process was predominantly a physisorption. The results reveal that cadmium (II) was considerably adsorbed on the *A. africana* shell and could serve as an economical method for the removal of cadmium from aqueous solutions.

Keywords: Adsorption isotherms, adsorption kinetics, *Afzelia africana*, biosorbent, biosorption.

Anish Saini¹, Rohini Kumar M.¹, Karthik Senan¹, Shakti Sagar¹, Vivekanandan K. E.² and Jabez Osborne W.^{1*}. (¹School of Biosciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India, ²CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608502, Tamil Nadu, India. Email: jabez.vit@gmail.com Tel: +91 9894204309). **Remediation of lead (Pb) by a novel *Klebsiella* sp. isolated from tannery effluent of Ranipet, Vellore district. African Journal of Biotechnology, Volume12(32) (2013): 5069-5074**

Lead is found to be one of the most toxic heavy metal according to American public health association (APHA). Vellore district is one of the most polluted sites in the world. It is more common for lead poisoning to build up slowly over time. Over time, even low levels of lead exposure can harm a child's mental development. Therefore new resources for the removal of lead are the need of the hour. Soil and effluent samples were obtained from common effluent treatment plant; Ranipet, Vellore district. The concentration of heavy metal was also assessed in

the collected samples and then isolated lead tolerant bacteria over lead containing mineral salt medium. The isolated desired bacteria was also tested for their ability to remediate other heavy metals like chromium (Cr), iron (Fe), zinc (Zn), cadmium (Cd) which are present in the tannery effluent. The one with good bioremediation activity was further characterized by sequencing 16S rRNA gene and it was found to be a novel species of *Klebsiella* genus.

Keywords: Lead tolerant bacteria, *Klebsiella*, heavy metal remediation.

Biotransformation

X.-Y. Yang^{1,2,†}, C. Huang^{1,3,†}, H.-J. Guo^{1,3,†}, L. Xiong^{1,3}, Y.-Y. Li^{1,3}, H.-R. Zhang^{1,3}, X.-D. Chen^{1,3,*}. (¹Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou, China, ²University of Chinese Academy of Sciences, Beijing, China, ³Key Laboratory of Renewable Energy, Chinese Academy of Sciences, Guangzhou, China). **Bioconversion of elephant grass (*Pennisetum purpureum*) acid hydrolysate to bacterial cellulose by *Gluconacetobacter xylinus*. Journal of Applied Microbiology. Volume 115(4)(2013): 995–1002**

To evaluate the possibility of elephant grass acid hydrolysate converting into bacterial cellulose (BC) produced by *Gluconacetobacter xylinus* CH001 and to characterize the morphology and structure of the cellulose produced.

Acid-hydrolysed and detoxified elephant grass acid hydrolysate was inoculated with *G. xylinus* CH001. After 14 days of static fermentation, about 6.4 g l⁻¹ of BC could be generated. Meanwhile, 60.4% (w/w) of BC yield on sugar consumption was obtained. Scanning electron micrographs illustrated that the network of cellulose fibres became denser, and the diameter changed with the growth. FT-IR spectra showed almost same results for all the BC samples collected on different culture time. X-ray diffractograms demonstrated that the crystalline form of BC was cellulose I, the crystallinity increased to 53-58%, and the crystallinity index reached up to 99%. Elephant grass acid hydrolysate could be utilized efficiently for BC production by *G. xylinus* CH001. Structure analysis on the cellulose produced showed its potential of being excellent material for further application.

Our studies for the first time examined the bioconversion of low-cost elephant grass into high-value BC and the changes in its morphology and structure following the culture time.

Keywords: acid hydrolysate; bacterial cellulose; elephant grass; fermentation; *Gluconacetobacter xylinus*

Abhishek Bhattacharya, Amitava Datta. (Department of Power Engineering, Jadavpur University, Salt Lake Campus, Kolkata, West Bengal 700098, India).. Effects of supplementary biomass firing on the performance of combined cycle power generation: A comparison between NGCC and IGCC plants. Biomass and Bioenergy, Volume 54(2013): 239–249

In the present work, effects of biomass supplementary firing on the performance of fossil fuel fired combined cycles have been analyzed. Both natural gas fired combined cycle (NGCC) and integrated coal gasification combined cycle (IGCC) have been considered in the study. The efficiency of the NGCC plant monotonically reduces with the increase in supplementary firing, while for the IGCC plant the maximum plant efficiency occurs at an optimum degree of supplementary firing. This difference in the nature of variation of the efficiency of two plants under the influence of supplementary firing has been critically analyzed in the paper. The ratings of different plant equipments, fuel flow rates and the emission indices of CO₂ from the plants at varying degree of supplementary firing have been evaluated for a net power output of 200 MW. The fraction of total power generated by the bottoming cycle increases with the increase in supplementary firing. However, the decrease in the ratings of gas turbines is much more than the increase in that of the steam turbines due to the low work ratio of the topping cycle. The NGCC plants require less biomass compared to the IGCC under identical condition. A critical degree of supplementary firing has been identified for the slag free operation of the biomass combustor. The performance parameters, equipment ratings and fuel flow rates for no supplementary firing and for the critical degree of supplementary biomass firing have been compared for the NGCC and IGCC plants.

Keywords: Combined cycle; Supplementary firing; Biomass; Efficiency; CO₂ emission

Michaela A. Dippold^{1, 3} and Yakov Kuzyakov^{2, 3}. (¹Department of Agroecosystem Research, University of Bayreuth, BayCEER, Universitätsstraße 30, 95447 Bayreuth, Germany, ²Department of Soil Science of Temperate Ecosystems, Georg-August-University of Göttingen, Göttingen, Germany, ³Department of Agricultural Soil Science, Georg-August-University of Göttingen, Göttingen, Germany. Email: midipp@gmx.de). Biogeochemical transformations of amino acids in soil assessed by position-specific labeling. Plant and Soil, Volume 373(1-2): 385-401

Amino acid turnover in soil is an important element of terrestrial carbon and nitrogen cycles. This study accounts for their driver - the microbial metabolism - by tracing them via the unique isotopic approach of position-specific labeling. Three ¹⁴C isotopomers of alanine at five concentration levels combined with selective sterilization were used to distinguish sorption mechanisms, exoenzymatic and microbial utilization of amino acids in soil.

Sorption and microbial uptake occurred immediately. Unspecific microbial uptake followed a linear kinetic, whereas energy-dependent uptake followed Michaelis-Menten. Less than 6 % of the initially added alanine was sorbed to soil, but after microbial transformation products were bound to the soil matrix at higher proportions (5–25 %). The carboxyl group (C-1) was rapidly oxidized by microorganisms, whereas C-2 and C-3 positions were preferentially incorporated into microbial biomass. Dependency of C metabolization on amino acid concentration reflected individual alanine transformation pathways for starvation, maintenance and growth conditions. This study demonstrates that position-specific labeling determines the mechanisms and rates of C cycling from individual functional groups. This approach reflected underlying metabolic

pathways and revealed the formation of new organic matter. We therefore conclude that position-specific labeling is a unique tool for detailed insights into submolecular transformation pathways and their regulation factors.

Keywords: Position-specific tracers, Amino acids stabilization, Sorption Exoenzyme and uptake kinetics, Metabolic tracing, Soil organic matter formation Sterilization and inhibition methods Biochemical pathways

Biomarker

Mingbao Feng¹, Ying Li¹, Ruijuan Qu¹, Liansheng Wang¹ and Zunyao Wang¹. (¹State Key Laboratory of Pollution Control and Resources Reuse, School of Environment, Nanjing University, Nanjing, 210046, Jiangsu, People's Republic of China. Zunyao Wang, Email: wangzun315cn@163.com). **Oxidative stress biomarkers in freshwater fish *Carassius auratus* exposed to decabromodiphenyl ether and ethane, or their mixture. *Ecotoxicology*, Volume 22(7) (2013): 1101-1110**

Decabromodiphenyl ether (BDE-209) and its commercial alternative decabromodiphenyl ethane (DBDPE) are two structurally similar brominated flame retardants, with evidence of their ubiquitous existence in aquatic ecosystems. The present study was conducted to investigate the hepatic oxidative stress inducing potential of BDE-209, DBDPE, and their mixture in *Carassius auratus* after exposure to different doses (10, 50 and 100 mg/kg) for 7, 14 and 30 days. Results showed that oxidative stress was evoked evidently for the experimental groups with longer exposure duration, as indicated by significant inhibition in the antioxidant enzymes activities and decrease in the reduced glutathione level, as well as simultaneous elevation of lipid peroxidation level measured by malondialdehyde content. In addition, it was found that BDE-209 possessed a higher oxidative stress inducing ability than DBDPE. Considering the more pronounced antioxidant responses in combined exposure, the interaction of BDE-209 and DBDPE was presumed to be additive action.

Keywords: Flame retardants Antioxidant enzymes, Lipid peroxidation Combined effect Fish liver

Maxime Pauwels^a, H el ene Fr erot^a, Dima Souleman^b, Franck Vandenbulcke^b. (^a Universit e Lille Nord de France, Laboratoire de G en tique et Evolution des Populations V eg etales, UMR CNRS 8198, B atiment SN2, F-59655 Villeneuve d'Ascq cedex, France, ^b Universit e Lille Nord de France, Laboratoire de G enie Civil et g eo-Environnement EA 4515 (LGCgE-Lille 1), Ecologie Num erique et Ecotoxicologie, F-59650 Villeneuve d'Ascq, France). **Using biomarkers in an evolutionary context: Lessons from the analysis of biological responses of oligochaete annelids to metal exposure. *Environmental Pollution*, Volume 179(2013) : 343–350**

Anthropogenic activities may lead to the accumulation of inorganic and organic compounds in topsoils. Biota living in close contact with contaminated soils may experience stress at different levels of biological organization throughout the continuum from molecular to community level. Biological responses observed at the individual or infra-individual level of biological

organization led to the development of biomarkers. The development of biomarkers consists often in evidencing biological modifications following a contaminant stress in laboratory conditions, using naïve organisms and it is sometime proposed to use the biological state of individuals from sentinel species collected in the field to evaluate the level of environmental exposure. However, considering the possibility of local adaptation following long-term exposure, organisms response sampled in the field may substantially differ from laboratory specimens. In this review, we discuss this point focusing on the definition and validity of molecular biomarkers of metal pollution using earthworms of the Lumbricidae family.

Keywords: Biomarker; Earthworms; Gene expression; Local adaptation; Metal pollution

Orlu, E.E. (Department of Applied and Environmental Biology, Rivers State University of Science and Technology, P.M.B.5080, Nkpolu, Rivers State, Nigeria). Seminal plasma protein as a biomarker for fertility and hatchability in the domestic fowl *Gallus domesticus*. Research Journal of Applied Sciences, Engineering and Technology, Volume 6(24) (2013): 4692-4696

This study was aimed at investigating the use of seminal plasma protein concentration to predict fertility and hatchability in the domestic fowl. Forty cocks with acceptable sperm motility and morphology were used and the seminal plasma protein concentration determined by the Biuret method. The result revealed a negative significant correlation between the seminal plasma protein concentration with sperm motility ($r = -0.5920$, $p < 0.05$), $Y = -0.3144X^2 + 2.1578X + 74.324$, $R^2 = 0.3506$ ($p < 0.05$). A significant positive correlation with percentage dead spermatozoa $r = 0.9743$ ($p < 0.01$), $R^2 = 0.9493$, $p < 0.01$), percent abnormal spermatozoa, $r = 0.8425$, $p < 0.01$), $R^2 = 0.7098$ ($p < 0.01$), $Y = 0.357X^2 + 1.5364X + 9.1886$. Egg fertility and Hatchability were negative but significantly correlated with seminal plasma protein concentration $r = -0.6302$ ($p < 0.01$) vs $r = -0.8438$ ($p < 0.01$) and $Y = 0.8977X^2 - 5.8289X + 81.628$ vs $Y = -0.2391X^2 + 3.3871X + 60.771$, respectively. There was low correlation with the quantitative semen characteristics. Prediction of Y_{max} from Seminal plasma protein concentration (X) showed that the best semen quality, highest percent fertility and hatchability would be obtained when the concentration of seminal plasma protein is >3.00 g/100 mL but <6.00 g/100 mL. It was concluded that seminal plasma protein concentration could be used as a biomarker for prediction of fertility and hatchability in the domestic fowl.

Keywords: Biomarker; Domestic fowl; Fertility; Hatchability; Seminal plasma protein

Meuwis, M.-A.^a, Vernier-Massouille, G.^b, Grimaud, J.C.^c, Bouhnik, Y.^d, Laharie, D.^e, Piver, E.^f, Seidel, L.^g, Colombel, J.F.^h, Louis, E.^a. (^a Hepato-Gastroenterology and Digestive Oncology Department, Liège University Hospital, CHU and GIGA-R, University of Liège, Liège, Belgium, ^b Department of Gastroenterology, Université Lille Nord de France and CHRU Lille, 59000 Lille, France, ^c Service de Gastro-Entérologie Hôpital Nord et Faculté de Médecine de Marseille, 13915 Cedex 20, Marseille, France, ^d Gastroenterology and IBD Department, Hôpital Beaujon-APHP-Paris-Diderot University, 92110 Clichy, France, ^e CHU de Bordeaux, Hôpital Haut-Lévêque, Service d'Hépatogastroentérologie, Université de Bordeaux, Laboratoire de Bactériologie, 33000 Bordeaux, France, ^f CHRU Tours Biochimie et Biologie Moléculaire, INSERM U966, Université François Rabelais de Tours, Faculté de Médecine, 10 Bd. Tonnellé, 37000 Tours, France, ^g Medical Informatics and Biostatistics, University of Liège, Belgium, ^h Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1069, New York, NY

10029, United States). Serum calprotectin as a biomarker for Crohn's disease. Journal of Crohn's and Colitis, Volume 7(12) (2013): 678-e683

Background and aims: In Crohn's disease, correlation between clinical assessment and disease activity at tissue level is weak. Our aim was to evaluate the value of serum calprotectin as a biomarker for Crohn's disease. Methods: The STORI trial patients (n. = 115) were studied at baseline, in clinical remission before infliximab withdrawal, or at the time of relapse after infliximab withdrawal. Forty healthy controls were also studied. Serum calprotectin level was measured by ELISA. Data were analyzed through correlation analyses, Kaplan Meier curves and Cox model, using available Crohn's Disease Activity Index (CDAI), Crohn's Disease Endoscopic Index of Severity (CDEIS), fecal calprotectin and C-reactive protein levels (hsCRP). Results: Median serum calprotectin was 8892. ng/mL (range: 410-125,000. ng/mL) in Crohn disease patients as compared with 1318. ng/mL (range: 215.8-3770. ng/mL) in controls (P<. 0.0001). Serum calprotectin was significantly higher for active disease (median. = 19,584. ng/mL) than for inactive disease (median. = 8353. ng/mL) (P<. 0.0001). Serum calprotectin correlated with hsCRP (r. = 0.4092, P<. 0.0001) and CDAI (r. = 0.4442, P<. 0.0001), but not with CDEIS, on the contrary to fecal calprotectin (r. = 0.6458, 0.5515, 0.2577 with P<. 0.0001, P<. 0.0001, P= 0.019 respectively). In multivariate analysis, serum calprotectin used as a discrete variable (threshold: 5675. ng/ml), appeared complementary to hsCRP (>. 5. mg/l) and fecal calprotectin (>. 250. µg/g) to predict relapse after infliximab withdrawal (P= 0.0173, 0.0024 and 0.0002; HR: 3.191, 3.561 and 4.120). Conclusions: As a CDbiomarker, serum calprotectin has a similar profile as hsCRP. It is also complementary to fecal calprotectin and hsCRP for prediction of relapse after infliximab withdrawal.

Keywords: Biomarker; Crohn's disease; Infliximab; Relapse prediction; Serum calprotectin

Biofertilizer

Phanit Nakayan¹, Asif Hameed¹, Satnam Singh¹, Li-Sen Young³, Mei-Hua Hung¹ and Chiu-Chung Young^{1, 2}. (¹Department of Soil & Environmental Sciences, College of Agriculture and Natural Resources, National Chung Hsing University, 250, Kuo Kuang Rd., Taichung 402, Taiwan, Republic of China, ²Biotechnology Center, National Chung Hsing University, Taichung 402, Taiwan, Republic of China, ³Department of Biotechnology, College of Art and Science, National Formosa University, No.64, Wunhua Rd., Huwei Township, Yunlin County 632, Taiwan, Republic of China. Email: ccyoung@mail.nchu.edu.tw). Phosphate-solubilizing soil yeast *Meyerozyma guilliermondii* CC1 improves maize (*Zea mays* L.) productivity and minimizes requisite chemical fertilization. *Plant and Soil*, Volume 373(1-2): 301-315

Phosphate-solubilizing yeasts have been under-exploited in eco-friendly maize cultivation. In this regard, soil yeasts *Meyerozyma guilliermondii* CC1, *Rhodotorula mucilaginosa* CC2 and *M. caribbica* CC3 were investigated for their plant growth-promoting (PGP) activities.

Soil yeasts were isolated and characterized. Maize (*Zea mays* L. cv. Tainong No.1) and Chinese cabbage (*Brassica rapa* L. cv. Pekinensis) were used for seed bioassay. Growth-promoting effects of yeasts under greenhouse conditions were evaluated using maize and lettuce (*Lactuca*

sativa L. cvs. Capitata and Taiwan sword leaf). Ultimately, *M. guilliermondii* CC1 was tested on field-grown maize; treatments included full-dose chemical fertilizers (CF), yeast (CC1), half-dose chemical fertilizers ($\frac{1}{2}$ CF), CC1 + $\frac{1}{2}$ CF and control. Nutrient uptake, growth, and yield of maize and rhizospheric soil microbes were estimated.

Strain *M. guilliermondii* CC1 exhibited better seed vigor index in maize and Chinese cabbage. CC1 + $\frac{1}{2}$ CF significantly improved the dry-weights, and nutrient uptakes of maize and sword leaf lettuce under greenhouse conditions. In field, CC1 + $\frac{1}{2}$ CF application exerted a pronounced effect on growth of maize, cob yield, nutrient-uptake and rhizospheric soil microbial counts. Our results validated superior biochemical potency and PGP traits of *M. guilliermondii* CC1 that reduced requisite chemical fertilizer application without affecting the optimal productivity in maize.

Keywords: Phosphate- solubilizing yeast, Indole-3- acetic acid, PGPR Inoculant Biocontrol, Rhizospheric soil microbial count

Zhaohai Bai¹, Haigang Li¹, Xueyun Yang², Baoku Zhou³, Xiaojun Shi⁴, Boren Wang⁵, Dongchu Li⁵, Jianbo Shen¹, Qing Chen¹, Wei Qin⁶, Oene Oenema⁶ and Fusuo Zhang¹. (¹Center for Resources, Environment and Food security (CREFS), China Agricultural University, Beijing, 100193, China, ²College of Resources and Environment, Northwest A & F University, 712100 Yangling, China, ³Institute of Soil and Fertilizer, Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, China, ⁴College of Resources and Environment, Southwest University, Chongqing, 400716, China, ⁵Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, 100081 Beijing, China, ⁶Department of Soil Quality, Wageningen University, P.O. Box 47, 6700 AA Wageningen, the Netherlands. Email: haigangli@cau.edu.cn). The critical soil P levels for crop yield, soil fertility and environmental safety in different soil types. *Plant and Soil*, Volume 372(1-2): 27-37

Sufficient soil phosphorus (P) is important for achieving optimal crop production, but excessive soil P levels may create a risk of P losses and associated eutrophication of surface waters. The aim of this study was to determine critical soil P levels for achieving optimal crop yields and minimal P losses in common soil types and dominant cropping systems in China.

Four long-term experiment sites were selected in China. The critical level of soil Olsen-P for crop yield was determined using the linear-plateau model. The relationships between the soil total P, Olsen-P and CaCl₂-P were evaluated using two-segment linear model to determine the soil P fertility rate and leaching change-point.

The critical levels of soil Olsen-P for optimal crop yield ranged from 10.9 mg kg⁻¹ to 21.4 mg kg⁻¹, above which crop yield response less to the increasing of soil Olsen-P. The P leaching change-points of Olsen-P ranged from 39.9 mg kg⁻¹ to 90.2 mg kg⁻¹, above which soil CaCl₂-P greatly increasing with increasing soil Olsen-P. Similar change-point was found between soil total P and Olsen-P. Overall, the change-point ranged from 4.6 mg kg⁻¹ to 71.8 mg kg⁻¹ among all the four sites. These change-points were highly affected by crop specie, soil type, pH and soil organic matter content. The three response curves could be used to access the soil Olsen-P status for crop yield, soil P fertility rate and soil P leaching risk for a sustainable soil P management in field.

Keywords: Critical level Response curve, P leaching Olsen-P CaCl₂-P

Peter J.Leggio. (Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EQ, UK.). Enhancing the Growth of Plants on Coal Waste Using a Biological Fertilizer. International Journal of Environment and Resource (IJER), Volume 2(3)(2013): 59-66

A biofertilizer, composed of a mixture of crushed zeolitic tuff and organic waste, has been used to grow plants on coal waste. The growth experiment is reported together with control experiments to demonstrate the efficacy of the biofertilizer. Plants used were: Brassica napus, Beta vulgaris, Linum usitatissimum and Zea mays which were grown in pots under controlled greenhouse conditions. Coal waste from a Nottinghamshire colliery in the English Midlands was used throughout the work as the plant substrate. Decomposition of the organic waste produces ammonium ions which are adsorbed by the zeolitic tuff, and when the mixture is added to a plant substrate, oxidizing ammonium micro-organisms function and sponsor nitrification (Leggo et al.,2009). The resulting growth enhancement is exceptional as shown by the shoot dry weight of the plants harvested at maturity.

Keywords: Coal Waste, Organic Waste, Decomposition, Ammonium Ions, Zeolitic tuff; Micro-Organisms, Enhanced Growth

Biocomposting

Caputo, M.C., De Girolamo, A.M., Volpe, A. (Consiglio Nazionale delle Ricerche, Istituto di Ricerca Sulle Acque, Viale F. De Blasio, 5, 70132 Bari, Italy). Soil amendment with olive mill wastes: Impact on groundwater. Journal of Environmental Management, Volume 131(2013): 216-221

Two sets of soil lysimeters were amended with solid and liquid olive mill wastes and the composition of leachate was analysed. Five treatments were carried out using: olive mill wastewater (OMW) at two different rates (80 and 320m³/ha); OMW pre-treated by catalytical digestion with MnO₂; compost obtained by exhausted olive pomace; freshwater as the control. Electric conductivity, pH, potassium, total polyphenols and nitrates were monitored in the leachate as indexes of potential groundwater contamination. The study demonstrated that the impact of all the selected amendments on groundwater was the minimum. OMW was safely applied to soil even at four times the rate allowed by the Italian law, and pre-treatment by catalytical digestion was not necessary to further reduce the impact on groundwater. The application of olive pomace compost was equally safe.

Keywords: Environmental impacts; Groundwater pollution; Lysimeters; Olive mill wastes; Soil application

Cáceres, R. , Marfà, O. (Biosystems Engineering and Agronomy Unit, Research and Technology, Food and Agriculture (IRTA), Carretera de Cabrils km 2, 08348 Cabrils, Barcelona, Spain). Diagnosis of the fertility of compost-based growing media: Method

comparison and monitoring in pot plant cultivation. *Scientia Horticulturae*, Volume 164(2013): 213-220

Using compost instead of peat as an ingredient in substrate mixtures is a way of increasing the sustainability of pot plant nurseries. However, this use should also include monitoring to assess crop fertility, given that compost generally has a high fertilizer content. Of the different monitoring methods, the most effective, objective and preventive one involves monitoring the nutrient status of the substrate. The main objective of this study was to provide evidence-based information on fast methods used in the field to characterize substrate fertility compared to a water extract method in order to apply these methods in cultures with unusual alternative substrates. We observed a high level of agreement between the concentration of nutrients measured using the IP method and the concentration using the fertility assessment methods most commonly used in horticulture (leachate and water extract methods). By using different compost-based substrates and comparing them to a control substrate, we have shown that the IP method is useful for monitoring substrate fertility in pot plants. Results show that the IP method indicates the nutrient composition of the functional part of the different substrates (root zone) and their development. The induced percolate method is a useful, nondestructive field method for quickly checking the nutrient content of the functional part of substrates.

Keywords: Cattle manure compost; Growing media; Induced percolate method; Nutrient status; Outdoor nurseries; Water extract method

Youssef, S.A. , Tartoura, K.A.H. (Botany Department, Suez Canal University, Ismailia, 41522, Egypt). Compost enhances plant resistance against the bacterial wilt pathogen *Ralstonia solanacearum* via up-regulation of ascorbate-glutathione redox cycle. *European Journal of Plant Pathology*, Volume 137(4) (2013): 2013, Pages 821-834

The interactions between the pathogen *Ralstonia solanacearum* and potato *Solanum tuberosum* plants were studied to investigate the reactive oxygen species metabolic system and ascorbate (ASC)-glutathione (GSH) redox cycle in response to compost application. Single potato eyepieces were germinated and grown in pots containing sandy soil with or without compost at a rate of 7.5 g kg⁻¹ soil. Non-compost- and compost-treated plants (CTP) were inoculated with *R. solanacearum* 25 days after planting and then analyzed after 10 days, unless otherwise stated. The present results revealed that pathogen infection caused a remarkable decrease in plant growth related parameters and productivity and an increase in disease incidence. However, under these conditions compost had substantially improved plant growth and decreased disease incidence and bacterial population. *R. solanacearum* resulted in significant enhancement in the activities of NADPH oxidase, lipoxygenase, the production rate of superoxide and hydroxyl radicals, levels of hydrogen peroxide, membrane lipid peroxidation, and protein oxidation indicating the induction of oxidative stress in potato roots. However, the pathogen-mediated enhancement in indices of oxidative stress was considerably decreased by compost application, which enhanced the activities of ascorbate peroxidase (APX, EC 1.11.1.11), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1) and glutathione reductase (GR, EC 1.6.4.2) in infected potato plants, implying a better ROS-scavenging activity. Data also indicated that there were general increases in ASC and GSH content in infected compost treated plants, but non-compost treated ones significantly had lower levels of such redox metabolites. In addition, significantly higher ratios of ASC/DHA (dehydroascorbate) and GSH/GSSG (glutathione disulphide) were generally found in CTP than in non-compost treated-ones. The obtained results suggest that compost provides effective

protection against the *Ralstonia* bacterial pathogen via up-regulation of the capacity of the ASC-GSH cycle and modulation of the cellular redox status, thereby eliminating ROS damage and sustaining membrane stability.

Keywords: Ascorbate-glutathione cycle enzymes; Compost; *Ralstonia solanacearum*; Reactive oxygen species; *Solanum tuberosum* L

Zhong, J.^a, Wei, Y.^a, Wan, H.^{ab}, Wu, Y.^b, Zheng, J.^a, Han, S.^c, Zheng, B.^b. (^a Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China, ^b Nanchang University, Nanchang 330031, China, ^c Institute of Atmospheric Physics, Chinese Academy of Sciences, Beijing 100029, China). Greenhouse gas emission from the total process of swine manure composting and land application of compost. *Atmospheric Environment*, Volume 81(2013) : 348-355

Greenhouse gas (GHG) emissions from animal manure management are of great concern in China. However, there are still great uncertainties about China's GHG inventory due to the GHG emission factors partly used default values from the Intergovernmental Panel of Climate Change (IPCC) guidelines. The purpose of this study was to use a case study in Beijing to determine the regional GHG emission factors based on the combination of swine manure composting and land application of the compost with both on-site examination and a life cycle assessment (LCA). The results showed that the total GHG emission factor was $240\text{kgCO}_2\text{eqtDS}^{-1}$ (dry solids), including the direct GHG emission factor of $115\text{kgCO}_2\text{eqtDS}^{-1}$ for swine manure composting and $48\text{kgCO}_2\text{eqtDS}^{-1}$ for land application of the compost. Among the total GHG emissions of $5.06\text{kgCH}_4\text{tDS}^{-1}$ and $0.13\text{kgN}_2\text{OtDS}^{-1}$, the swine manure composting contributed approximately 89% to CH_4 emissions while land application accounted for 92% of N_2O emission. Meanwhile, the GHG emission profile from the full process in Beijing in 2015 and 2020 was predicted by the scenario analysis. The composting and land application is a cost-effective way for animal manure management in China considering GHG emissions.

Keywords: Compost; Greenhouse gas emission; Land application; Life cycle assessment; Pig manure

Price, G.W.^a, Zeng, J.^a, Arnold, P.^b. (^a Department of Engineering, Faculty of Agriculture, Dalhousie University, PO Box 550, Truro, NS, B2N 5E3, Canada, ^b Ivan Curry School of Engineering Acadia University, Wolfville, NS, B4P 2R6, Canada). Influence of agricultural wastes and a finished compost on the decomposition of slaughterhouse wastecomposts. *Journal of Environmental Management*, Volume 130(2013): 248-254

The objective of this study was to evaluate the efficacy of combining agricultural wastes or a finished compost (wheat straw, horse manure and bedding, sheep manure, and a wheat straw-SHW finished compost) as compost feedstocks with cattle slaughterhouse wastes (SHW) on a field-scale. The composts were managed in covered bins over 200 days and physico-chemical parameters related to organic matter bio-degradation were measured over time. Thermophilic temperatures were maintained above 55°C for 12-46 days to meet the Canadian Council of Ministers of the Environment (CCME) guidelines for pathogen control. Final C:N ratios were highest in a horse manure and bedding:SHW compost at 23:1 but ranged from 18.5 to 20.5:1 for the remaining three treatments, representing a wheat straw:SHW compost and different

combinations of horse manure and bedding, SHW, and/or sheep manure. Average reduction in mass of total carbon across all the composts in the current study was 54.2%. Maturity tests at the end of the study determined that the CO₂-C evolution rate in all compost products was less than 1mgg⁻¹ organic matterday⁻¹ suggesting highly stable final compost products. Compost mass reductions all responded as exponential decay functions with R² values ranging from 0.84 to 0.99 regardless of compost feedstock composition. Agricultural by-products and composts are suitable feedstocks for use with SHW to generate a stable final product while meeting regulatory parameters to achieve conventional pathogen control.

Keywords: Compost stabilization; Horse bedding (HB); PH; Slaughterhouse wastes (SHW); Total carbon and nitrogen

Biopesticides

Ingvar Sundh¹ and Mark S. Goettel². (¹Department of Microbiology, Uppsala BioCenter, Swedish University of Agricultural Sciences, P.O. Box 7025, 75007 Uppsala, Sweden, ²Lethbridge Research Centre, Agriculture & Agri-Food Canada, P.O. Box 3000, Lethbridge, AB, Canada. Corresponding author, Email: ingvar.sundh@slu.se). Regulating biocontrol agents: a historical perspective and a critical examination comparing microbial and macrobial agents. *BioControl*, Volume 58(5) (2013): 575-593

Ever since the inclusion of microbial biocontrol agents (MBCAs) within the regulatory frameworks initially designed for chemical pesticides, there has been awareness that these frameworks are not optimal for assessment and registration of new microbial biocontrol products. It is often claimed that the regulatory situation has contributed to a relatively slow uptake of microbial biocontrol in practice. In contrast to the MBCAs, non-indigenous invertebrate biocontrol agents (IBCAs) are regulated in many countries through quarantine and other biosecurity related legislation for prevention of introduction of alien organisms, whereas use of indigenous IBCAs are generally unregulated. In this study, we investigate what scientific support there is for performing evaluations of these two main groups of biocontrol agents (BCAs) within different frameworks. We compare potential risks of MBCAs and IBCAs, present a retrospective analysis of the development and implementation of the regulatory frameworks, and compare current requirements for MBCAs with those for other applications with microorganisms. One conclusion is that the ecological risks are of similar types between the two groups of BCAs, and that for both groups the environmental safety is most pertinently evaluated according to biological and ecological principles. The main difference between MBCAs and IBCAs with respect to human health is that the former may cause infectious disease. However, we found no evidence that this hazard is more serious for microorganisms for biocontrol than for microbes used in other types of applications, which generally have substantially lower regulatory demands than those for MBCAs. Several international initiatives have produced helpful guidelines and recommendations for simplified assessments and authorisations of BCAs. Still, we conclude that as long as MBCAs are evaluated within systems initially developed for chemicals, the risk for inappropriate emphasis of chemical hazards and therefore unnecessarily complicated assessments will be maintained. Therefore, this study supports the idea that development of new systems for the regulatory oversight of MBCAs, possibly a mutual framework covering all living BCAs, should be considered. Research issues that need to be

further explored are to what extent utilisation of MBCAs actually results in increased exposure of non-targets to microorganisms, the biogeography and microbial ecology of representative MBCAs, and finally development of better methodology for determining potential human toxicity and pathogenicity of candidate MBCAs.

Keywords: Microbial control Macrobial control Biocontrol regulations Biocontrol safety Biocontrol legislation

Kadir Ilhan¹ and Ozgur Akgun Karabulut¹. (¹Department of Plant Protection, Faculty of Agriculture, Uludag University, 16059 Gorukle-Bursa, Turkey. Email: ozgurk@uludag.edu.tr). **Efficacy and population monitoring of bacterial antagonists for gray mold (*Botrytis cinerea* Pers. ex. Fr.) infecting strawberries. *BioControl*, Volume 58(4) (2013): 457-470**

In this study, the efficacy of bacterial antagonists was tested in both pre- and post-harvest stages against *Botrytis cinerea*, which causes one of the major diseases of strawberries. In total, 219 bacterial antagonists were obtained from various parts of strawberry plants, and their efficacies against *B. cinerea* were determined. Two isolated bacteria, which were identified as *Bacillus megaterium* and *Pseudomonas vesicularis*, were found to be effective and otherwise suitable as biological control agents. Moreover, an isolate of *Pseudomonas fluorescens* from pea plants was successfully used against *B. cinerea* in the experiments. These three antagonist isolates were field- tested in both the pre- and post-harvest periods in two consecutive years. The field studies demonstrated that all three isolates were as effective as a synthetic fungicide. In the first year field tests, the isolates reduced the decay incidence to 41.08–43.03 % compared with 55.48 % in the control. Similar results were obtained in the second year. In the post-harvest stage, all the bacterial isolates also controlled the disease effectively. The bacterial population was monitored in both the pre- and post-harvest periods using a spontaneous antibiotic-resistant mutant and the RAPD-PCR method. The RAPD-PCR technique alone proved insufficient to determine the population levels of the bacterial antagonists.

Keywords: Strawberry *Botrytis cinerea* Antagonist bacteria Biological control

Duriya Chantasigh^{a,b}, Supattra Kitikhun^a, Nemat O. Keyhani^c, Katewade^c Boonyapakron^a, Honglada Thoetkiattikul^a, Kusol Pootanakit^b, Lily Eurwilaichitr^a. (^aEnzyme Technology Laboratory, Bioresources Technology Unit, National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Phahonyothin Rd., Khlong, Luang, Pathum Thani 12120, Thailand, ^b Institute of Molecular Biosciences, Mahidol University, Salaya Campus, Nakorn Pathom 73170, Thailand, ^c Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611, USA). **Identification of catalase as an early up-regulated gene in *Beauveria bassiana* and its role in entomopathogenic fungal virulence. *Biological Control*, Volume 67(2) (2013): 85–93**

The ability of entomopathogenic fungi to infect insects is a complex process involving differential expression of numerous genes some of which are up-regulated when the fungus is in contact with or exposed to insect cuticles. In this report, we identified a set of differentially expressed genes in the entomopathogenic fungus *Beauveria bassiana* BCC2659 in response to *Spodoptera exigua* larvae.

PCR-select suppression subtractive hybridization (PCR-SSH) was used to identify genes differentially expressed during the initial aspects of the fungal-insect interaction, i.e. up to a 2 h post-infection model. Ten fungal genes identified by PCR-SSH were confirmed to be up-regulated by semi-quantitative RT-PCR. Of these genes, a catalase (*catE7*), implicated in stress resistance, was chosen for further characterization in order to probe its role in *B. bassiana* pathogenesis and to determine whether overexpression would result in a more virulent strain. To investigate this, a transgenic *B. bassiana* strain, overexpressing *CatE7* was constructed. Fungal transformant lines with extra *catE7* copies (*Bb::BbcatE7*) showed 2-fold higher catalase activity than the wild type. *Bb::BbcatE7* strains germinated faster than the wild-type parent and exhibited significantly higher virulence against *S. exigua* larvae. Although the *Bb::BbcatE7* strains were no better than wild type in terms of vegetative growth in the presence of exogenous H_2O_2 concentrations, conidial germination rates were higher in the *Bb::BbcatE7* strain in the presence of H_2O_2 . These results suggest that responses mediated by catalases play an important role in the fungal-insect infection process and the manipulation of catalase expression can lead to more effective fungal strains for insect control.

Keywords: Insect fungi; Beauveria; bassiana; Biocontrol; Catalase; Suppression subtractive hybridization

María Ángeles González-Sánchez^a, Antonio de Vicente^b, Alejandro Pérez-García^b, Rosa Pérez-Jiménez^a, Diego Romero^b, Francisco M. Cazorla^b. (^a IFAPA-CICE-Málaga, CAP-Junta de Andalucía, Cortijo de la Cruz s/n, Churriana, Málaga, Spain, ^b Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora”, IHSM-UMA-CSIC, Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Campus Universitario de Teatinos, Bulevar Louis Pasteur 31, 29071-Málaga, Spain). **Evaluation of the effectiveness of biocontrol bacteria against avocado white root rot occurring under commercial greenhouse plant production conditions. Biological Control, Volume 67(2) (2013): 94–100**

Avocado white root rot (WRR) is a disease of significant importance in the Mediterranean area and is caused by the emerging pathogen *Rosellinia necatrix*. Five *Pseudomonas* spp. and three *Bacillus subtilis* strains were selected for this study based on their rhizospheric soil survival, antagonistic and biocontrol activities under experimental conditions. After a single inoculation, *Pseudomonas* spp. showed higher persistence in rhizospheric soil, and *B. subtilis* strains were not detected for longer than ten days. In biocontrol assays, *B. subtilis* CB115 protected the plants against WRR under experimental growth chamber conditions at a similar level to the biocontrol reference strain *Pseudomonas chlororaphis* PCL1606. The strains *B. subtilis* CB115, *P. chlororaphis* CB254 and PCL1606 and *Pseudomonas fluorescens* CB306 were further selected to explore their biocontrol abilities under commercial greenhouse conditions. *B. subtilis* CB115 showed consistent biocontrol with protection levels similar to the biocontrol strain *P. chlororaphis* PCL1606. Although *B. subtilis* CB115 did not show persistence features, repeated applications by direct soil drenching led to the detection of a stable population that was mainly composed of spores in the avocado rhizospheric soil after 74 days. Our results indicate that both *P. chlororaphis* PCL1606 and *B. subtilis* CB115 can help control *R. necatrix* under commercial greenhouse conditions. This is the first study investigating the performance of biocontrol bacteria against avocado WRR under commercial greenhouse settings.

Keywords: *Rosellinia necatrix*; Antagonism; *Persea americana*; *Pseudomonas chlororaphis*; *Bacillus subtilis*

Alexandra Bernal^a, Trevor Williams^b, Estrella Hernández-Suárez^c, Aurelio Carnero^c, Primitivo Caballero^{a,d}, Oihane Simón^a. (^a Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Mutilva Baja 31192, Navarra, Spain, ^b Instituto de Ecología A.C. Xalapa, Veracruz 91070, Mexico, ^c Instituto Canario de Investigaciones Agrarias, Valle de Guerra 38200, San Cristóbal de la Laguna, Tenerife, Spain, ^d Dpto. Producción Agraria, Universidad Pública de Navarra, Campus Arrosadía s/n 31006, Pamplona, Navarra, Spain). **A native variant of *Chrysodeixis chalcites* nucleopolyhedrovirus: The basis for a promising bioinsecticide for control of *C. chalcites* on Canary Islands' banana crops. *Biological Control*, Volume 67(2) (2013): 101–110**

Chrysodeixis chalcites (Lepidoptera: Noctuidae) larvae cause up to 30% production loss in banana crops in the Canary Islands. Larvae of this species are susceptible to a nucleopolyhedrovirus (ChchNPV). This study aimed at evaluating the genetic diversity and bioinsecticidal activity of ChchNPV isolates collected from *C. chalcites* larvae in the Canary Islands. From a total 97 isolates collected in different banana greenhouses, restriction endonuclease analysis identified five genetic variants that differed slightly from ChchNPV isolates from Netherlands (ChchSNPV-NL) and Almería, Spain (ChchNPV-SP1). Physical maps revealed minimal differences at the genome level, mostly due to variation in the position/existence of restriction sites. ChchSNPV-TF1 was the most prevalent variant, representing 78% of isolates examined, and was isolated at all Canary Island sampling sites. This isolate was the most pathogenic isolate against *C. chalcites* second instars in terms of concentration-mortality metrics, compared to homologous variants or two heterologous viruses *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Anagrapha falcifera* multiple nucleopolyhedrovirus (AnfaMNPV). ChchSNPV-TF1 was also one of the fastest killing variants although no differences were observed in occlusion body production among the different variants in second instars. We conclude that ChchSNPV-TF1 merits further evaluation as the basis for a biological insecticide for control of *C. chalcites* in banana crops in the Canary Islands.

Keywords: *Chrysodeixis chalcites*; Banana crop; Nucleopolyhedrovirus; Field isolates; Insecticidal characteristics;

Alejandro Tena, Elena Llácer, Alberto Urbaneja. (Unidad Asociada de Entomología UJI-IVIA-CIB CSIC, Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain). **Biological control of a non-honeydew producer mediated by a distinct hierarchy of honeydew quality. *Biological Control*, Volume 67(2) (2013): 117–122**

Parasitoids feed commonly on honeydew. However, a high content of energy reserves in honeydew-fed parasitoids does not imply an increase of their fitness because honeydew may be relatively unsuitable. Herein, we studied the feeding behavior and fitness (longevity and fecundity) of a parasitoid, *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), which host does not produce honeydew, when it had access to honeydew excreted by five common hemipterans in citrus: *Aleurothrix floccosus* (Maskell) (Aleyrodidae), *Aphis spiraecola* Patch

(Aphididae), *Coccus hesperidum* L. (Coccidae), *Icerya purchasi* Maskell (Monophlebidae) and *Planococcus citri* (Risso) (Pseudococcidae). *A. melinus* females accepted equally and spent the same time feeding on the five honeydews. The first intake of these honeydews prolonged *A. melinus* longevity but only between 1 and 2 days, independently of the honeydew source. However, when they had continuous access to honeydew, *A. melinus* fitness entirely depended on the honeydew source. The longevity and realized fecundity of females with access to honeydew excreted by *A. spiraecola* was similar to unfed females. Contrarily, *C. hesperidum* and *I. purchasi* excreted the honeydew with highest nutritional value. In addition, *A. melinus* discriminated between honeydews with high and poor nutritional quality in a choice-test. Our results demonstrate that honeydew excreted by hemipterans in citrus show a distinct hierarchy of quality and *A. melinus* females are able to recognize it. These results indicate that the presence of different types of honeydews in agroecosystems should be taken into account separately in this and future biological control programs to design proper measures.

Keywords: *Aonidiella aurantii*; *Aphytis melinus*; Citrus; Nutritional ecology; Hemiptera

Laifeng Lu^{a,b}, Changzhou Ye^a, Shuanghuan Guo^a, Kuang Sheng^a, Lingxiao Shao^a, Tao Zhou^a, Ting Yu^{a,b}, Xiaodong Zheng^{a,b}. (^a Department of Food Science and Nutrition, Zhejiang University, Hangzhou 310058, People's Republic of China, ^b Fuli Institute of Food Science, Zhejiang University, Hangzhou 310058, People's Republic of China). **Preharvest application of antagonistic yeast *Rhodosporidium paludigenum* induced resistance against postharvest diseases in mandarin orange. *Biological Control*, Volume 67(2) (2013): 130–136**

Preharvest application of biocontrol agents has been employed as a preferred strategy to minimize a wide range of postharvest decays. *Rhodosporidium paludigenum* as a novel biocontrol yeast significantly inhibited the development of various postharvest fruit diseases. However, whether preharvest treatment with *R. paludigenum* might suppress postharvest pathogens has not been investigated. This study was conducted to evaluate the efficacy of preharvest applications of *R. paludigenum* against postharvest diseases in mandarin orange. Our results showed that preharvest applications of *R. paludigenum* significantly reduced postharvest decays of mandarin orange caused by *Penicillium digitatum* and *Penicillium italicum*, by 20.8% and 30.7% reduction in disease percentage, and by 14.6% and 37.3% reduction in disease severity, respectively. The population growth of *R. paludigenum* on the mandarin orange surface did not show any significant changes compared with the initial concentration during 20 days of storage after harvest. Moreover, it was found that the activities of some defense-related enzymes, comprising β -1,3-glucanase, phenylalanine ammonia-lyase, peroxidase and polyphenoloxidase, increased significantly in response to preharvest application of *R. paludigenum*. In addition, preharvest treatment of *R. paludigenum* did not impair fruit quality during postharvest storage. Therefore, the preharvest application of yeast antagonist *R. paludigenum* would be a useful biocontrol strategy to reduce the fungal diseases of harvested mandarin orange.

Keywords: *Rhodosporidium paludigenum*; Mandarin orange; Biological control; Disease resistance; *Penicillium digitatum*; *Penicillium italicum*

Ignazio Graziosi, Lynne K. Rieske. (Department of Entomology, University of Kentucky, S225 Ag North, Lexington, KY 40546-0091, USA). **Response of *Torymus sinensis*, a parasitoid of the gallforming *Dryocosmus kuriphilus*, to olfactory and visual cues. *Biological Control*, Volume 67(2) (2013): 137–142**

Torymus sinensis (Hymenoptera: Torymidae) has been manipulated extensively in biological control programs targeting the globally invasive Asian chestnut gall wasp, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae). The life cycle of *T. sinensis* is synchronized with gall wasp larval development to allow effective gall wasp population suppression and a reduction in gall formation. In spite of its extensive use for biological control, relatively little is known about its host location and host acceptance behavior. We investigated *T. sinensis* host location behavior using a Y-tube olfactometer. Adult females were tested for their response to olfactory and visual cues associated with *D. kuriphilus* galls and chestnut foliage. Adult parasitoids were not attracted by the odor of fresh galls alone, and had a negative response to the visual cues of galls and chestnut foliage when odor cues were not provided. However, the combination of olfactory and visual stimuli provided by a fresh gall coupled with chestnut foliage elicited a strongly positive response. This positive response persisted even when the fresh gall was replaced by an inert surrogate gall, provided the visual stimulus remained and the olfactory cues from fresh galls were available. Our results indicate that both visual and olfactory cues are required to enable *T. sinensis* to successfully find suitable hosts. These findings improve our understanding of the stimuli that influence *T. sinensis* host location behaviors leading to successful gall wasp parasitization, and may enhance our ability to manipulate *T. sinensis* for gall wasp management.

Keywords: Host location; Olfactometer; Asian chestnut gall wasp; Biological control

J. Nawrocka, U. Małolepsza. (Department of Plant Physiology and Biochemistry, University of Lodz, Banacha nr 12/16, 90-237 Łódź, Poland). Diversity in plant systemic resistance induced by *Trichoderma*. Biological Control, Volume 67(2) (2013): 149–156

Trichoderma species includes many important in agriculture strains, known as effective biological control agents (BCAs). While their capability of mycoparasitism and strong position as antagonists of pathogenic microorganisms are quite well understood, there are still many questions about the process of systemic resistance induced in plants by these fungi. During plant – *Trichoderma* interaction, numerous elicitors released by the *Trichoderma* hyphae may induce different types of signals transmitted within the plant e.g. by salicylic acid (SA), jasmonic acid (JA) or reactive oxygen species (ROS), triggering expression of defense proteins. As a result of gene activation, the plant produces enzymes involved in direct suppression of pathogens and enhancing the biochemical and structural barriers in plant organism. Depending on the *Trichoderma* strain, plant species as well as biotic and abiotic conditions, the defensive reactions activated by fungi may oscillate between the two types of systemic resistance: induced systemic resistance (ISR) and systemic acquired resistance (SAR). Different pathways of ISR are investigated both at the biochemical and molecular level, however there are still many issues that need clarification. The main objective of this paper is to present an overview of information about the influence of *Trichoderma* on the diversity of systemic resistance induction in plants and the possible development of this process.

Keywords: *Trichoderma* induced resistance; Induced systemic resistance; Systemic acquired resistance; Plant defense response

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Western Australia 6231, Australia, ^c School of Agricultural and Resource Economics, University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia). Natural pest control in citrus as an ecosystem service: Integrating ecology, economics and management at the farm scale. *Biological Control*, Volume 67(2) (2013): 170–177

While we were completing a year-long survey of soil invertebrates in eight citrus orchards in South Australia, there was an outbreak of Kelly's citrus thrips (*Pezothrips kellyanus*). Four growers in our survey reported their orchards were free of thrips, while the others reported suffering serious economic damage. A retrospective analysis, using data from the invertebrate survey, showed that orchards without thrips damage all had dense ground cover (perennial grasses, diverse forbs and with a deep litter layer), whereas orchards with thrips damage all had patchy ground cover (bare mineral soil with scattered annual weeds or a sparse monoculture of lucerne or oats and no litter layer). Orchards with dense ground cover and no thrips damage had far denser populations of predatory mesostigmatid mites (mean $6471 \pm 692 \text{ m}^{-2}$ 1 SE) compared with orchards with patchy ground cover and thrips damage ($1097 \pm 126 \text{ m}^{-2}$). Most Mesostigmata (total 17 spp.) were generalist predators, capable of feeding on thrips larvae when they move from the fruit to the soil to pupate. We suggest the absence of thrips damage is causally related to the presence of a diverse, abundant fauna of natural enemies, enhanced by good quality ground cover habitat and that growers with no thrips damage are benefitting from the ecosystem service of natural pest control. Using three scenarios of increasing severity of thrips damage (10%, 20% and 40%), we estimated the mean value of natural pest control of thrips as an ecosystem service was A\$ 2640, A\$ 4610 and A\$ 8540 per hectare for those orchards that benefited from the service, whereas those orchards that received no such benefit potentially lost A\$ 1970, A\$ 3260 and A\$ 5850 respectively. Our findings led to the planting of improved ground cover as habitat for predators by three growers, and the development of a commercial predator biocontrol agent for thrips by a fourth, based on mites harvested from his orchard. Growers who had effective natural pest control of thrips are more likely to have greater economic resilience in relation to price volatility shocks than those growers who do not benefit from this ecosystem service.

Keywords: Conservation biological control; Thrips; Habitat management; Ground cover; Economic resilience; Replacement cost valuation

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To determine the potential role of non-crop vegetation extrafloral nectaries (EFNs) in the presence and feeding of *Amitus fuscipennis*, a parasitoid of *Trialeurodes vaporariorum*, non-crop vegetation in and around ten fields of common beans (*Phaseolus vulgaris*) was studied in the Central Andes of Colombia. Using 0.25 m² quadrants located randomly both within the bean fields and at a distance of 3 m from the crop, the non-crop vegetation was identified and counted. The abundance of both *T. vaporariorum* and *A. fuscipennis* was also determined. Furthermore, plants separated by more than 3 m from the crop were examined for 30 min to determine the

presence of *A. fuscipennis*. There were 92 plant species associated with bean crops: 46 within the crop, 73 at 3 m from the crop border, and an additional 18 species at more than 3 m from the crop. The occurrence of *A. fuscipennis* was influenced by the presence of EFNs and whitefly nymphs. Some plants allowed reproduction of both insect species. Experimental data confirmed that *A. fuscipennis* fed on EFNs. The role of the crop itself as a sugar source for the parasitoid was studied. It was confirmed that *P. vulgaris* stipels are EFNs and a source of sugar for *A. fuscipennis*. Several non-crop plant species in the agroecosystem and within the bean crop play a role in the whitefly/parasitoid interaction as alternate hosts for *T. vaporariorum* and as a source of concentrated sugar in EFNs for *A. fuscipennis*.

Keywords: *Phaseolus vulgaris*; Conservation biological control; Extra floral nectaries; Anthrone; Parasitoids

Yong-Gen Lou^a, Gu-Ren Zhang^b, Wen-Qing Zhang^b, Yang Hu^c, Jin Zhang^a. (^a State Key Laboratory of Rice Biology, Institute of Insect Science, Zhejiang University, Hangzhou 310058, China, ^b State Key Laboratory of Biocontrol, Institute of Entomology, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China, ^c Research and Development Center of Rice Production Technology, China National Rice Research Institute, Hangzhou 310006, China). **Biological control of rice insect pests in China. Biological Control, Volume 67(1) (2013): 8–20**

Rice is one of the most important food crops in the world. China has the second largest area of the rice growing in the world and the highest yield of rice produced. Infestation by insect pests, especially rice planthoppers, stem borers and leaf folders, is always a serious challenge to rice production in China. Current methods for controlling insect pests in China mainly include good farming practices, biological control, breeding and growing resistant varieties, and the use of chemical insecticides. However, for farmers, the favorite method for insect pest control is still the application of chemical insecticide, which not only causes severe environmental pollution and the resurgence of herbivores but also reduces populations of the natural enemies of herbivores. To control insect pests safely, effectively and sustainably, strategies encouraging biological control are currently demanded. Here we review the progress that has been made in the development and implementation of biological controls for rice in China since the 1970s. Such progress includes the species identification of the natural enemies of rice insect pests, the characterization of their biology, and the integration of biological controls in integrated pest management. To develop effective ecological engineering programs whose aim is to implement conservation biological controls, further research, including the evaluation of the roles of plants in non-crop habitats in conservation biological controls, volatiles in enhancing efficiency of natural enemies and natural enemies in manipulating insect pests, and education to increase farmers' knowledge of biological controls, is proposed.

Keywords: Rice; Insect pests; Biological control; Ecological engineering; China

Henok Kurabachew, Kerstin Wydra¹. (Gottfried Wilhelm Leibniz Universität Hannover, Institute of Plant Diseases and Plant Protection, Herrenhäuser Straße 2, Hannover D-30419, Germany). **Characterization of plant growth promoting rhizobacteria and their potential as bioprotectant against tomato bacterial wilt caused by *Ralstonia solanacearum*. Biological Control, Volume 67(1) (2013): 75–83**

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most destructive bacterial diseases of tomato and other economically important crops. To develop a biological control strategy against the pathogen, 150 isolates of rhizobacteria were isolated and screened for *in vitro* antibiosis. Thirteen isolates inhibited the growth of *R. solanacearum* and were identified with Fatty Acid Methyl Ester (GC-FAME) and biochemical methods as *Pseudomonas* spp., *Serratia marcescens* and *Bacillus cereus*. These isolates were further characterized for their plant growth promoting traits and production of the quorum sensing signal molecule acyl-homoserine lactones (AHL). Based on the *in vitro* antibiosis, four isolates, viz. *B. cereus* BC1AW, BC2BA, BC3AW, BC4SS and *Pseudomonas putida* PP3WT were selected for *ad planta* tests. Isolates BC1AW and PP3WT significantly reduced bacterial wilt incidence in tomato genotypes King Kong 2 (moderately resistant) in the pot experiments by 46.8% and 44.7% and in L390 (susceptible) by 33.6% and 30%, respectively. While in split root experiments they reduced wilt incidence by 48.7%, 43.2% and 25.7% and 20.1% in King Kong 2 and L390, respectively. Shoot dry weight also increased in plants treated with BC1AW and PP3WT and reduced the number of *R. solanacearum* cells by in mid-stems of both tomato genotypes. Hence, BC1AW and PP3WT were selected as promising biocontrol isolates whose effectiveness under field conditions and mode of action at molecular level should be investigated.

Keywords: Hydrogen cyanide; Induced resistance; Quorum sensing; *R. solanacearum*; Rhizobacteria; Siderophore

David Maxwell Suckling. (The New Zealand Institute of Plant & Food Research Ltd., PO Box 4704, Christchurch 8140, New Zealand). Benefits from biological control of weeds in New Zealand range from negligible to massive: A retrospective analysis. Biological Control, Volume 66(1) (2013): 27–32

Emerging concern highlighting non-target impacts in classical biological control of arthropods and weeds has heightened awareness of these risks but raised the risk of obscuring beneficial effects. This review applied a retrospective assessment of the benefits from weed biological control in New Zealand, using the framework designed for pre-clearance assessment of classical biological control. Of those agents released which can be assessed because of sufficient passage of time ($n = 33$), their impact has been assessed according to the modern criteria for judging beneficial effects used by New Zealand's Environmental Protection Authority (negligible, minimal, minor, moderate, major and massive). Cases with negligible benefit ($n = 12$) included failures to establish self-sustaining populations, while cases with minimal benefit ($n = 11$) included some where predation reduced the realized benefit of established organisms. The remaining cases offered massive ($n = 2$), major ($n = 1$), moderate ($n = 5$) or minor ($n = 2$) benefit. Suppression of ragwort (*Jacobaea vulgaris* Gaertn, 1754), and St. Johns wort (*Hypericum perforatum* L.) were considered to be massive in magnitude, offering long term ecosystem benefits of controlling invasive weeds. Improved clarity around risk and benefit could help improve the quality of debate on biological control, and the five step scale used in New Zealand may prove more widely useful elsewhere.

Keywords: Biological control, Weeds, Non-target, Risk, Benefit, Ecosystem

Samuel Julio Martins, Flavio Henrique Vasconcelos de Medeiros, Ricardo Magela de Souza, Mário Lúcio Vilela de Resende, Pedro Martins Ribeiro Junior. (Universidade Federal de Lavras, Departamento de Fitopatologia, 37200-000 Lavras, MG, Brazil).

Biological control of bacterial wilt of common bean by plant growth-promoting rhizobacteria. Biological Control, Volume 66(1) (2013): 65–71

Bacterial wilt (BW) caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*) is an emerging, seed-transmitted disease of common bean (*Phaseolus vulgaris*) in Brazil, and plant growth-promoting rhizobacteria (PGPR) have the potential to be used in disease management. The present work aimed at determining the potential of selected PGPR on the biological control of BW through seed treatment, growth promotion and induced resistance. Bean seeds cv. 'Pérola' were artificially inoculated with *Cff*, immersed in a PGPR suspension, and sown in 4 L pots containing a soil: sand mixture (2:1). Plants were assessed for seedling emergence (SE), speed emergence index (SEI), relative growth index (RGI), root dry weight (RDW), shoot dry weight (SDW), as well as biochemical plant responses in the presence or absence of *Cff*. The disease control ranged from 42% to 76%, respectively, for *Bacillus subtilis* UFLA285 and ALB629 compared to the untreated control. PGPR treatments also increased RGI, SDW, and RDW. Upon *Cff* inoculation, UFLA285 increased phenolics' content and ALB629 in the lignin accumulation compared to the untreated control. Without the pathogen inoculation, both PGPR promoted an increase in phenylalanine ammonia lyase activity and total phenolics content and UFLA285 in the lignin accumulation. Our findings demonstrated the potential of selected PGPR for disease control, enhancement of the RGI and biomass accumulation. Surprisingly, instead of a priming effect of PGPR, *Cff* apparently blocks the defense response development although the overall phenotype is disease control, suggesting there is a complementary and/or compensatory mode of action involved.

Keywords: Seed treatment; Resistance suppression; PGPR; ISR; POX; PAL

Fabio Andres Castillo Martinez^a, Eduardo Marcos Balciunas^a, Attilio Converti^b, Paul D. Cotter^c, Ricardo Pinheiro de Souza Oliveira^a. (^a Biochemical and Pharmaceutical Technology Department, Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Lineu Prestes 580, São Paulo 05508-900, Brazil, ^b Department of Civil, Chemical and Environmental Engineering, Pole of Chemical Engineering, Genoa University, I-16145 Genoa, Italy, ^c Teagasc Food Research Centre, Moorepark, Fermoy and Alimentary Pharmabiotic Centre, Cork, Ireland). **Bacteriocin production by *Bifidobacterium* spp. A review. Biotechnology Advances, Volume 31(4) (2013): 482–488**

Bacteriocins are ribosomally-synthesized antibacterial peptides. These compounds are produced by a broad variety of different bacteria belonging mainly to the genus *Bifidobacterium*, to which health promoting properties have frequently been attributed. However, despite the fact that the identification of *Bifidobacterium*-associated bacteriocins was first reported in 1980 and that they exhibit antimicrobial activity against pathogenic microorganisms such as *Listeria monocytogenes*, *Clostridium perfringens*, and *Escherichia coli*, relatively little information is still available about the antimicrobial compounds produced by strains of this genus. More detailed understanding of the action mechanisms of these antimicrobials could allow us to determine the extent to which their production contributes to the probiotic properties of specific bifidobacteria strains and, potentially, be of crucial significance for ultimate preservation of functional foods or pharmaceutical applications. Here we review what is already known about their structure, classification, mode of action, functionality, immunity, production and purification.

Keywords: Bacteriocins; *Bifidobacterium* spp.; Antimicrobial compounds; Lactic acid bacteria

Fang Chen^{1, 2}, Min Wang², Yu Zheng², Shuju Li³, Huizhe Wang³, Deduo Han¹ and Shangjing Guo¹. (¹School of Pharmaceutical, Liaocheng University, Liaocheng, 252059, Shandong, China, ²Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin, 300457, China, ³Tianjin Kernel Cucumber Research Institute, Tianjin, 300192, China). **The Effect of Biocontrol Bacteria on Rhizosphere Bacterial Communities Analyzed by Plating and PCR-DGGE. *Current Microbiology*, Volume 67(2) (2013): 177-182**

Bacillus subtilis B579, which was isolated from rhizosphere of cucumber, exhibited an excellent biocontrol activity on soil-born pathogens under greenhouse conditions. It could colonize in rhizosphere of cucumber with large number of populations after inoculated in plant growth season. To reveal the effect of high level colonization of *B. subtilis* B579 on rhizobacteria community structure, cultivation-based analysis coupled with denaturing gradient gel electrophoresis (DGGE) analysis were used to profile the changes of rhizobacteria community structure sampling at 1 week interval. Cultivation-based and DGGE fingerprinting analysis showed significant plant-dependent and seasonal shifts in rhizobacteria populations. Only minimal and transient effects were observed at 4–9 weeks after sowing in samples of B579 treatment, without the pathogen inoculation and showed the best plant growth potential. Sequencing of dominant bands excised from the gel revealed that *Streptomyces* sp. was the dominate species in soils before and after sowing. *Burkholderia* sp. was the dominate species in bulk soil, while *Bacillus* sp. was dominated in rhizosphere within the growth season. *Arthrobacter ramosus* and *Nocardioides* sp. were identified as the specific species in samples treated by B579 at the maturity and flowering stages of cucumber.

Aino M. Marttinen¹, Anna L. Haukioja¹, Mutlu Keskin² and Eva M. Söderling¹. (¹Institute of Dentistry, University of Turku, Lemminkäisenkatu 2, FI-20520 Turku, Finland, ²Periodontology Department, Faculty of Dentistry, Istanbul University, Fatih, 34390 Istanbul, Turkey. Email: esoder@utu.fi). **Effects of *Lactobacillus reuteri* PTA 5289 and *L. paracasei* DSMZ16671 on the Adhesion and Biofilm Formation of *Streptococcus mutans*. *Current Microbiology*, Volume 67(2) (2013): 193-199**

Probiotics have decreased the counts of salivary mutans streptococci (MS) in clinical studies. The aim of this study was to compare the effects of *Lactobacillus reuteri* PTA 5289 and *L. paracasei* DSMZ16671 on the adhesion of a reference strain and a clinical isolate of *Streptococcus mutans* and on the counts of MS in a biofilm. The adhesion of *S. mutans* Ingbritt and the clinical isolate *S. mutans* 2366 to a smooth glass surface and saliva-coated hydroxyapatite (SHA) were studied in the presence of and without the lactobacilli. A three-species biofilm formed on saliva-coated hydroxyapatite discs was used in the biofilm experiments. The lactobacilli did not affect adhesion to the glass surface but interfered with binding to SHA. No effects of the lactobacilli were detected on the MS levels in the three-species biofilms. The results of the SHA binding experiments best reflected the results of the existing clinical studies.

Baitian Xu¹, Hongyin Zhang², Keping Chen¹, Qin Xu², Yao Yao² and Hui Gao². (¹Institute of Life Sciences, Jiangsu University, Zhenjiang, 212013, Jiangsu, People's Republic of China, ²College of Food and Biological Engineering, Jiangsu University, Zhenjiang, 212013, Jiangsu, People's Republic of China. Email: dzxy_xubaitian@163.com). **Biocontrol**

of Postharvest *Rhizopus* Decay of Peaches with *Pichia caribbica*. *Current Microbiology*, Volume 67(2) (2013); 255-261

A new yeast antagonist, *Pichia caribbica*, isolated in our laboratory from the soil collected from unsprayed orchards, was evaluated for its biocontrol capability against *Rhizopus stolonifer* on peaches and the possible mechanisms involved. The decay incidence and lesion diameter of *Rhizopus* decay of peaches treated by *P. caribbica* were significantly reduced compared with the control fruits, and the higher the concentration of *P. caribbica*, the better the efficacy of the biocontrol. Rapid colonization of the yeast in peach wounds stored at 25 °C was observed. In peaches, the activities of peroxidase (POD), catalase (CAT), and phenylalanine ammonia-lyase (PAL) were significantly induced by *P. caribbica* treatment compared to those of the control fruits. All these results indicated that *P. caribbica* has a great potential for the development of commercial formulations to control postharvest *Rhizopus* decay of peaches. Its modes of action were based on competition for space and nutrients with pathogens, inducement of activities of defense-related enzymes such as POD, CAT, and PAL of peaches.

Biodegradation

Christopher J. Anderson¹. (¹School of Forestry and Wildlife Sciences, Auburn University, 301 Forestry and Wildlife Sciences Building, Auburn, AL 36849, USA. Email: andercj@auburn.edu). Degradation and Composition of Polycyclic Aromatic Hydrocarbons (PAHs) Following Oil Exposure in Experimental Salt Marshes. *Water, Air, & Soil Pollution*, Volume 224(2013):1608

Using mesocosms sodded with *Juncus roemerianus*, this study examined the change in concentration and composition of polycyclic aromatic hydrocarbons (PAHs) immediately following oil exposure. Wetland mesocosms were exposed to oil pre-treated through varying weathering and dosages to induce a range of PAH concentrations. Six trials (consisting of two or three mesocosms) were established using different combinations of oil dosages and weathering durations to elicit a range of initial PAH concentrations in the soil. Wetland soils were sampled per trial and analyzed 2 and 10 weeks following oil exposure for PAHs. Based on initial concentrations (2 weeks post-oil exposure), a total of 42 analytes (dominated by three-, four-, and five-ring compounds) were consistently detected across all trials. The \sum PAH concentration of wetland trials ranged from 108.1 to 906.2 mg kg⁻¹ dry weight. Individual PAH analyte reduction and degradation between sampling dates was calculated as the total percent change in analyte concentration and the percent change in analyte concentration relative to the concentration of biomarker hopane, respectively. All trials showed a significant relationship between PAH analyte molecular weight and its percent reduction and percent degradation. Trials with the highest initial \sum PAH concentration (659.4 and 906.2 mg kg⁻¹ dry weight) showed the most PAH loss with significantly greater reduction of high-molecular-weight analytes (four- and five-rings) than the other trials. However, when evaluated based on degradation, all trials showed similar trends of percent degradation relative to molecular weight. Our results suggest that oil loss through hydrologic export (i.e., washout from tidal action) may be important after initial oil exposure, and salt marsh soils may have a certain capacity to retain PAHs in soil.

Keywords: Polycyclic aromatic hydrocarbons, Oil spill, Salt marshes, *Juncus roemerianus* Mesocosms, Degradation Water washing

Shilpa K. Sonar¹, Reshma V. Wagh¹, Prashant S. Niphadkar¹, Praphulla N. Joshi¹, Shilpa S. Deshpande¹ and Shobhana V. Awate¹. (¹Catalysis & Inorganic Chemistry Division, CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pashan, Pune, 411008, India. Email: sv.awate@ncl.res.in). **Enhanced Dual-Effect of Adsorption and Photodegradation of SiO₂ Embedded TiO₂ Hybrid Catalyst for Improved Decolourization of Methylene Blue. *Water, Air, & Soil Pollution*, Volume 224(2013):1726**

Dual-effects of adsorption and photodegradation over titania, silica embedded titania, silica and commercial Degussa P-25 samples were studied for the decolourization of methylene blue in aqueous medium. Silica embedded titania and silica were prepared using inexpensive polymeric version of ethyl silicate as a source of silica. Catalysts were characterized by X-ray diffraction, scanning electron microscopy, UV-Vis spectroscopy and low temperature (77 K) nitrogen adsorption measurements. Among all the catalysts, silica embedded titania has exhibited faster decolourization of methylene blue solution on account of the enhancement of adsorption followed by degradation. An amount of the catalyst and the initial dye concentration of MB solution were found to influence the decolourization activity. Compared to titania catalyst, silica embedded titania and Degussa P-25 have shown the red shift in their UV-Vis spectrum. The experimental data of the reaction fitted well to the pseudo first order kinetic model. In present studies, the adsorption mechanism for the decolourization of MB solution was found to be applicable for an intra particle diffusion model.

Keywords: Adsorption, Photodegradation Silica embedded titania, Methylene blue Kinetic model

Behzad Mortazavi^{a,b,1}, Agota Horel^{a,b}, Jennifer S. Anders^{a,b}, Arsalan Mirjafari^{c,1}, Melanie J. Beazley^a, Patricia A. Sobecky^a. (^a Department of Biological Sciences, Box 870344, University of Alabama, Tuscaloosa, AL 35487, USA, ^b Dauphin Island Sea Lab, Dauphin Island, AL 36528, USA, ^c Department of Chemistry and Mathematics, Florida Gulf Coast University, Fort Myers 33965, USA). **Enhancing the biodegradation of oil in sandy sediments with choline: A naturally methylated nitrogen compound. *Environmental Pollution*, Volume 182(2013) : 53–62**

We investigated how additions of choline, a naturally occurring methylated nitrogen-containing compound, accelerated hydrocarbon degradation in sandy sediments contaminated with moderately weathered crude oil (4000 mg kg⁻¹ sediment). Addition of lauroylcholine chloride (LCC) and tricholine citrate (TCC) to oil contaminated sediments resulted in 1.6 times higher hydrocarbon degradation rates compared to treatments without added choline derivatives. However, the degradation rate constant for the oil contaminated sediments amended with LCC was similar to that in contaminated sediments amended with inorganic nitrogen, phosphorus, and glucose. Additions of LLC and TCC to sediments containing extensively weathered oil also resulted in enhanced mineralization rates. Cultivation-free 16S rRNA analysis revealed the presence of an extant microbial community with clones closely related to known hydrocarbon degraders from the *Gammaproteobacteria*, *Alphaproteobacteria*, and *Firmicutes* phyla. The results demonstrate that the addition of minimal amounts of organic compounds to oil contaminated sediments enhances the degradation of hydrocarbons.

Keywords: Crude oil; Biodegradation; Choline; Gulf of Mexico; Remediation

Berith Elkær Knudsen^{a, b, 1}, Lea Ellegaard-Jensen^{a, b, 1}, Christian Nyrop Albers^a, Søren Rosendahl^b, Jens Aamand^a. (^a Department of Geochemistry, Geological Survey of Denmark and Greenland (GEUS), Øster Voldgade 10, DK-1350 Copenhagen K, Denmark, ^b Department of Biology, Copenhagen University, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark). **Fungal hyphae stimulate bacterial degradation of 2,6-dichlorobenzamide (BAM). *Environmental Pollution*, Volume 181(2013) : 122–127**

Introduction of specific degrading microorganisms into polluted soil or aquifers is a promising remediation technology provided that the organisms survive and spread in the environment. We suggest that consortia, rather than single strains, may be better suited to overcome these challenges.

Here we introduced a fungal–bacterial consortium consisting of *Mortierella* sp. LEJ702 and the 2,6-dichlorobenzamide (BAM)-degrading *Aminobacter* sp. MSH1 into small sand columns. A more rapid mineralisation of BAM was obtained by the consortium compared to MSH1 alone especially at lower moisture contents. Results from quantitative real-time polymerase chain reaction (qPCR) demonstrated better spreading of *Aminobacter* when *Mortierella* was present suggesting that fungal hyphae may stimulate bacterial dispersal. Extraction and analysis of BAM indicated that translocation of the compound was also affected by the fungal hyphae in the sand. This suggests that fungal–bacterial consortia are promising for successful bioremediation of pesticide contamination.

Keywords: 2,6-dichlorobenzamide (BAM); Consortium; Bacterial dispersal; Pesticide biodegradation; Fungal–bacterial interactions

Katarzyna H. Kucharzyk^{1, 4}, Terence Soule³ and Thomas F. Hess^{1, 2}. (¹Environmental Biotechnology Institute, Environmental Science Program, University of Idaho, Moscow, ID 83844, USA, ²Department of Biological & Agricultural Engineering, University of Idaho, Moscow, ID 83844, USA, ³Department of Computer Science, University of Idaho, Janssen Engineering Building, Moscow, ID 83844, USA, ⁴Present address: Civil and Environmental Engineering, Duke University, Durham, NC 27709, USA). **Maximizing microbial perchlorate degradation using a genetic algorithm: consortia optimization. *Biodegradation*, Volume 24(5) (2013): 583-596**

Microorganisms in consortia perform many tasks more effectively than individual organisms and in addition grow more rapidly and in greater abundance. In this work, experimental datasets were assembled consisting of all possible selected combinations of perchlorate reducing strains of microorganisms and their perchlorate degradation rates were evaluated. A genetic algorithm (GA) methodology was successfully applied to define sets of microbial strains to achieve maximum rates of perchlorate degradation. Over the course of twenty generations of optimization using a GA, we saw a statistically significant 2.06 and 4.08-fold increase in average perchlorate degradation rates by consortia constructed using solely the perchlorate reducing bacteria (PRB) and by consortia consisting of PRB and accompanying organisms that did not degrade perchlorate, respectively. The comparison of kinetic rates constant in two types of microbial consortia additionally showed marked increases.

Keywords: Genetic algorithm, Perchlorate degradation, Consortium Resazurin

Giuseppe Toscano¹, Lucia Cavalca³, M. Letizia Colarieti^{1,2}, Rosalia Scelza^{2, 4}, Riccardo Scotti⁴, Maria A. Rao⁴, Vincenza Andreoni³, Sonia Ciccazzo^{2,3} and Guido Greco^{1,2}. (¹DIC, Università degli Studi di Napoli Federico II, Naples, Italy, ²AMRA s.c.a.r.l., Naples, Italy, ³DiSTAM, Università degli Studi di Milano, Milan, Italy, ⁴DiSSPA, Università degli Studi di Napoli Federico II, Naples, Italy). **Aerobic biodegradation of propylene glycol by soil bacteria. *Biodegradation*, Volume 24(5) (2013): 603-613**

Propylene glycol (PG) is a main component of aircraft deicing fluids and its extensive use in Northern airports is a source of soil and groundwater contamination. Bacterial consortia able to grow on PG as sole carbon and energy source were selected from soil samples taken along the runways of Oslo Airport Gardermoen site (Norway). DGGE analysis of enrichment cultures showed that PG-degrading populations were mainly composed by *Pseudomonas* species, although *Bacteroidetes* were found, as well. Nineteen bacterial strains, able to grow on PG as sole carbon and energy source, were isolated and identified as different *Pseudomonas* species. Maximum specific growth rate of mixed cultures in the absence of nutrient limitation was 0.014 h^{-1} at 4°C . Substrate C:N:P molar ratios calculated on the basis of measured growth yields are in good agreement with the suggested values for biostimulation reported in literature. Therefore, the addition of nutrients is suggested as a suitable technique to sustain PG aerobic degradation at the maximum rate by autochthonous microorganisms of unsaturated soil profile.

Keywords: Aircraft deicing fluids, Propylene glycol, Soil bioremediation, Biodegradation kinetics, *Pseudomonas*.

Deepak Singh¹ and Gurunath Ramanathan¹. (¹Department of Chemistry, Indian Institute of Technology, Kanpur, 208016, India. Email: gurunath@iitk.ac.in). **Biomining of 3-nitrotoluene by *Diaphorobacter* species. *Biodegradation*, Volume 24(5) (2013): 645-655**

Three bacterial strains utilizing 3-nitrotoluene (3-NT) as a sole source of carbon, nitrogen and energy were isolated from an industrial wastewater treatment plant. Biochemical tests and 16S rDNA sequence analysis revealed that the isolated strains belonged to *Diaphorobacter* sp. Detailed studies were carried out with *Diaphorobacter* sp. strain DS2. Degradation of 3-NT by *Diaphorobacter* sp. strain DS2 was accompanied by the release of nitrite in the culture broth with increase in biomass. Total organic carbon analysis confirmed the extensive mineralization of 3-NT. The strain could degrade 3-methylcatechol, 4-methylcatechol and catechol easily suggesting that the degradation pathway could involve these as possible intermediates. Successful PCR amplification of the oxygenase large subunit and the presence of high activity for catechol 2,3-dioxygenase in the crude cell lysate further confirmed that the degradation of 3-NT occurred through (methyl)catechol intermediates in strain DS2. The strain DS2 was found to degrade other isomers of mononitrotoluene (2-NT and 4-NT) and nitrobenzene as well.

Keywords: Nitrotoluene, Nitrobenzene, Biomining, Dioxygenase, *Diaphorobacter* sp.

Indumathy Jayamani¹ and Alison M. Cupples¹. (¹A135 Research Engineering Complex, Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI 48824, USA. Email: cupplesa@msu.edu). **Effect of isobutanol on toluene biodegradation in nitrate amended, sulfate amended and methanogenic enrichment microcosms. *Biodegradation*, Volume 24(5) (2013): 657-663**

Isobutanol is an alternate fuel additive that is being considered because of economic and lower emission benefits. However, future gasoline spills could result in co-contamination of isobutanol with gasoline components such as benzene, toluene, ethyl-benzene and xylene. Hence, isobutanol could affect the degradability of gasoline components thereby having an effect on contaminant plume length and half-life. In this study, the effect of isobutanol on the biodegradation of a model gasoline component (toluene) was examined in laboratory microcosms. For this, toluene and isobutanol were added to six different toluene degrading laboratory microcosms under sulfate amended, nitrate amended or methanogenic conditions. While toluene biodegradation was not greatly affected in the presence of isobutanol in five out of the six different experimental sets, toluene degradation was completely inhibited in one set of microcosms. This inhibition occurred in sulfate amended microcosms constructed with inocula from wastewater treatment plant activated sludge. Our data suggest that toluene degrading consortia are affected differently by isobutanol addition. These results indicate that, if co-contamination occurs, in some cases the in situ half-life of toluene could be significantly extended.

Keywords: Toluene, Isobutanol, Anaerobic biodegradation, Methanogenic Nitrate/sulfate reducing

Kazunari Sei^{1, 2}, Keiko Miyagaki¹, Takashi Kakinoki¹, Kunihiro Fukugasako¹, Daisuke Inoue^{1, 2} and Michihiko Ike¹. (¹Division of Sustainable Energy and Environmental Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan, ²Department of Health Science, School of Allied Health Sciences, Kitasato University, 1-15-1 Kitasato, Sagami-hara-Minami, Kanagawa 252-0373, Japan). **Isolation and characterization of bacterial strains that have high ability to degrade 1,4-dioxane as a sole carbon and energy source. *Biodegradation*, Volume 24(5) (2013): 665-674**

Four novel metabolic 1,4-dioxane degrading bacteria possessing high ability to degrade 1,4-dioxane (designated strains D1, D6, D11 and D17) were isolated from soil in the drainage area of a chemical factory. Strains D6, D11 and D17 were allocated to Gram-positive actinomycetes, similar to previously reported metabolic 1,4-dioxane degrading bacteria, whereas strain D1 was allocated to Gram-negative *Afipia* sp. The isolated strains could utilize a variety of carbon sources, including cyclic ethers, especially those with carbons at position 2 that were modified with methyl- or carbonyl-groups. The cell yields on 1,4-dioxane were relatively low (0.179–0.223 mg-protein (mg-1,4-dioxane)⁻¹), which was likely due to requiring energy for C–O bond fission. The isolated strains showed 2.6–13 times higher specific 1,4-dioxane degradation rates (0.052–0.263 mg-1,4-dioxane (mg-protein)⁻¹ h⁻¹) and 2.3–7.8 fold lower half saturation constants (20.6–69.8 mg L⁻¹) than the most effective 1,4-dioxane degrading bacterium reported to date, *Pseudonocardia dioxanivorans* CB1190, suggesting high activity and affinity toward 1,4-dioxane degradation. Strains D1 and D6 possessed inducible 1,4-dioxane degrading enzymes, whereas strains D11 and D17 possessed constitutive ones. 1,4-Dioxane degradation (100 mg L⁻¹) by *Afipia* sp. D1 was not affected by the co-existence of up to 3,000 mg L⁻¹ of ethylene glycol. The effects of initial pH, incubation temperature and NaCl concentration on 1,4-dioxane degradation by the four strains revealed that they could degrade 1,4-dioxane under a relatively wide range of conditions, suggesting that they have a certain adaptability and applicability for industrial wastewater treatment.

Keywords: 1,4-dioxane Metabolic 1,4-dioxane degrading bacteria, Isolation and characterization, Wastewater treatment

William M. Moe¹, Weili Hu¹, Trent A. Key¹ and Kimberly S. Bowman¹. (¹Department of Civil and Environmental Engineering, Louisiana State University, 3515B Patrick Taylor Hall, Baton Rouge, LA 70803, USA. Email: moemwil@lsu.edu). **Removal of the sesquiterpene β -caryophyllene from air via biofiltration: performance assessment and microbial community structure. *Biodegradation*, Volume 24(5) (2013): 685-698**

Experiments were conducted in a laboratory-scale biofilter to assess the ability of a fixed-film biological process to treat an air stream containing β -caryophyllene, a sesquiterpene emitted by a variety of conifer trees as well as industrial wood processing operations. Treatment performance was evaluated under a variety of pollutant loading conditions and nutrient supply rates over an operational period lasting more than 240 days. At empty bed contact times (EBCTs) as low as 10 s and daily average pollutant loading rate as high as 24.2 g C/(m³ h) (grams pollutant measured as carbon per cubic meter packed bed volume per hour), removal efficiencies in excess of 95 % were observed when sufficient nutrients were supplied. Results demonstrate that, as with biofilters treating other compounds, biofilters treating β -caryophyllene can experience local nutrient limitations that result in diminished performance. The biofilter successfully recovered high removal efficiency within a few days after resumption of pollutant loading following a 14-day interval of no contaminant loading. Construction of a 16S rRNA gene library via pyrosequencing revealed the presence of a high proportion of bacteria clustering within the genera *Gordonia* (39.7 % of the library) and *Rhodanobacter* (37.6 %). Other phylotypes detected at lower relative abundances included *Pandoraea* (6.2 %), unclassified *Acetobacteraceae* (5.5 %), *Dyella* (3.3 %), unclassified *Xanthomonadaceae* (2.6 %), *Mycobacterium* (1.8 %), and *Nocardia* (0.6 %). Collectively, results demonstrate that β -caryophyllene can be effectively removed from contaminated gas streams using biofilters.

Keywords: Biofilter, Biofiltration, β -Caryophyllene, Community structure, Conifer, Lumber *Gordonia* Pinene, Sesquiterpene Shut down, Terpene Wood

Maricy Raquel Lindenbah Bonfá¹, Matthew James Grossman¹, Francine Piubeli¹, Encarnación Mellado² and Lucia Regina Durrant¹. (¹Departamento de Ciência de Alimentos-FEA, Universidade Estadual de Campinas-UNICAMP, Rua Monteiro Lobato, 80 CEP, Campinas, SP, 13083-862, Brazil, ²Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, Sevilla, Spain). **Phenol degradation by halophilic bacteria isolated from hypersaline environments. *Biodegradation*, Volume 24(5) (2013): 699-709**

Phenol is a toxic aromatic compound used or produced in many industries and as a result a common component of industrial wastewaters. Phenol containing waste streams are frequently hypersaline and therefore require halophilic microorganisms for efficient biotreatment without dilution. In this study three halophilic bacteria isolated from different saline environments and identified as *Halomonas organivorans*, *Arhodomonas aquaeolei* and *Modicisalibacter tunisiensis* were shown to be able to grow on phenol in hypersaline media containing 100 g/L of total salts at a concentration of 3 mM (280 mg/L), well above the concentration found in most waste streams. Genes encoding the aromatic dioxygenase enzymes catechol 1,2 dioxygenase and protocatechuate 3,4-dioxygenase were present in all strains as determined by PCR amplification using primers specific for highly conserved regions of the genes. The gene for protocatechuate

3,4-dioxygenase was cloned from the isolated *H. organivorans* and the translated protein was evaluated by comparative protein sequence analysis with protocatechuate 3,4-dioxygenase proteins from other microorganisms. Although the analysis revealed a wide range of sequence divergence among the protocatechuate 3,4-dioxygenase family, all of the conserved domain amino acid structures identified for this enzyme family are identical or conservatively substituted in the *H. organivorans* enzyme.

Keywords: Phenol Biodegradation, Hypersaline, Halophile, Halomonas, Arhodomonas, Modicisalibacter, Catechol 1,2 dioxygenase, Protocatechuate 3,4-dioxygenase

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The effect of the terpenes α -pinene, eucalyptol, and limonene, individually and as mixtures, on atrazine (ATZ) biodegradation and on biological activity in a biobed biomixture was evaluated. Additionally, terpenes emitted from the biomixture were captured using solid-phase microextraction. Terpenes added individually at relatively low concentrations ($50 \mu\text{g kg}^{-1}$) significantly enhanced ATZ degradation and biological activity during the first incubation days. No significant effect on ATZ degradation was found from adding the terpene mixture, and, interestingly, an inhibitory effect on phenoloxidase activity was found during the first 20 days of incubation when mixed terpenes were present at $100 \mu\text{g kg}^{-1}$. Capturing terpenes demonstrated that during the first hour of incubation a significant fraction of the terpenes was volatilized. These results are the first to demonstrate the feasibility of using terpenes to enhance the degradation of a pesticide. However, successive applications of terpenes or the addition of materials that slowly release terpenes could sustain the ATZ degradation enhancement.

Keywords: Biobeds, Atrazine. Biomixture. Biodegradation. Terpenes

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While bioremediation of total petroleum hydrocarbons (TPH) is in general a robust technique, heterogeneity in terms of contaminant and environmental characteristics can impact the extent of biodegradation. The current study investigates the implications of different soil matrix types (anthropogenic fill layer, peat, clay, and sand) and bioavailability on bioremediation of an aged

diesel contamination from a heterogeneous site. In addition to an uncontaminated sample for each soil type, samples representing two levels of contamination (high and low) were also used; initial TPH concentrations varied between 1.6 and 26.6 g TPH/kg and bioavailability between 36 and 100 %. While significant biodegradation occurred during 100 days of incubation under biostimulating conditions (64.4–100 % remediation efficiency), low bioavailability restricted full biodegradation, yielding a residual TPH concentration. Respiration levels, as well as the abundance of *alkB*, encoding mono-oxygenases pivotal for hydrocarbon metabolism, were positively correlated with TPH degradation, demonstrating their usefulness as a proxy for hydrocarbon biodegradation. However, absolute respiration and *alkB* presence were dependent on soil matrix type, indicating the sensitivity of results to initial environmental conditions. Through investigating biodegradation potential across a heterogeneous site, this research illuminates the interplay between soil matrix type, bioavailability, and bioremediation and the implications of these parameters for the effectiveness of an in situ treatment.

Keywords: Biodegradation, Soil matrix, Bioavailability, Aged contamination Microbial respiration Mono-oxygenase

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The potential of kenaf (*Hibiscus cannabinus* L.) and corn (*Zea mays* L.) for accumulation of cadmium and zinc was investigated. Plants have been grown in lysimetres containing dredging sludge, a substratum naturally rich in trace metals. Biomass production was determined. Sludge and water percolating from lysimeters were analyzed by atomic absorption spectrometry. No visible symptoms of toxicity were observed during the three- month culture. Kenaf and corn tolerate trace metals content in sludge. Results showed that Zn and Cd were found in corn and kenaf shoots at different levels, 2.49 mg/kg of Cd and 82.5 mg/kg of Zn in kenaf shoots and 2.1 mg/kg of Cd and 10.19 mg/kg in corn shoots. Quantities of extracted trace metals showed that decontamination of Zn and Cd polluted substrates is possible by corn and kenaf crops. Tolerance and bioaccumulation factors indicated that both species could be used in phytoremediation.

Keywords: Dredging sludge, Cadmium Zinc, Bioaccumulation factor

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Very little is known about the influence of bacterial-fungal ecological interactions on polycyclic aromatic hydrocarbon (PAH) dissipation in soils. *Fusarium solani* MM1 and *Arthrobacter oxydans* MsHM11 can dissipate PAHs in vitro. We investigated their interactions and their effect

on the dissipation of three PAHs—phenanthrene (PHE), pyrene (PYR) and dibenz(a,h)anthracene (DBA)—in planted microcosms, in sterile sand or non-sterile soil. In sterile sand microcosms planted with alfalfa, the two microbes survived and grew, without any significant effect of co-inoculation. Co-inoculation led to the dissipation of 46 % of PHE after 21 days. In soil microcosms, whether planted with alfalfa or not, both strains persisted throughout the 46 days of the experiment, without any effect of co-inoculation or of alfalfa, as assessed by real-time PCR targeting taxon-level indicators, i.e. Actinobacteria 16S rDNA and the intergenic transcribed spacer specific to the genus *Fusarium*. The microbial community was analyzed by temporal temperature gradient electrophoresis and real-time PCR targeting bacterial and fungal rDNA and PAH-ring hydroxylating dioxygenase genes. These communities were modified by PAH pollution, which selected PAH-degrading bacteria, by the presence of alfalfa and, concerning the bacterial community, by inoculation. PHE and PYR concentrations significantly decreased (91 and 46 %, respectively) whatever the treatment, but DBA concentration significantly decreased (30 %) in planted and co-inoculated microcosms only.

Keywords: *Arthrobacter oxydans*, *Fusarium solani*, PAH, Soil remediation, Rhizosphere microcosms

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Biofouling is a serious problem on filter membranes of water purification systems due to formation of bacterial biofilms, which can be detrimental to the membrane performance. Biofouling occurs on membrane surface and therefore greatly influences the physical and chemical aspects of the surface. Several membranes including microfiltration, ultrafiltration, and reverse osmosis (RO) membranes were used to learn about the anti-biofouling properties of vanillin affecting the membrane performances. Vanillin has been recognized as a potential quorum quenching compound for *Aeromonas hydrophila* biofilms. The initial attachment and dynamics of biofilm growth were monitored using scanning electron microscopy and confocal laser scanning microscopy. Biofilm quantities were measured using a plate count method and total protein determinations. Vanillin addition was effective in the prevention of biofilm formation on the tested membrane surfaces. Among the membranes, RO membranes made with cellulose acetate showed the most substantial reduction of biofilm formation by addition of vanillin. The biofilm reduction was confirmed by the results of surface coverage, biomass and protein accumulation. The HPLC spectrum of the spent culture with vanillin addition showed that vanillin may interfere with quorum sensing molecules and thus prevent the formation of the biofilms.

Keywords: Anti-biofouling, *Aeromonas hydrophila*, RO membrane, Vanillin Quorum sensing

Cory D. Penn¹ and Steven L. Daniel¹. (¹Department of Biological Sciences, Eastern Illinois University, 600 Lincoln Avenue, Charleston, IL 61920, USA). **Salicylate Degradation by the Fungal Plant Pathogen *Sclerotinia sclerotiorum*. Current Microbiology, Volume 67(2) (2013): 218-225**

The fungal plant pathogen *Sclerotinia sclerotiorum* was studied to determine its ability to degrade salicylate, an important defense-signaling molecule in plants. *S. sclerotiorum* D-E7 was grown at 25 °C in an undefined medium (50 ml) containing minerals, 0.1 % soytone, 50 mM MES buffer (pH 6.5), 25 mM glucose, and 1 mM salicylate. Glucose, oxalate, and salicylate concentrations were monitored by HPLC. *S. sclerotiorum* D-E7 was found to be active in salicylate degradation. However, salicylate alone was not growth supportive and, at higher levels (10 mM), inhibited glucose-dependent growth. Biomass formation (130 mg [dry wt] of mycelium per 50 ml of undefined medium), oxalate concentrations (~10 mM), and culture acidification (final culture pH approximated 5) were essentially the same in cultures grown with or without salicylate (1 mM). Time-course analyses revealed that salicylate degradation and glucose consumption were complete after 7 days of incubation and was concomitant with growth. Trace amounts of catechol, a known intermediate of salicylate metabolism, were detected during salicylate degradation. Overall, these results indicated that *S. sclerotiorum* has the ability to degrade salicylate and that the presence of low levels of salicylate did not affect growth or oxalate production by *S. sclerotiorum*.

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The toxic textile dye, Disperse Brown 118, was degraded by *Brevibacillus laterosporus*. 96 % decolorization was achieved within 48 h at pH 7, 40 °C at 50 mg dye l⁻¹ accompanied by significant increases in the activities of veratryl alcohol oxidase, tyrosinase and NADH-DCIP reductase. HPTLC and FT-IR spectroscopy confirmed biodegradation after dye decolorization. As identified by GC-MS, biodegradation products of Disperse Brown 118 were N-carbamoyl-2-[(8-chloroquinazolin-4-yl)oxy] acetamide and N-carbamoyl-2-(quinazolin-4-yloxy)acetamide which were much less toxic than parent dye as evidenced by phytotoxicity tests.

Keywords: Biodegradation, Bioremediation, Biotransformation, Detoxification, Phytotoxicity

Atul Nayak¹ and Diganta Bhusan Das¹. (¹Department of Chemical Engineering, Loughborough University, Loughborough, LE11 3TU, UK. Email: d.b.das@lboro.ac.uk). **Potential of biodegradable microneedles as a transdermal delivery vehicle for lidocaine. Biotechnology Letters, Volume 35(9) (2013): 1351-1363**

There has been an increasing interest in applying biotechnology in formulating and characterising new and innovative drug delivery methods, e.g., drug-loaded biodegradable microneedles within the area of transdermal delivery technology. Recently, microneedles have been proposed for use in pain management, e.g., post-operative pain management through

delivery of a local anaesthetic, namely, lidocaine. Lidocaine is a fairly common, marketed prescription-based, local anaesthetic pharmaceutical, applied for relieving localised pain and lidocaine-loaded microneedles have been explored. The purpose of this review is to evaluate the properties of biodegradable polymers that may allow the preparation of microneedle systems, methods of preparing them and pharmacokinetic conditions in considering the potential use of lidocaine for delivery through the skin.

Keywords: Biodegradable, Drug delivery, Lidocaine, Microneedles, Substrate, Tensile strength

Maria Dimarogona¹, Evangelos Topakas¹ and Paul Christakopoulos². (¹Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 5 Iroon Polytechniou Str, Zografou Campus, 15700 Athens, Greece, ²Biochemical and Chemical Process Engineering, Division of Sustainable Process Engineering, Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, 97187 Luleå, Sweden. Email: paul.christakopoulos@ltu.se). **Recalcitrant polysaccharide degradation by novel oxidative biocatalysts. Applied Microbiology and Biotechnology, Volume 97(19) (2013): 8455-8465**

The classical hydrolytic mechanism for the degradation of plant polysaccharides by saprophytic microorganisms has been reconsidered after the recent landmark discovery of a new class of oxidases termed lytic polysaccharide monoxygenases (LPMOs). LPMOs are of increased biotechnological interest due to their implication in lignocellulosic biomass decomposition for the production of biofuels and high-value chemicals. They act on recalcitrant polysaccharides by a combination of hydrolytic and oxidative function, generating oxidized and non-oxidized chain ends. They are copper-dependent and require molecular oxygen and an external electron donor for their proper function. In this review, we present the recent findings concerning the mechanism of action of these oxidative enzymes and identify issues and questions to be addressed in the future.

Keywords: Lytic polysaccharide monoxygenases CBM33, Cellobiose dehydrogenase GH61, Bioethanol, Cellulose

Yukiko Shinozaki¹, Yoshihiro Kikkawa², Shun Sato³, Tokuma Fukuoka³, Takashi Watanabe¹, Shigenobu Yoshida¹, Toshiaki Nakajima-Kambe⁴ and Hiroko K. Kitamoto¹. (¹National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba Ibaraki, 305-8604, Japan, ²Electronics and Photonics Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 4, 1-1-1 Higashi, Tsukuba Ibaraki, 305-8562, Japan, ³Research Institute for Innovation in Sustainable Chemistry, AIST, Tsukuba Central 5-2, 1-1-1 Higashi, Tsukuba Ibaraki, 305-8565, Japan, ⁴Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba Ibaraki, 305-8572, Japan. Email: kitamoto@affrc.go.jp). **Enzymatic degradation of polyester films by a cutinase-like enzyme from *Pseudozyma antarctica*: surface plasmon resonance and atomic force microscopy study. Applied Microbiology and Biotechnology, Volume 97(19) (2013): 8591-8598**

Enzymatic degradation of polyester films by a cutinase-like enzyme from *Pseudozyma antarctica* JCM10317 (PaE) was analyzed by surface plasmon resonance (SPR). The adsorption

of PaE and the degradation rate for polyester films were quantitatively monitored by a positive and negative SPR signal shifts, respectively. The decrease in SPR signal and the erosion depth of amorphous poly(L-lactide) (a-PLLA) film measured by atomic force microscopy (AFM) had a linear relationship, and the weight loss was estimated from the AFM data combined with a density of a-PLLA film. Furthermore, SPR sensorgrams for various polyester films showed that degradation rate of poly(ϵ -caprolactone) and poly(butylene succinate-*co*-adipate) which contain C6 units was higher than that of other polyesters such as poly(butylene succinate) and a-PLLA. These results suggest that C6 is the preferred chain length as substrates for PaE.

Keywords: Atomic force microscopy, Biodegradable, Cutinase Polyesters, Surface plasmon resonance

Weiwei Zhang¹, Kun Yin^{1, 2} and Lingxin Chen¹. (¹Key Laboratory of Coastal Zone Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research(YIC), Chinese Academy of Sciences (CAS), Shandong Provincial Key Laboratory of Coastal Zone Environmental Processes, YICCAS, 17 Chunhui Road, Yantai, Shandong, 264003, People's Republic of China, ²University of Chinese Academy of Sciences, Beijing, 100049, People's Republic of China. Email: lxchen@yic.ac.cn). **Bacteria-mediated bisphenol A degradation. Applied Microbiology and Biotechnology, Volume 97(13) (2013): 5681-5689**

Bisphenol A (BPA) is an important monomer in the manufacture of polycarbonate plastics, food cans, and other daily used chemicals. Daily and worldwide usage of BPA and BPA-contained products led to its ubiquitous distribution in water, sediment/soil, and atmosphere. Moreover, BPA has been identified as an environmental endocrine disruptor for its estrogenic and genotoxic activity. Thus, BPA contamination in the environment is an increasingly worldwide concern, and methods to efficiently remove BPA from the environment are urgently recommended. Although many factors affect the fate of BPA in the environment, BPA degradation is mainly depended on the metabolism of bacteria. Many BPA-degrading bacteria have been identified from water, sediment/soil, and wastewater treatment plants. Metabolic pathways of BPA degradation in specific bacterial strains were proposed, based on the metabolic intermediates detected during the degradation process. In this review, the BPA-degrading bacteria were summarized, and the (proposed) BPA degradation pathway mediated by bacteria were referred.

Keywords: Bisphenol A, Bacteria Degradation, Degradation pathway

Julia Giebler^{*}, Lukas Y. Wick, Antonis Chatzinotas, Hauke Harms. (Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany. Correspondence: Julia Giebler, Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, Permoserstrasse 15, 04318 Leipzig, Germany. Tel.: +49 (0)341 235 1374; fax: +49 (0)341 235 1351; e-mail: julia.giebler@ufz.de). **Alkane-degrading bacteria at the soil–litter interface: comparing isolates with T-RFLP-based community profiles. FEMS Microbiology Ecology, Volume 86(1) (2013): 45–58**

Alkane-degrading bacteria were isolated from uncontaminated soil microcosms, which had been incubated with maize litter as natural alkane source. The isolates served to understand spatio-temporal community changes at the soil–litter interface, which had been detected using *alkB* as a

functional marker gene for bacterial alkane degraders. To obtain a large spectrum of isolates, liquid subcultivation was combined with a matrix-assisted enrichment (Teflon membranes, litter). Elevated cell numbers of alkane degraders were detected by most probable number counting indicating enhanced alkane degradation potential in soil in response to litter treatment. Partial 16S rRNA gene sequencing of 395 isolates revealed forty different phylogenetic groups [operational taxonomic units (OTUs)] and spatio-temporal shifts in community composition. Ten OTUs comprised so far unknown alkane degraders, and five OTUs represented putative new bacterial genera. The combination of enrichment methods yielded a higher diversity of isolates than liquid subcultivation alone. Comparison of 16S rRNA gene T-RFLP profiles indicated that many alkane degraders present in the enrichments were not detectable in the DNA extracts from soil microcosms. These possibly rare specialists might represent a seed bank for the alkane degradation capacity in uncontaminated soil. This relevant ecosystem function can be fostered by the formation of the soil–litter interface.

Keywords: soil alkane degradation potential; cultivation; growth matrix-based enrichment; *alkB* T-RFLP; seed bank; rare biosphere

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Phenoxyacetic acids can be degraded by diverse soil microorganisms. Nevertheless, we miss information about the succession of 4-chloro-2-methylphenoxyacetic acid (MCPA) degraders in micro-environments of soils as well as specific functions of different microbial groups during MCPA degradation. We studied MCPA degradation at the soil–litter interface in a microcosm experiment and followed the succession of different degrader populations by quantifying the abundance of 16S rRNA genes as well as, the fungal ITS fragment and the functional genes *tfdA* (in total and divided into three classes) and *cadA*. Adjacent to the litter layer, a dynamic depletion zone of MCPA indicated that the litter effect on MCPA degradation depends on substrate availability and the affected soil volume. The increase of the *tfdA* class III and *cadA* genes was linked to MCPA mineralisation. Total abundance of *tfdA* genes was dominated by class I MCPA degraders and did not reflect MCPA degradation potential of the soil. Litter addition induced the development of pioneer and late-stage fungal communities, which were probably both involved in MCPA degradation. The results underline the importance of the ecological behaviour of different degrader populations for the understanding of herbicide degradation in soils.

Keywords: *tfdA* diversity; *cada* ; TaqMan probe-based qPCR; substrate availability

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To assess the involvement of the genus *Variovorax* and the linuron hydrolase gene *libA* in *in situ* linuron degradation in agricultural fields, changes in *Variovorax* community size and composition, in *libA* abundance and in linuron mineralization capacity were monitored in field soil plots either treated or not with a linuron-containing herbicide mixture. Changes in *Variovorax* community composition, due to the proliferation of a hereto unknown *Variovorax* phylotype D, and increases in *libA* numbers occurred concomitant to increases in linuron mineralization capacity in the plot treated with the herbicide mixture. The observations suggest that *Variovorax* and *libA* proliferated as a response to linuron and hence their contribution to *in situ* linuron degradation. The involvement of *Variovorax* phylotype D and *libA* in linuron degradation in the examined soil was supported by laboratory soil microcosm experiments. Attempts to enrich in suspended cultures and isolate the organism corresponding to phylotype D from the soil were unsuccessful as the enrichment resulted in replacement of *Variovorax* phylotype D by other *Variovorax* phylotypes. This illustrates that linuron-degrading strains isolated by liquid enrichment cultures are not always representatives of those responsive to linuron in the field, although the genus specificity of linuron degradation was retained.

Keywords: phenylurea herbicide; pesticide biodegradation; enrichment bias; biomarkers

Terrence H. Bell^{1,2}, Etienne Yergeau², Dave F. Juck², Lyle G. Whyte¹, Charles W. Greer^{2,*}. (¹Department of Natural Resource Sciences, McGill University, Sainte-Anne-de-Bellevue, QC, Canada, ²National Research Council Canada, EME-Montreal, Montreal, QC, Canada. *Correspondence: Charles Greer, National Research Council Canada, EME-Montreal, 6100 Royalmount Avenue, Montreal, QC, Canada H4P 2R2. Tel.: (514) 496 6182; fax: (514) 496 6265; e-mail: charles.greer@cnrc-nrc.gc.ca). Alteration of microbial community structure affects diesel biodegradation in an Arctic soil. *FEMS Microbiology Ecology*, Volume 85(1) (2013): 51–61

A wide range of microbial taxa are active in hydrocarbon-contaminated Arctic soils, and many are capable of hydrocarbon metabolism. The most effective hydrocarbon degraders may not naturally dominate following contamination events, so shifts in microbial abundance could potentially increase hydrocarbon biodegradation. In this study, we contaminated an Arctic soil with diesel and used gentamicin and vancomycin to inhibit distinct portions of the microbial community. We measured diesel loss using gas chromatography, bacterial and fungal abundance with qPCR, and assessed bacterial diversity and community composition through Ion Torrent sequencing of 16S rRNA gene amplicons. The combined addition of both antibiotics increased diesel biodegradation significantly relative to the no-antibiotic treatment, despite reduced bacterial and fungal abundance; however, this effect was not observed when nutrients were also added. All treatments produced unique bacterial communities, and both *Xanthomonadaceae* and *Micrococcineae* were dominant in the dual antibiotic treatment. The bacterial communities

resulting from dual gentamicin and vancomycin addition were similar both with and without nutrients, although nutrient addition produced a much larger fungal population, which may partly explain the differences in biodegradation between these two treatments. These results suggest that the most efficient hydrocarbon-degrading community may not always be promoted naturally in contaminated soils.

Keywords: community composition; Arctic; bioremediation; hydrocarbons; Ion Torrent; soil microorganisms

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The aim of the present study is to constitutively express heterologous oxalate decarboxylase (OxdC) in *Lactobacillus plantarum* and to examine its ability to degrade oxalate *in vitro* for their future therapy against enteric hyperoxaluria.

In this study, we generated a recombinant strain of *Lb. plantarum* to constitutively overexpress *B. subtilis* oxalate decarboxylase (*oxdC*) using a host lactate dehydrogenase promoter (P_{ldhL}). The recombinant *Lb. plantarum* was able to degrade more than 90% oxalate compared to 15% by the wild type. In addition, the recombinant strain also had higher tolerance up to 500 mmol I^{-1} oxalate.

We developed a recombinant *Lb. plantarum* NC8 that constitutively expressed heterologous oxalate decarboxylase and degraded oxalate efficiently under *in vitro* conditions.

The long-term aim is to develop an efficient strain for future therapy against oxalosis.

Keywords: hyperoxaluria; oxalate degradation; probiotic potential; recombinant *Lactobacillus plantarum*

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In order to accelerate the bioconversion process of press-shredded empty fruit bunches (EFB), the effect of high-pressure steam pre-treatment (HPST) in degrading the lignocellulosic structure was investigated. HPST was carried out under various sets of temperature/pressure conditions such as 170/0.82, 190/1.32, 210/2.03, and 230°C/3.00 MPa. It was noted that after HPST, the surface texture, color, and mechanical properties of the treated EFB had obviously altered. Scanning electron micrographs of the treated EFB exhibited effective surface erosion that had occurred along the structure. Moreover, the Fourier transform infrared and thermogravimetric analyses showed the removal of silica bodies and hemicellulose ingredients. X-ray diffraction profiles of the treated EFB indicated significant increases in crystallinity. These results reveal that HPST is an effective pre-treatment method for altering the physicochemical properties of the EFB and enhancing its biodegradability characteristics for the bioconversion process.

Keywords: Empty fruit bunches; High-pressure steam; Selective degradation; Lignocellulosic structure; Pre-treatment

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The enzymatic hydrolysis of cassava starch (*Mannihot esculenta*) to glucose was studied; considering various process parameters such as temperature, pH, inoculum age, cell loading and substrate concentration. *Aspergillus niger* was isolated from deteriorated groundnut seeds and used as amylase source. After characterization, amylase exhibited optimum activity at pH 4.7 and temperature 58°C. The cells appear most viable on the 5th day. An initial rate method was employed in evaluating the kinetic parameters using Michaelis-Menten model. The K_m and r_{max} was found to be 0.2248 mmol/l and 2.055×10^{-4} mmol/l.min, respectively. Cassava starch was selected for the study because of its relative abundance in Nigeria compared to other starchy materials.

Keywords: *Aspergillus niger*, amylase, enzymatic hydrolysis, glucose syrup, cassava starch.

Ekundayo, F. O.^{1*} and Osunla, C. A.². (¹Department of Microbiology, Federal University of Technology, P. M. B. 704, Akure, Ondo State, Nigeria, ²Department of Biological Sciences, Achievers' University, P.M.B. 1030, Owo, Ondo State, Nigeria. Email: foekundayo2002@yahoo.com). Phytase activity of fungi from oil polluted soils and their ability to degrade bonnylight crude oil. African Journal of Biotechnology, Volume 12(36) (2013): 5540-5548

Fungi were isolated from contaminated soil samples taken from three selected automobile workshops, screened for phytase activities and biodegradative abilities. Physicochemical and total petroleum hydrocarbon analyses were carried using standard chemical and gas chromatography procedures, respectively. There was significant increase (at $P \leq 0.05$) in the potassium, sodium, calcium, magnesium, pH and organic matter of all contaminated soil samples. The fungi isolated were *Aspergillus niger*, *Aspergillus saprophyticus*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Trichoderma viride*, *Penicillium italicum*, *Articulospora inflata* and *Neurospora crassa*. Of all the fungal isolates, *A. flavus* had the maximum phytase

activity at the 48 h of incubation while *N. crassa* produced the least phytase activity at all the hour of incubation. Phytase activity of *A. flavus* and *A. saprophyticus* were found to be most active at pH 5.0 and 50°C. *A. niger* had the highest degrading ability on crude oil and spent engine oil at all days of incubation while *N. crassa* had the least degrading ability on crude and spent engine oil. The high total petroleum hydrocarbon (TPH) concentration in contaminated soil may be as a result of consistent exposure of the soil to spent engine oil which could make the soil conditions unsatisfactory for microbial growth.

Keywords: Fungi, biodegradation, bonny light crude oil, phytase.

Biosensor

Blessing E. Obinaju, Francis L. Martin. (Centre for Biophotonics, Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster LA1 4YQ, UK). Novel biospectroscopy sensor technologies towards environmental health monitoring in urban environments. Environmental Pollution, Volume 183(2013) : 46–53

Biospectroscopy is an emerging inter-disciplinary field that exploits the application of sensor technologies [*e.g.*, Fourier-transform infrared spectroscopy, Raman spectroscopy] to lend novel insights into biological questions. Methods involved are relatively non-destructive so samples can subsequently be analysed by more conventional approaches, facilitating deeper mechanistic insights. Fingerprint spectra are derived and these consist of wavenumber–absorbance intensities; within a typical biological experiment, a complex dataset is quickly generated. Biological samples range from biofluids to cytology to tissues derived from human or sentinel sources, and analyses can be carried out *ex vivo* or *in situ* in living tissue. A reference range of a designated normal state can be derived; anything outside this is potentially atypical and discriminating chemical entities identified. Computational approaches allow one to minimize within-category confounding factors. Because of ease of sample preparation, low-cost and high-throughput capability, biospectroscopy approaches herald a new greener means of environmental health monitoring in urban environments.

Keywords: Biochemical-cell fingerprint; Biospectroscopy; Computational analysis; Fourier-transform infrared; Infrared spectra; Sentinel organism

Lindsay Seed^a, Pat Wolseley^b, Laura Gosling^c, Linda Davies^c, Sally A. Power^{a,d}. (^a Imperial College London, Division of Biology, Silwood Park, Ascot, Berkshire SL5 7PY, UK, ^b Natural History Museum, Cromwell Rd, London SW7 5BD, UK, ^c Centre for Environmental Policy, Imperial College London, London SW7 1NA, UK, ^d Hawkesbury Institute for the Environment, University of Western Sydney, Locked Bag 1797, Penrith, 2751 NSW, Australia). Modelling relationships between lichen bioindicators, air quality and climate on a national scale: Results from the UK OPAL air survey. Environmental Pollution, Volume 182(2013): 437–447

Air pollution has many negative effects on the natural environment, from changes in plant growth patterns to loss of ecosystem function. This study uses citizen science to investigate national-scale patterns in the distribution and abundance of selected lichen species on tree trunks

and branches, and to relate these to air pollution and climate. Volunteers collected data for nine lichen indicators on 19,334 deciduous trees. Submitted data provided information on species-level patterns, and were used to derive composite lichen indices. Multiple linear regression and ANCOVA were used to model the relationships between lichen response variables on *Quercus* spp. and pollution, climate and location. The study demonstrated significant relationships between patterns in indicator lichens and levels of N- and S-containing pollutants on trunks and twigs. The derived lichen indices show great potential as a tool to provide information on local, site-specific levels of air quality.

Keywords: Citizen science; OPAL; Air pollution index; Nitrogenous air pollutants; Bioindicator species

Tapan Sarkar¹, Yingning Gao¹ and Ashok Mulchandani¹. (¹Department of Chemical and Environmental Engineering, University of California Riverside, Riverside, CA 92521, USA). Carbon Nanotubes-Based Label-Free Affinity Sensors for Environmental Monitoring. *Applied Biochemistry and Biotechnology*, Volume 170(5) (2013): 1011-1025

Nanostructures, such as nanowires, nanobelts, nanosprings, and nanotubes, are receiving growing interest as transducer elements of bio/chemical sensors as they provide high sensitivity, multiplexing, small size, and portability. Single-walled carbon nanotubes (SWNTs) are one such class of nanostructure materials that exhibit superior sensing behavior due to its large-surface carbon atoms that are highly responsive to surface adsorption events. Further, their compatibility with modern microfabrication technologies and facile functionalization with molecular recognition elements make them promising candidates for bio/chemical sensors applications. Here, we review recent results on nanosensors based on SWNTs modified with biological receptors such as aptamers, antibodies, and binding proteins, to develop highly sensitive, selective, rapid, and cost-effective label-free chemiresistor/field-effect transistor nanobiosensors for applications in environmental monitoring.

Keywords: 1-D nanostructures, Carbon nanotubes, Label-free biosensor, Chemiresistor Field-effect transistor, Pathogen Explosives, Toxins Nerve agents

E.V. Usachev¹, O.V. Usacheva², I.E. Agranovski^{1,*}. (¹Griffith School of Engineering, Griffith University, Brisbane, Qld, Australia, ²Department of Molecular Genetics, The D.I. Ivanovsky Institute of Virology of The Ministry of Health and Social Development of The Russian Federation, Moscow, Russia. *Correspondence: Igor E. Agranovski, Griffith School of Engineering, Griffith University, Brisbane, 4111 Qld, Australia. E-mail: i.agranovski@griffith.edu.au). Surface plasmon resonance-based real-time bioaerosol detection. *Journal of Applied Microbiology*, Volume 115(3) (2013): 766-773

Rapid and precise bioaerosol detection in different environments has become an important research and technological issue over last decades. Previously, we employed a real-time PCR protocol in conjunction with personal bioaerosol sampler for rapid detection of airborne viruses. The approach has been proved to be specific and sensitive. However, a period of time required for entire procedure was in manner of hours. Some new developments are required to decrease the detection time down to real-time protocols.

Presently, a surface plasmon resonance (SPR)-based immunosensor that coupled with a specific antigen-antibody reaction could offer sensitive, specific, rapid and label-free detection. This

study describes the possibility of combining the personal sampler with SPR technology for qualitative and extremely rapid detection of airborne micro-organisms. Common viral surrogate MS2 bacteriophage, frequently used in bioaerosol studies, was employed as a model organism. The results of the sensor functionalizing procedure with monoclonal anti-MS2 antibody and optimization of the chip performance are presented. The SPR-based detection of the airborne virus was found to be very fast; the viral presence was detected in less than 2 min, and the entire procedure (sampling and analysis) was undertaken in 6 min, which could be considered as real-time detection for this type of measurements.

The combination of SPR with the personal sampler targeted towards bioaerosol detection was proven to be feasible. The SPR sensor was found to be highly stable and suitable for multiple utilizations without significant decrease in response. The suggested approach opens new possibilities for the development of portable and rapid (almost real time) bioaerosol monitors.

This technology is the first in the world real-time bioaerosol monitor. This outcome would be of strong interest to individuals representing public health, biosecurity, defence forces, environmental sciences and many others.

Keywords: detection; environmental; identification; microbial contamination; rapid methods

Yating Chai, Shin Horikawa, Suiqiong Li, Howard C. Wikle, Bryan A. Chin. (Materials Research and Education Center, Auburn University, Auburn, AL 36849, USA). A surface-scanning coil detector for real-time, *in-situ* detection of bacteria on fresh food surfaces. *Biosensors and Bioelectronics*, Volume 50(2013) : 311–317

Proof-in-principle of a new surface-scanning coil detector has been demonstrated. This new coil detector excites and measures the resonant frequency of free-standing magnetoelastic (ME) biosensors that may now be placed outside the coil boundaries. With this coil design, the biosensors are no longer required to be placed inside the coil before frequency measurement. Hence, this new coil enables bacterial pathogens to be detected on fresh food surfaces in real-time and *in-situ*. The new coil measurement technique was demonstrated using an E2 phage-coated ME biosensor to detect *Salmonella typhimurium* on tomato surfaces. Real-time, *in-situ* detection was achieved with a limit of detection (LOD) statistically determined to be lower than 1.5×10^3 CFU/mm² with a confidence level of difference higher than 95% ($p < 0.05$).

Keywords: Surface-scanning coil detector; Magnetoelastic biosensor; Real-time detection; *In-situ* detection; *Salmonella typhimurium*; Fresh food surfaces

Sang-Gyu Kim^{a,1}, Hee-Jo Lee^{b,1}, Jung-Hyun Lee^b, Hyo-Il Jung^b, Jong-Gwan Yook^a. (^a School of Electrical and Electronic Engineering, Yonsei University, Seoul, South Korea, ^b School of Mechanical Engineering, Yonsei University, Seoul, South Korea). A highly sensitive and label free biosensing platform for wireless sensor node system. *Biosensors and Bioelectronics*, Volume 50(2013): 362–367

In this paper, we propose a radio-frequency (RF) biosensor platform based on oscillation frequency deviation at 2.4 GHz. Its feasibility is experimentally demonstrated with the well-known biomolecular binding systems such as biotin–streptavidin and deoxyribonucleic acid

(DNA) hybridization. For a basic principle of our biosensing system, the impedance of a resonator with the biomolecular immobilization is at first varied so that the corresponding change results in frequency change of an oscillator. Especially, to enhance the sensitivity of the proposed system, a surface acoustic wave (SAW) filter having a high-Q factor (~2000) is utilized. From the resulting component, even a small change of oscillation frequency can be transformed into a large output amplitude variation. According to the experimental results, it is found that our system shows the low detectable limit (~1 ng/ml) and fast response time (~real-time) for different target biomolecules, i.e. streptavidin and complementary DNA (cDNA). As a result, we find that our device is an effective biosensing system that can be used for a label-free and real-time measurement of the biomolecular binding events.

Keywords: Biosensor; Biomolecule; Radio-frequency; Surface acoustic wave filter; Wireless sensor node

Juan C. Vidal^a, Laura Bonel^b, Alba Ezquerro^a, Susana Hernández^a, Juan R. Bertolín^a, Carlota Cubel^a, Juan R. Castillo^a. (^a Analytical Spectroscopy and Sensors Group (GEAS), Institute of Environmental Sciences (IUCA), University of Zaragoza, c/ Pedro Cerbuna, 12, 50009-Zaragoza, Spain, ^b CAPHER IDI S.L., c/ Ermesinda de Aragón, 4, c-116, 50012-Zaragoza, Spain). **Electrochemical affinity biosensors for detection of mycotoxins: A review. Biosensors and Bioelectronics, Volume 49(2013): 146–158**

This review discusses the current state of electrochemical biosensors in the determination of mycotoxins in foods. Mycotoxins are highly toxic secondary metabolites produced by molds. The acute toxicity of these results in serious human and animal health problems, although it has been only since early 1960s when the first studied aflatoxins were found to be carcinogenic. Mycotoxins affect a broad range of agricultural products, most important cereals and cereal-based foods. A majority of countries, mentioning especially the European Union, have established preventive programs to control contamination and strict laws of the permitted levels in foods. Official methods of analysis of mycotoxins normally requires sophisticated instrumentation, e.g. liquid chromatography with fluorescence or mass detectors, combined with extraction procedures for sample preparation. For about sixteen years, the use of simpler and faster analytical procedures based on affinity biosensors has emerged in scientific literature as a very promising alternative, particularly electrochemical (i.e., amperometric, impedance, potentiometric or conductimetric) affinity biosensors due to their simplicity and sensitivity. Typically, electrochemical biosensors for mycotoxins use specific antibodies or aptamers as affinity ligands, although recombinant antibodies, artificial receptors and molecular imprinted polymers show potential utility. This article deals with recent advances in electrochemical affinity biosensors for mycotoxins and covers complete literature from the first reports about sixteen years ago.

Keywords: Mycotoxins; Electrochemical affinity biosensors; Immunosensor; Aptasensor; Amperometry; Electrochemical impedance spectroscopy

Khalil Heileman^{a,1}, Jamal Daoud^{a,1}, Maryam Tabrizian^{a,b}. (^a Department of Biomedical Engineering, Faculty of Medicine, McGill University, 3775 University Street, Montreal, Quebec, Canada H3A 2B4, ^b Faculty of Dentistry, McGill University, Strathcona Anatomy & Dentistry Building, 3640 University Street, Montreal, Quebec, Canada). **Dielectric spectroscopy as a viable biosensing tool for cell and tissue characterization and analysis. Biosensors and Bioelectronics, Volume 49(2013): 348–359**

The use of dielectric spectroscopy to carry out real time observations of cells and to extract a wealth of information about their physiological properties has expanded in recent years. This popularity is due to the simple, easy to use, non-invasive and real time nature of dielectric spectroscopy. The ease of integrating dielectric spectroscopy with microfluidic devices has allowed the technology to further expand into biomedical research. Dielectric spectra are obtained by applying an electrical signal to cells, which is swept over a frequency range. This review covers the different methods of interpreting dielectric spectra and progress made in applications of impedance spectroscopy for cell observations. First, methods of obtaining specific electrical properties of cells (cell membrane capacitance and cytoplasm conductivity) are discussed. These electrical properties are obtained by fitting the dielectric spectra to different models and equations. Integrating models to reduce the effects of the electrical double layer are subsequently covered. Impedance platforms are then discussed including electrical cell substrate impedance sensing (ECIS). Categories of ECIS systems are divided into microelectrode arrays, interdigitated electrodes and those that allow differential ECIS measurements. Platforms that allow single cell and sub-single cell measurements are then discussed. Finally, applications of impedance spectroscopy in a range of cell observations are elaborated. These applications include observing cell differentiation, mitosis and the cell cycle and cytotoxicity/cell death. Future applications such as drug screening and in point of care applications are then covered.

Keywords: Dielectric spectroscopy; Impedance monitoring; Microelectrodes; Dielectric biosensors; Microfluidic impedance; Dielectric cell models

Tianxing Wang^{a, b}, Ning Hu^a, Jiayue Cao^a, Jieying Wu^b, Kaiqi Su^a, Ping Wang^a. (^a Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, PR China, ^b ACEA Bio (Hangzhou) Co., Ltd., West Lake Technical & Economic Park, Hangzhou 310030, PR China). **A cardiomyocyte-based biosensor for antiarrhythmic drug evaluation by simultaneously monitoring cell growth and beating. Biosensors and Bioelectronics, Volume 49(2013): 9–13**

Drug-induced cardiotoxicity greatly endangers the human health and results in resource waste. Also, it is a leading attribution to drug withdrawal and late-stage attrition in pharmaceutical industry. In the study, a dual function cardiomyocyte-based biosensor was introduced for rapid drug evaluation with xCELLigence RTCA Cardio system. The cardiomyocyte-based biosensor can monitor the cardiomyocyte growth and beating status simultaneously under the drug effects. Two typical cardiovascular drug, verapamil and flecainide were selected as treatment agents to test the performance of this biosensor. The experiment results showed that the performance of cardiomyocyte-based biosensor verified the basic drug effects by beating status and also tested the drug cytotoxicity by the cell index curves of cardiomyocyte growth. Based on the advanced sensor detection technology and cell culture technology, this cardiomyocyte-based biosensor will be a utility platform for the drug preclinical assessment.

Keywords: Real time cell analysis; Cell-based biosensor; Primary neonatal rat cardiomyocyte; Pharmacological assays; Cardiotoxicity

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engineering, Yunnan University, Kunming 650091, China, ^c Division of Electron Microscopy, Kunming Medical University, Kunming 650500, China). Acetylcholinesterase biosensor based on SnO₂ nanoparticles–carboxylic graphene–nafion modified electrode for detection of pesticides. *Biosensors and Bioelectronics*, Volume 49(2013): 25–31

A sensitive amperometric acetylcholinesterase (AChE) biosensor, based on SnO₂ nanoparticles (SnO₂NPs), carboxylic graphene (CGR) and nafion (NF) modified glassy carbon electrode (GCE) for the detection of methyl parathion and carbofuran has been developed. The nanocomposites of SnO₂ NPs and CGR was synthesized and characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR), respectively. Chitosan (CS) was used to immobilize AChE on SnO₂NPs–CGR–NF/GCE and to improve electronic transmission between AChE and SnO₂ NPs–CGR–NF/GCE. NF was used as the protective membrane for the AChE biosensor. The SnO₂ NPs–CGR–NF nanocomposites with excellent conductivity, catalysis and biocompatibility offered an extremely hydrophilic surface for AChE adhesion. The AChE biosensor showed favorable affinity to acetylthiocholine chloride (ATCl) and could catalyze the hydrolysis of ATCl with an apparent Michaelis–Menten constant value of 131 μM. The biosensor detected methyl parathion in the linear range from 10⁻¹³ to 10⁻¹⁰ M and from 10⁻¹⁰ to 10⁻⁸ M. The biosensor detected carbofuran in the linear range from 10⁻¹² to 10⁻¹⁰ M and from 10⁻¹⁰ to 10⁻⁸ M. The detection limits of methyl parathion and carbofuran were 5×10⁻¹⁴ M and 5×10⁻¹³ M, respectively. The biosensor exhibited low applied potential, high sensitivity and acceptable stability, thus providing a promising tool for analysis of pesticides.

Keywords: SnO₂ nanoparticle; Carboxylic graphene; Acetylcholinesterase; Amperometric biosensor; Stability

Philani Mashazi^{a, b}, Phumlani Tetyana^a, Sibulelo Vilakazi^a, Tebello Nyokong^b. (a Nanotechnology Innovation Centre, Advanced Materials Division, Mintek, Private Bag X3015, Randburg 2125, South Africa, b Nanotechnology Innovation Centre—Sensors, Chemistry Department, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa). Electrochemical impedimetric immunosensor for the detection of measles-specific IgG antibodies after measles infections. *Biosensors and Bioelectronics*, Volume 49(2013): 32–38

The detection of measles-specific primary antibodies (IgG) using electrochemical impedimetric immunosensors is reported. The optimum conditions for electrode saturation were reached after 40 min for 1 μg ml⁻¹ antibody concentrations. Surface roughness using AFM increased with each immobilization or antigen-antibody reaction step clearly confirming the surface modification and recognition between antigen and antibody. The human serum (HS) and newborn calf serum (NCS) spiked with antigen-specific antibody were studied to mimic the real sample analysis. The HS and NCS sera containing antibodies due to measles exhibited correlation between the increasing antibody serum concentrations and the charge-transfer resistance (electrochemically measured). This work clearly showed the potential use of impedance as the preferred electrochemical method for detecting measles-antibodies in label-free manner.

Keywords: Measles-antigen; Measles-specific antibodies; Impedance; Immunosensors

Huanshun Yin^{a,1}, Yunlei Zhou^{a,b,1}, Zhenning Xu^a, Mo Wang^a, Shiyun Ai^a. (^a College of Chemistry and Material Science, Shandong Agricultural University, 271018, Taian, Shandong, PR China, ^b Key Laboratory of Cell Proliferation and Regulation Biology of Ministry of Education, College of Life Science, Beijing Normal University, 100875, Beijing, PR China). Ultrasensitive electrochemical immunoassay for DNA methyltransferase activity and inhibitor screening based on methyl binding domain protein of MeCP2 and enzymatic signal amplification. *Biosensors and Bioelectronics*, Volume 49(2013): 39–45

In this work, we fabricated a novel electrochemical immunosensor for detection of DNA methylation, analysis of DNA MTase activity and screening of MTase inhibitor. The immunosensor was on the basis of methyl binding domain protein of MeCP2 as DNA CpG methylation recognition unit, anti-His tag antibody as “immuno-bridge” and horseradish peroxidase labeled immunoglobulin G functionalized gold nanoparticles (AuNPs–IgG–HRP) as signal amplification unit. In the presence of *M. SssI* MTase, the symmetrical sequence of 5'-CCGG-3' was methylated and then recognized by MeCP2 protein. By the immunoreactions, anti-His tag antibody and AuNPs–IgG–HRP was captured on the electrode surface successively. Under the catalysis effect of HRP towards hydroquinone oxidized by H₂O₂, the electrochemical reduction signal of benzoquinone was used to analyze *M. SssI* MTase activity. The electrochemical reduction signal demonstrated a wide linear relationship with *M. SssI* concentration ranging from 0.05 unit/mL to 90 unit/mL, achieving a detection limit of 0.017 unit/mL (S/N=3). The most important advantages of this method were its high sensitivity and good selectivity, which enabled the detection of even one-base mismatched sequence. In addition, we also verified that the developed method could be applied for screening the inhibitors of DNA MTase and for developing new anticancer drugs.

Keywords: DNA methylation; Methyl binding domain protein; Anti-histidine tag antibody; Horseradish peroxidase labeled immunoglobulin G; Electrochemical detection

S. Michaelis, J. Wegener, R. Robelek. (Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Regensburg, Germany). Label-free monitoring of cell-based assays: Combining impedance analysis with SPR for multiparametric cell profiling. *Biosensors and Bioelectronics*, Volume 49(2013): 63–70

Label-free approaches to monitor cell-based assays provide an unprecedented, time-resolved and non-invasive view on the response of mammalian cells to chemical, biological or physical stimuli. The most widespread techniques are impedance analysis and optical sensing using evanescent waves like SPR. This study describes the combination of both in one experimental setup so that a given cell population can be monitored simultaneously for electrical and optical changes. The device is based on commercial SPR chips that are processed by photolithography to provide electrodes for impedance analysis and gold spots for surface plasmon excitation on the same substrate. Simultaneous recordings do not interfere with each other but provide independent, time-resolved information on cell shape changes (impedance) and dynamic mass redistribution (SPR) as they occur during exposure of the cells to drugs or toxins or along their normal life cycle. This study provides proof-of-concept experiments of the dual biosensor platform in two experimental settings: signals are recorded and analyzed (i) during cell attachment, spreading and differentiation of initially suspended cells and (ii) during the exposure of the mature cells to an actin cytoskeleton disrupting drug. Impedance and SPR recordings

provide complementary information that can be used to trace and assign intracellular mechanisms of action.

Keywords: Surface plasmon resonance; Electric cell-substrate impedance sensing; Real time monitoring; Label-free detection; Wholistic sensors; Whole cell biosensors

Shuang Zhou^a, Yunfeng Zhao^a, Michael Mecklenburg^c, Dajin Yang^a, Bin Xie^b. (^a China National Center for Food Safety Risk Assessment, Key Laboratory of Food Safety Risk Assessment, Ministry of Health, Beijing 100021, PR China, ^b Department of Pure and Applied Biochemistry, Lund University, Getingevägen 60, Box 124, S-22100 Lund, Sweden, ^c Biomedical Technology AB, Lund, Sweden). **A novel thermometric biosensor for fast surveillance of β -lactamase activity in milk. *Biosensors and Bioelectronics*, Volume 49(2013): 99–104**

Regulatory restrictions on antibiotic residues in dairy products have resulted in the illegal addition of β -lactamase to lower antibiotic levels in milk in China. Here we demonstrate a fast, sensitive and convenient method based on enzyme thermistor (ET) for the surveillance of β -lactamase in milk. A fixed amount of penicillin G, which is a specific substrate of β -lactamase, was incubated with the milk sample, and an aliquot of the mixture was directly injected into the ET system to give a temperature change corresponding to the remained penicillin G. The amount of β -lactamase present in sample was deduced by the penicillin G consumed during incubation. This method was successfully applied to quantify β -lactamase in milk with the linear range of 1.1–20 U mL⁻¹ and the detection limit of 1.1 U mL⁻¹. The recoveries ranged from 93% to 105%, with relative standard deviations (RSDs) below 8%. The stability of the column equipped in ET was also studied, and only 5% decrease of activity was observed after 60 days of use. Compared with the conventional culture-based assay, the advantages of high throughput, timesaving and accurate quantification have made this method an ideal alternative for routine use.

Keywords: β -lactamase; Antibiotics; Milk; Enzyme thermistor; Enzyme immobilization

Hanping He^a, Jingping Xia^a, Xiaoqian Peng^a, Gang Chang^b, Xiuhua Zhang^a, Yafen Wang^a, Kazuhiko Nakatani^c, Zhaowen Lou^a, Shengfu Wang^a. (^a College of Chemistry and Chemical Engineer, Hubei Collaborative Innovation Center for Advanced Organic Chemical Materials, Ministry of Education Key Laboratory for the Synthesis and Application of Organic Functional Molecule, Hubei University, Youyi Road 368, Wuchang, Wuhan, Hubei 430062, PR China, ^b College of Materials Science and Engineering, Ministry-of-Education Key Laboratory for the Green Preparation and Application of Functional Materials, Hubei University, Youyi Road 368, Wuchang, Wuhan, Hubei 430062, PR China, ^c Department of Regulatory Bioorganic Chemistry, The Institute of Scientific and Industrial Research (ISIR), Osaka University, 8-1 Mihogaoka, Ibaraki 567-0047, Japan). **Facile electrochemical biosensor based on a new bifunctional probe for label-free detection of CGG trinucleotide repeat. *Biosensors and Bioelectronics*, Volume 49(2013): 282–289**

We have developed a simple and label-free electrochemical assay to detect CGG trinucleotide repeat. For this purpose, a new bifunctional probe (FecNCD2) was developed, in which a recognition part (naphthyridine carbamate dimmer, NCD) was connected with an electro-active part (ferrocenyl group) using a chain of –CO–NH–CH₂–CH₂–. The results of circular dichroismic measurements indicated that FecNCD2 exhibited a superior performance for

selective binding to CGG trinucleotide repeats compared to a previous bifunctional electrochemical probe connected with shorter linker $-\text{CH}_2-$ (FecNCD1). Then, the electrochemical properties of FecNCD2 were evaluated and were found to show a good redox response due to the ferrocene moiety. Owing to the high performances of FecNCD2, the label-free electrochemical biosensor for CGG repeats was constructed by immobilizing them onto gold disk electrode and by using FecNCD2 as an electrochemical probe in solution. Further CGG repeats in solution were confirmed to be detectable using the CGG modified biosensor in competitive experiments, i.e., by treating it in test solutions containing FecNCD2 and $\text{d}(\text{CGG})_{10}$ or others. No interference of ct-DNA on the CGG detection was also confirmed with this approach. The strategy should have significant potential for the development of versatile and low-cost biosensor for early diagnosis and treatment of neurodegenerative diseases associated with trinucleotide repeats.

Keywords: Electrochemical biosensor; Trinucleotide repeat; Recognition of CGG; Ferrocenyl modified naphthyridine derivative

Aurélie Bouchet-Spinelli^a, Bertrand Reuillard^{a,b}, Liliane Coche-Guérente^b, Sylvie Armand^a, Pierre Labbé^b, Sébastien Fort^a. (^a Centre de Recherches sur les Macromolécules Végétales (CERMAV-CNRS), affiliated with Université de Grenoble, Member of the Institut de Chimie Moléculaire de Grenoble (ICMG) and Member of the PolyNat Carnot Institute, BP 53, 38041 Grenoble cedex 9, France, ^b Département de Chimie Moléculaire, UMR CNRS-UJF 5250, Affiliated with Université de Grenoble, Member of the Institut de Chimie Moléculaire de Grenoble (ICMG), BP 53, 38041, Grenoble, France). **Oligosaccharide biosensor for direct monitoring of enzymatic activities using QCM-D. Biosensors and Bioelectronics, Volume 49(2013): 290–296**

Enzymatic modification of saccharidic biomass is a subject of intensive research with potential applications in plant or human health, design of biomaterials and biofuel production. Bioengineering and metagenomics provide access to libraries of glycoside hydrolases but the biochemical characterization of these enzymes remains challenging, requiring fastidious colorimetric tests in discontinuous assays. Here, we describe a highly sensitive carbohydrate biosensor for the detection and characterization of glycoside hydrolases. Immobilization of oligosaccharides was achieved using copper-catalyzed azide-alkyne cycloaddition of maltoheptaose-modified probes onto self-assembled monolayers bearing azide reactive groups. This biosensor allowed detection of glycoside hydrolase activities at the picomolar level using quartz-crystal microbalance with dissipation monitoring (QCM-D). To our knowledge, this protocol provides the best performance to date for the detection of glycoside hydrolase activities. For each enzyme tested, we could determine the kinetic constant from the QCM-D data, and derive conclusions that correlated well with those of standard colorimetric tests. This opens the way to a new generation of rapid and direct tests characterizing functionally carbohydrate-active enzymes.

Keywords: Carbohydrate-active enzyme; Carbohydrate biosensor; Oligosaccharides; Self-assembled monolayers; Quartz-crystal microbalance with dissipation monitoring (QCM-D).

Li-li Tong, Lu Li, Zhenzhen Chen, Qian Wang, Bo Tang. (College of Chemistry, Chemical Engineering and Materials Science, Engineering Research Center of Pesticide and

Medicine Intermediate Clean Production, Ministry of Education, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Normal University, Jinan 250014, PR China). Stable label-free fluorescent sensing of biothiols based on ThT direct inducing conformation-specific G-quadruplex. Biosensors and Bioelectronics, Volume 49(2013): 420–425

In this work, a new, label-free, turn-on fluorescent sensor for biothiols detection based on ThT direct inducing conformation-specific G-quadruplex is developed. The sensing approach is based on a conformational switch of oligonucleotide controlled by Hg^{2+} and a commercially available water-soluble fluorescent dye, Thioflavin T (ThT). A noticeable fluorescence light-up in ThT on binding to the G-quadruplex grants the sensor excellent sensitivity. The specific quadruplex conformation induced directly by ThT and pronounced structural selectivity of ThT for G-quadruplexes could generate more stable luminescence and make sure high specificity in complex biological samples. The present assay allows for the selective determination of cysteine and glutathione in the range of 2.0×10^{-8} – 2.5×10^{-6} M and 3.0×10^{-8} – 2.0×10^{-6} M with a detection limit of 8.4 nM and 13.9 nM respectively. The diagnostic capability and potential in practical applications of this method have been demonstrated by detecting biothiols in human blood serum.

Keywords: G-quadruplex; Thioflavin T (ThT); Fluorescent sensing; Biothiols; Mercury ions

Jingting Luo^a, Pingxiang Luo^b, Min Xie^b, Ke Du^c, Bixia Zhao^c, Feng Pan^d, Ping Fan^a, Fei Zeng^d, Dongping Zhang^a, Zhuanghao Zheng^a, Guangxing Liang^a. (^a Institute of Thin Film Physics and Applications, Shenzhen Key Laboratory of Sensor Technology, Shenzhen University, Shenzhen 518060, China, ^b Maternity and Child Care Centers in Fujian Province, Fuzhou 350001, China, ^c Affiliated Nanhua Hospital of University of South China, Hunan 421002, China, ^d Laboratory of Advanced Materials, Department of Materials Science and Engineering, Tsinghua University, Beijing 100084, China). A new type of glucose biosensor based on surface acoustic wave resonator using Mn-doped ZnO multilayer structure. Biosensors and Bioelectronics, Volume 49(2013): 512–518

This work reports a high-performance Mn-doped ZnO multilayer structure Love mode surface acoustic wave (SAW) biosensor for the detection of blood sugar. The biosensor was functionalized via immobilizing glucose oxidase onto a pH-sensitive polymer which was attached on Mn-doped ZnO biosensor. The fabricated SAW glucose biosensor is highly sensitive, accurate and fast with good anti-interference. The sensitivity of the SAW glucose biosensor is 7.184 MHz/mM and the accuracy is 6.96×10^{-3} mM, which is sensitive and accurate enough for glucose monitoring. A good degree of reversibility and stability of the glucose sensor is also demonstrated, which keeps a constant differential frequency shift up to 32 days. Concerning the time response to human serum, the glucose sensor shows a value of 4.6 ± 0.4 min when increasing glucose concentrations and 7.1 ± 0.6 min when decreasing, which is less than 10 min and reach the fast response requirement for medical applications. The Mn-doped ZnO Love mode SAW biosensor can be fully integrated with CMOS Si chips and developed as a portable, passive and wireless real time detection system for blood sugar monitoring in human serum.

Keywords: Glucose; Surface acoustic wave (SAW); ZnO; Glucose oxidase functional immobilization; Biosensor

Ai-Xian Zheng, Zhong-Xiao Cong, Jin-Ru Wang, Juan Li, Huang-Hao Yang, Guo-Nan Chen. (The Key Lab of Analysis and Detection Technology for Food Safety of the MOE, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350108, PR China). Highly-efficient peroxidase-like catalytic activity of graphene dots for biosensing. Biosensors and Bioelectronics, Volume 49(2013): 519–524

In recent years, considerable efforts have been devoted to the construction of efficient enzyme mimetics, which have significant advantages of simple synthesis, good stability and design flexibility. In this paper, we described that graphene dots (GDs) possess highly-efficient peroxidase-like catalytic activity, and its activity is much higher than graphene oxide (GO) with large size. They can catalyze the oxidation of peroxidase substrate 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H₂O₂ to produce a blue product, which can be used for H₂O₂ detection by measuring the absorbance change. This catalytic reaction can be also used for other analyte detection by monitoring the generation or consumption of H₂O₂, such as glucose and reduced glutathione (GSH). The GDs-based system permits detection of as low as 10 nM H₂O₂, which is much lower than that of other nanomaterials-catalyzed methods. Meanwhile, the detection limit of this system is 0.5 μM for glucose and 0.5 μM for GSH, respectively. Furthermore, the proposed system also shows high selectivity and is capable of sensing in complicated biological samples such as cell lysate. Due to their high catalytic activity, high diffusion and excellent biocompatibility, GDs can be expected to be applied in various fields, such as biotechnology, medical diagnostics and environmental monitoring.

Keywords: Peroxidase-like catalytic activity; Graphene dots; Hydrogen peroxide; Glucose; Reduced glutathione

Jin Ha Choi^a, Hyun Soo Kim^a, Jeong-Woo Choi^a, Jong Wook Hong^b, Young-Kee Kim^c, Byung-Keun Oh^a. (^a Department of Chemical & Biomolecular Engineering, Sogang University, #1 Shinsu-Dong Mapo-Gu, Seoul 121-742, Republic of Korea, ^b Materials Research and Education Center, Department of Mechanical Engineering, Auburn University, Auburn, Alabama 36849, United states, ^c Department of Chemical Engineering, Hankyong National University, Sukjong-dong 67, Ansong, Kyonggi-do 456-749, Republic of Korea). A novel Au-nanoparticle biosensor for the rapid and simple detection of PSA using a sequence-specific peptide cleavage reaction. Biosensors and Bioelectronics, Volume 49(2013): 415–419

PSA (prostate-specific antigen) is one of the most widely used proteins for the diagnosis of breast and prostate cancer. Of note, PSA displays enzymatic activity for the specific peptide sequence HSSKLQ, which it recognizes and cleaves. In this study, we developed a site-specific enzymatic-cleavage-reaction-based biosensor for the detection of PSA using fluorescein isothiocyanate (FITC)/peptide-conjugated gold (Au) nanoparticle complexes (FPANs). The FPANs do not initially fluoresce in the spectral region associated with the fluorophore, due to the quenching effect of the Au nanoparticles. When PSA was added to a solution containing the FPANs, PSA recognized and cleaved the specific sequence of the peptides attached to the Au nanoparticles. As a result, FITCs were separated from the Au nanoparticles and emitted strong fluorescence in their spectral region. Using this detection method, PSA was successfully detected as a function of concentration (10 pM—100 nM). This approach is superior to the

immunoassay with respect to the performance of sensor, which is very rapid, simple, and one-step method for the detection of PSA and other protein markers can be measured for the early detection of several diseases.

Keywords: Prostate specific antigen (PSA); Au nanoparticle; Nanobiosensor; Enzymatic cleavage reaction; Protein detection

Ji Young Choi^{a,1}, Gun-Hee Kim^{b,1}, Zhiqian Guo^{a,c,1}, Hye Yeon Lee^a, K.M.K. Swamy^{a,d}, Jaeyoung Pai^b, Seunghoon Shin^c, Injae Shin^b, Juyoung Yoon^a. (^a Department of Chemistry and Nano Science and Department of Bioinspired Science (WCU), Ewha Womans University, Seoul 120-750, Republic of Korea, ^b National Creative Research Initiative Center for Biofunctional Molecules, Department of Chemistry, Yonsei University, Seoul 120-749, Republic of Korea, ^c Key Laboratory for Advanced Materials and Institute of Fine Chemicals, East China University of Science & Technology, Shanghai 200237, PR China, ^d Department of Pharmaceutical Chemistry, V. L. College of Pharmacy, Raichur 584103, India, ^e Department of Chemistry, Hanyang University, Seoul 133-791, Republic of Korea). **Highly selective ratiometric fluorescent probe for Au³⁺ and its application to bioimaging☆. Biosensors and Bioelectronics, Volume 49(2013): 438–441**

The 4-propargylamino-1,8-naphthalimide based fluorescent probe **1** has been explored as a sensor for selective detection of Au³⁺. 4-Amino-1,8-naphthalimides, that possess typical intramolecular charge transfer (ICT) electronic characteristics, have been widely used as versatile platforms for fluorescent probes. The newly designed probe **1** contains a propargylamine moiety at C-4 of the naphthalimide chromophore that reacts with Au³⁺ to generate a product that has distinctly different electronic properties from **1**. Specifically, the probe undergoes a remarkable change in its absorption spectrum upon addition of Au³⁺ that is associated with a distinct color change from yellow to light pink. In addition, a blue shift of ca. 56 nm also takes place in the emission spectra of the probe. Consequently, **1** serves as a reaction-based sensor or so called chemodosimeter for Au³⁺. Importantly, surfactants enhance the rate of reaction of **1** with Au³⁺, thus, enhancing its use as a real time sensor. Finally, the results of studies probing its application to bioimaging of Au³⁺ in live cells show that the probe **1** has a unique ability to sense Au³⁺ in cells and, in particular, in lipid droplets within cells.

Keywords: Au³⁺ sensing; Fluorescent probe; Cell imaging; Lipid droplet; Naphthalimide

Zhengbo Che^e, Liang Chen, He Ma, Tong Zhou, Xiaoxiao Li. (Department of Chemistry, Capital Normal University, Beijing 100048, China). **Aptamer biosensor for label-free impedance spectroscopy detection of potassium ion based on DNA G-quadruplex conformation. Biosensors and Bioelectronics, Volume 48(2013): 108–112**

Herein, a label-free and highly sensitive electrochemical impedance spectroscopy (EIS) aptasensor for the detection of potassium ion (K⁺) was developed based on a conformational change in which a K⁺-stabilized single stranded DNA (ssDNA) with G-rich sequence was used as the recognition element. In the measurement of K⁺ ions, the change in interfacial electron transfer resistance (R_{ct}) of the sensor using a redox couple of [Fe(CN)₆]^{3-/4-} as the probe was monitored. In the presence of K⁺, the G-rich DNA folded into the G-quadruplex structure, and then K⁺ can bind to the G-quadruplex structure, leading to an increase in the R_{ct} . The R_{ct} increased with K⁺ concentration, and the plot of R_{ct} against the logarithm of K⁺ concentration is linear over the range from 0.1 nM to 1 mM with a detection limit of 0.1 nM.

Other metal ions, such as Ca^{2+} , Mg^{2+} , Na^+ , Li^+ , Al^{3+} , Zn^{2+} , Cu^{2+} , and Ni^{2+} caused no notable interference on the detection of K^+ . The scheme reported herein is applicable to the detection of other kinds of G-rich aptamer-binding chemicals and biomolecules.

Keywords: Aptamer; Electrochemical impedance spectroscopy; Potassium ion; G-quadruplex

Yang Zhang, Hong Zhao', Zhijiao Wu, Ying Xue, Xiaofang Zhang, Yujian He, Xiangjun Li, Zhuobin Yuan. (School of Chemistry and Chemical Engineering, University of Chinese Academy of Sciences, 19A YuQuan Road, 100049 Beijing, China). A novel graphene-DNA biosensor for selective detection of mercury ions. *Biosensors and Bioelectronics*, Volume 48(2013): 180–187

A novel electrochemical biosensor for sensitive and selective detection of mercury (II) ions (Hg^{2+}) based on a DNA grafted graphene is proposed. Graphene oxide (GO) was reduced by dopamine, and then the single-strand probe DNA modified at the 5'-end with an alkylamino modifier (NH_2 -ssDNA) was grafted on the reduced graphene oxide (RGO) surface via Michael addition reaction. In the presence of Hg^{2+} , the target DNA with four thymine–thymine (T–T) mismatches would hybridize with the probe DNA on the glassy carbon electrode (GCE) through T– Hg^{2+} –T coordination chemistry. The hybridization of the two oligonucleotides leads to the increase in the peak currents of $[\text{Ru}(\text{NH}_3)_6]^{3+}$, which could be used for electrochemical sensing of Hg^{2+} . The difference in the value of the peak currents of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ before and after DNA hybridization was linear with the concentration of Hg^{2+} in the range from 8.0×10^{-9} to 1.0×10^{-7} M with a linear coefficient of 0.996. The detection limit was 5.0×10^{-9} M (S/N=3). The proposed electrochemical biosensor is rapid, convenient and low-cost for effective sensing of Hg^{2+} . Particularly, the proposed method was applied successfully to the determination of Hg^{2+} in real environmental samples.

Keywords: Mercury ions; Graphene; Michael addition; Biosensor; Nucleic acids

Amandeep Kaur^a, Jung Rae Kim^{b, c}, Iain Michie^b, Richard M. Dinsdale^a, Alan J. Guwy^a, Giuliano C. Premier^b. (Sustainable Environment Research Centre (SERC), ^a Faculty of Health, Sport and Science, University of Glamorgan, Pontypridd, Mid-Glamorgan CF37 1DL, UK, ^b Faculty of Advanced Technology, University of Glamorgan, Pontypridd, Mid-Glamorgan CF37 1DL, UK, ^c School of Chemical and Biomolecular Engineering, Pusan National University, Jangjeon-Dong, Geumjeong-gu, Busan 609-735, Korea). Microbial fuel cell type biosensor for specific volatile fatty acids using acclimated bacterial communities. *Biosensors and Bioelectronics*, Volume 47(2013): 50–55

Volatile fatty acid (VFA) concentration is one of the most important parameters for monitoring bio-processes such as anaerobic digestion and microbial fuel cells. In this study the correlation between VFA concentration and current/voltage responses and electrochemical properties by using the MFC technology was evaluated. The discrimination between different species of VFA by using two methods i.e., coulombic efficiency and cyclic voltammetry was investigated. Coulombic efficiency gave a slow response of greater than 20 h, particularly at concentration levels of 20 mg l^{-1} . By using cyclic voltammetry to measure the oxidation peak at a consistent scan rate showed linear correlation to VFA concentration and peak current produced, up to $<40 \text{ mg l}^{-1}$) in a rapid response time of 1–2 min. The results presented showed good correlations

between the individual VFA species concentration and charge, and also current generated. A MFC based biosensor array was produced capable of measuring individual acetate, propionate and butyrate concentrations with sensitivity down to 5 mg l^{-1} and up to 40 mg l^{-1} .

Keywords: Microbial fuel cell; Volatile fatty acids; Biosensor; VFA monitoring; Bioelectrochemical system (BES)

Leonardo Pires^a, Kai Sachsenheimer^a, Tanja Kleintschek^b, Ansgar Waldbaur^a, Thomas Schwartz^b, Bastian E. Rapp^a. (^a Institute of Microstructure Technology (IMT), Karlsruhe Institute of Technology (KIT), 76344 Eggenstein-Leopoldshafen, Germany, ^b Institute of Functional Interfaces (IFG), Karlsruhe Institute of Technology (KIT), 76344 Eggenstein-Leopoldshafen, Germany). **Online monitoring of biofilm growth and activity using a combined multi-channel impedimetric and amperometric sensor. Biosensors and Bioelectronics, Volume 47(2013): 157–163**

Biofilms are ubiquitous in water interfaces and therefore influence our daily lives in an ambivalent manner. In medicine, infections can be attributed to biofilm formation. In technical systems, biofilms are causative agents for biocorrosion, contamination, and clogging processes and are responsible for shear force modification in marine systems. To control and manipulate biofilm formation advanced technologies are needed.

This paper reports on a novel real-time biofilm monitoring system using custom-made electronics. The system is able to monitor four electrochemical impedance spectroscopy (EIS) electrodes and three amperometric sensors in two microfluidic channels assessing biofilm growth and activity in parallel using *Pseudomonas aeruginosa* as a model system. The biofilm was characterized during its seeding and growth stages as well as during different injection intervals of a biocide (sodium azide) which allowed monitoring biofilm destabilization and deactivation effects in real time. The results obtained were confirmed by fluorescence microscopy after live/dead cell staining of the bacteria in the measured biofilm.

Keywords: Biosensors; Impedance spectroscopy; Amperometric detection; Electrochemistry; Biofilms

Ming Soon Cheng^a, Jia Shin Ho^a, Suk Hiang Lau^b, Vincent T.K. Chow^b, Chee-Seng Toh^a. (^a Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore, ^b Department of Microbiology, Yong Loo Ling School of Medicine, 5 Science Drive 2, National University of Singapore, Singapore 117597, Singapore). **Impedimetric microbial sensor for real-time monitoring of phage infection of *Escherichia coli*. Biosensors and Bioelectronics, Volume 47(2013): 340–344**

We describe an impedimetric microbial sensor for real-time monitoring of the non-lytic M13 bacteriophage infection of *Escherichia coli* cells using a gold electrode covalently grafted with a monolayer of lipopolysaccharide specific antibody. After infection, damage to the lipopolysaccharide layer on the outer membrane of *E. coli* causes changes to its surface charge and morphology, resulting in the aggregation of redox probe, $\text{Fe}(\text{CN})_6^{3-/4-}$ at the electrode surface and thereby increases its electron-transfer rate. This consequent decrease of electron-transfer resistance in the presence of bacteriophage can be easily monitored using Faradaic impedance spectroscopy. Non-lytic bacterium–phage interaction which is hardly observable

using conventional microscopic methods is detected within 3 h using this impedimetric microbial sensor which demonstrates its excellent performance in terms of analysis time, ease and reduced reliance on labeling steps during *in-situ* monitoring of the phage infection process.

Keywords: Biosensor; Virus; Pathogen–host interaction; *E. coli*; Bacteria; Impedance spectroscopy

Chi-Hua Nieh, Yuki Kitazumi, Osamu Shirai, Kenji Kano. (Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan). Sensitive D-amino acid biosensor based on oxidase/peroxidase system mediated by pentacyanoferrate-bound polymer. *Biosensors and Bioelectronics*, Volume 47(2013): 350–355

A sensitive D-amino acid oxidase (DAAO)/peroxidase (POD) bienzyme biosensor is constructed, in which pentacyanoferrate-bound poly(1-vinylimidazole) polymer (PVI[Fe(CN)₅]) is selected as a mediator. Reductive current of PVI[Fe(CN)₅] related to the H₂O₂ concentration generated in the DAAO reaction was measured at –0.1 V vs. Ag|AgCl with DAAO/POD/PVI[Fe(CN)₅]-modified electrode. The result revealed that PVI[Fe(CN)₅] is suitable as a mediator for this bienzyme system due to its appropriate formal potential and its extremely low reactivity against DAAO. The stability of DAAO was improved by adding free flavin adenine dinucleotide and the electrode composition was optimized for the detection of D-alanine. Nafion and ascorbate oxidase-immobilized films worked successfully to prevent severe interference from uric acid and ascorbic acid. The low detection limits of D-alanine (2 μM) and D-serine (2 μM) imply its possibility for the determination of extremely low concentration of D-amino acids in physiological fluids. The proposed bienzyme biosensor is proved to be capable of detecting D-amino acids in urine.

Keywords: Pentacyanoferrate-bound polymer; D-Amino acid oxidase; Peroxidase; Ascorbate oxidase; Urine

Xia Xu^{a,b}, Xiangjiang Liu^{a,b}, Yanbin Li^{a,c}, Yibin Ying^{a,b}. (^a College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, PR China, ^b Key Laboratory of Equipment and Informatization in Environment Controlled Agriculture, Ministry of Agriculture, PR China, ^c Department of Biological & Agricultural Engineering, University of Arkansas, Fayetteville, AR 72701, USA). A simple and rapid optical biosensor for detection of aflatoxin B1 based on competitive dispersion of gold nanorods. *Biosensors and Bioelectronics*, Volume 47(2013): 361–367

This report illustrates a promising one-step and label-free optical biosensor for determination of aflatoxin B1 (AFB1) that is most commonly found in foods and highly dangerous even at very low concentrations. In this research, gold nanorods (GNRs) were employed as a sensing platform, which showed high stability under high ionic strength conditions without addition of any stabilizing agent. GNR-AFB1–BSA (bovine serum albumin) conjugates aggregated after mixing with free antibodies, resulting in significant changes in absorption intensity. At the same time the existence of AFB1 molecules in samples caused dispersion of nanorods, as a result of competitive immune-reaction with antibodies. By taking advantages of the competitive dispersion of GNRs, the developed method could effectively reduce false results caused by

undesirable aggregation, which is a big problem for spherical gold nanoparticles. Absorption intensity of UV–vis spectra served as the sensing indicator, with dynamic light scattering (DLS) measurement as another sensing tool. The designed biosensing system could detect AFB1 in a linear range from 0.5 to 20 ng mL⁻¹, with a good correlation coefficient of 0.99. And the limit of detection (LOD) was 0.16 ng mL⁻¹, indicating an excellent sensitivity with absorbance result. The recoveries of the spiked AFB1 in real peanut samples ranged from 94.2% to 117.3%. Therefore the proposed nano-biosensor was demonstrated to be sensitive, selective, and simple, providing a viable alternative for rapid screening of toxins in agriculture products and foods.

Keywords: Aflatoxin B1; Gold nanorods; Optical Biosensor; Competitive immunoassay; Dispersion

Ajay Kumar Yagati^a, Taek Lee^b, Junhong Min^c, Jeong-Woo Choi^{a, b}. (^a Research Center for Integrated Biotechnology, Sogang University, 35 Baekbeom-ro (Sinsu-dong), Mapo-gu, Seoul 121-742, Republic of Korea, ^b Department of Chemical and Biomolecular Engineering, Sogang University, 35 Baekbeom-ro (Sinsu-dong), Mapo-gu, Seoul 121-742, Republic of Korea, ^c School of Integrative Engineering, Chung-Ang University, Heukseok-dong, Dongjak-gu, Seoul 156-756, Republic of Korea). **An enzymatic biosensor for hydrogen peroxide based on CeO₂ nanostructure electrodeposited on ITO surface. Biosensors and Bioelectronics, Volume 47(2013): 385–390**

In this study, an enzymatic biosensor for amperometric detection of hydrogen peroxide was developed based on the direct electrochemistry of myoglobin (Mb) on a porous cerium dioxide (CeO₂) nanostructured film. The developed film accomplished with large surface area was electrodeposited on an indium tin oxide (ITO) substrate. Surface morphological studies revealed that the formed CeO₂ film has a large specific surface area with a unique nanostructure on the ITO surface. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were employed to demonstrate the electrochemical behavior of Mb immobilized on the fabricated film, which exhibited facile, direct electrochemistry and good electrocatalytic performance without any electron mediator. The electrode displayed a pair of quasi-reversible reduction–oxidation peaks at –0.3 and –0.2 V, respectively, due to the Mb [Fe³⁺/Fe²⁺] redox couple, which is a surface-controlled electrochemical process with one electron transfer. This reagent-less biosensor showed good stability and high sensitivity for detecting H₂O₂ without any influence of intermediate compounds. This protein-based biosensor was capable of detecting H₂O₂ as low as 0.6 μM with linearity up to 3 mM and a response time of ~8 s, compared to those of other modified electrodes. Hence, porous CeO₂ is a possible candidate material for fabricating enzymatic sensors or devices.

Keywords: Electrodeposition; Cerium oxide; Electrochemistry; Myoglobin; Biosensor; Hydrogen peroxide

Saurabh R. Nirantar, Kun Song Yeo, Sharon Chee, David P. Lane, Farid J. Ghadessy. (p53Lab (ASTAR), 8A Biomedical Grove, #06-04/05 Neuros, Singapore 138648, Singapore). **A generic scaffold for conversion of peptide ligands into homogenous biosensors. Biosensors and Bioelectronics, Volume 47(2013): 421–428**

Numerous peptide ligands including protease recognition sequences, peptides mediating protein–protein interactions, peptide epitopes of antibodies and mimotopes are available which bind molecules of interest. However, there is currently no facile method for the incorporation of these

peptides into homogenous detection systems. We present a generalizable method for the incorporation of such peptides into a novel fusion protein framework comprising an enzyme and its inhibitor. The incorporated peptide functions as an allosteric hinge, linking enzyme to its inhibitor. Upon interaction with its cognate analyte, the peptide mediates dissociation of the inhibitor from the enzyme, and facilitates one-step signal generation. Likewise, cleavage of the peptide by a specific protease also causes enzyme-inhibitor dissociation, leading to signal generation. Using the β -lactamase Tem1 and its inhibitor protein as a model scaffold, we show both specific and sensitive (between low nanomolar and mid-picomolar) colorimetric detection of proteases and antibodies within minutes in a homogenous one-step reaction visible to the naked eye. The same scaffold affords in vivo detection of antibody binding and protease function by linking activity to a selectable phenotype in *E. coli*.

Keywords: Biosensor; Generic; Scaffold; Protein; Peptide ligand

Shaolin Mu, Qiaofang Shi. (Department of Chemistry, Yangzhou University, Yangzhou 225002, Jiangsu Province, PR China). Xanthine biosensor based on the direct oxidation of xanthine at an electrogenerated oligomer film. Biosensors and Bioelectronics, Volume 47(2013): 429–435

Poly(*o*-aminophenol-*co*-pyrogallol) (PAP) was first synthesized via the electrochemical copolymerization of *o*-aminophenol and pyrogallol in the acidic solution, using a reduced graphene oxide/glassy carbon (RGO/GC) electrode as a working electrode. Reduced graphene oxide played an important role in increasing PAP amount deposited on the RGO/GC electrode compared to that on the bare GC electrode, which is due to that RGO has the large specific surface area. The results from the spectra of IR, ¹H NMR and ESR and the measurement of molecular weight demonstrated that PAP is an oligomer with the free radicals and exhibited good redox activity in a wide pH range from pH<1–9.0 and can effectively catalyze xanthine oxidation due to the presence of the free radicals and the reversible redox groups in the copolymer chain. On the basis of the direct oxidation of xanthine on PAP, the PAP/RGO/GC electrode was used as a xanthine biosensor. The biosensor showed a linear range from 1.0 to 120 μ M xanthine at pH 6.0 with a correction coefficient of 0.9965 and fast response to xanthine oxidation. The peak potential of xanthine oxidation shifted from 0.814 to 0.668 V as pH increased from 5.0 to 7.5.

Keywords: Poly(*o*-aminophenol-*co*-pyrogallol) oligomer; Oligomer characterization; Reduced graphene oxide; Novel xanthine catalyst; Xanthine biosensor

Margriet Roelse^a, Norbert C.A. de Ruijter^b, Elwin X. Vrouwe^c, Maarten A. Jongsma^{a, d}. (^a Plant Research International, Wageningen UR, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands, ^b Lab. of Cell Biology, Wageningen UR, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands, ^c Micronit Microfluidics BV, Colosseum 15, 7521 PV Enschede, The Netherlands, ^d NanoNextNL, P.O. Box 3021, 3502 GA Utrecht, The Netherlands). A generic microfluidic biosensor of G protein-coupled receptor activation—monitoring cytoplasmic [Ca²⁺] changes in human HEK293 cells. Biosensors and Bioelectronics, Volume 47(2013): 436–444

Cell lines expressing recombinant G-protein coupled receptors (GPCRs) are activated by specific ligands resulting in transient [Ca²⁺] rises that return to basal levels in 30–60 s. Yellow Cameleon

3.6 (YC3.6) is a genetically encoded calcium indicator which can be co-expressed to monitor these cytosolic $[Ca^{2+}]$ changes in real-time using Förster (Fluorescence) resonance energy transfer (FRET). On this basis, we designed the prototype of a generic microfluidic biosensor of GPCR activation, imaging $[Ca^{2+}]$ changes in recombinant human HEK293 cells, which express a combination of a GPCR (Neurokinin 1) and YC3.6. An internal reference for non-specifically induced $[Ca^{2+}]$ changes were YC3.6 cells without GPCR but expressing a red fluorescent protein (mCherry) for identification. These cell lines were grown as a mixed population in a flow cell with a volume of $\sim 50 \mu\text{l}$ and a flow cell surface of 170 mm^2 . Cells were activated by brief exposures to specific and non-specific analytes using an injection valve with a flexible sample volume (tested range $5\text{--}100 \mu\text{l}$) at a flow speed of $100 \mu\text{l}/\text{min}$. A flow cell surface of 0.2 mm^2 with 50 cells was imaged every $2\text{--}4 \text{ s}$ to obtain signal kinetics. The lower limit of detection was 30 pM Substance P (SP, $2 \text{ pg}/50 \mu\text{l}$), and reproducible responses to repeated injections every 3 min were obtained at 1 nM SP. This biosensor was designed for ~ 50 cells for statistical reasons, but at a lower limit of 1 receptor- and 1 reference-cell, specific ligand detection is still feasible.

Keywords: G protein-coupled receptor; $[Ca^{2+}]$ imaging; HEK293 cells; FRET; Dynamic; Microfluidic flow cell; Cameleon

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Graphene oxide (GO) has been widely used to develop fluorescence resonance energy transfer (FRET) biosensors for tumor markers (e.g., matrix metalloproteinases, MMPs) due to its superior fluorescence quenching capacity and unique adsorption characteristics for biomolecules. In this study, fluorescein isothiocyanate-labeled peptide (Pep-FITC) was assembled onto the GO surface through covalent binding to construct a GO-Pep-FITC FRET sensor for sensitive, rapid, and accurate detection of MMP-2 in complex serum samples. Compared to similar GO-based FRET sensors fabricated through physical adsorption, the as prepared ones via covalent binding are significantly more stable under physiological conditions, enabling their detection of MMP-2 with high sensitivity (detection limit: $2.5 \text{ ng}/\text{mL}$). More importantly, it allows for rapid MMP-2 detection (within 3 h) even in complex biological samples with satisfactory accuracy and the relative standard deviation $\leq 7.03\%$. Our studies further suggest that such a platform developed here for sensitive, rapid, and accurate detection of biomarkers holds great promise for clinical diagnosis of protease-related diseases.

Keywords: Graphene oxide; Fluorescence resonance energy transfer; Matrix metalloproteinase; Protease; Sensor; Tumor

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Education, Jiangnan University, Wuxi 214122, PR China). Fabrication of a colorimetric biosensing platform for the detection of protein–DNA interaction. *Biosensors and Bioelectronics*, Volume 46(2013): 108–112

Protein–DNA interaction plays important roles in many cellular processes, and there is an urgent demand for valid methods to monitor the interaction. In view of this, we propose a simple label-free colorimetric platform for the detection of protein–DNA interaction. Protein–DNA couples together with peroxidase-mimicking DNAzyme and exonuclease are elaborately incorporated into an integrated biosensing system. Besides the simplicity and efficiency, the strategy also has a great advantage for its universality in the detection of different protein–DNA couples. In our experiments, effective validation of our approach can be supported by two different protein–DNA couples (estrogen receptor α and nuclear factor kappa B). Experimental results show that the DNAzyme is competent to give rise to evident readout signals to monitor protein–DNA couples. Furthermore, with the substitution of DNA binding sequence in the probe, this method could be extended to a general platform for the detection of protein–DNA interaction.

Keywords: Protein–DNA interaction; G-quadruplex; Colorimetric; Detection

Bioengineering

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Solid waste production is inevitable and its unsanitary disposal in the environment is of public and environmental health concern. Leachate, generated due to the infiltration of water/precipitation through the waste mass and the wastes biodegradation, is a mixture of dissolved organic matter, inorganic macro-components, heavy metals, xenobiotic organic compounds and microorganisms. Several studies have reported the acute toxicity of leachate using different end points, while evidences are accumulating on their potentials to induce genetic damage. In this wise, different short-term *in vivo* and *in vitro* bioassays are being utilized in the evaluations of genotoxicity and mutagenicity of leachates; and the possible mechanisms of genetic damage. This paper reviews reports on leachate-induced genetic damage. There is need for a shift from waste disposal to sustainable waste management. Awareness on possible health impacts or consequences of exposure to solid waste should also be created through health education.

Key words: Solid waste leachate, genotoxicity, mutagenicity, environmental pollution.

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²Knowledge Research and Development Center, Inteqc Feed Co., Ltd., Samutsakhorn 74000, Thailand, ³Department of Chemical Engineering, Faculty of Engineering, Mahidol University, Nakornpathom 73170, Thailand, ⁴Department of Botany, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok, 10330, Thailand, ⁵Biofuels by Biocatalysts Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand. Email: warawut.c@chula.ac.th Tel: +66 22185482. Fax: +66 22185482). Biodiesel production from *Jatropha curcas* oil catalyzed by whole cells of *Aureobasidium pullulans* var. *melanogenum* SRY 14-3. *African Journal of Biotechnology* Vol.12(27)(2013): 4380-4386

The main obstacle to using lipase as a catalyst in industrial scale biodiesel production is the cost and availability of the enzyme. To overcome this obstacle, the potential of using a whole cell biocatalyst (for at least partial *in situ* lipase production) was evaluated as a means to reduce the cost of the lipase. The reaction conditions for biodiesel production via transesterification between *Jatropha curcas* (physic nut) oil and methanol when catalyzed in the presence of lipase-producing *Aureobasidium pullulans* yeast cells was investigated. The appropriate conditions for optimal biodiesel production were found to be 1:3 oil:methanol molar ratio at 30°C with constant stirring at 250 rpm. Under these conditions a maximum fatty acid methyl ester (biodiesel) production level of 71.8% was obtained after 72 h.

Key words: Lipase, *Aureobasidium pullulans*, physic nut oil, biodiesel, green energy.

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We present a microfabricated paper-based microbial fuel cell (MFC) generating a maximum power of 5.5 $\mu\text{W}/\text{cm}^2$. The MFC features (1) a paper-based proton exchange membrane by infiltrating sulfonated sodium polystyrene sulfonate and (2) micro-fabricated paper chambers by patterning hydrophobic barriers of photoresist. Once inoculum and catholyte were added to the MFC, a current of 74 μA was generated immediately. This paper-based MFC has the advantages of ease of use, low production cost, and high portability. The voltage produced was increased by 1.9 \times when two MFC devices were stacked in series, while operating lifetime was significantly enhanced in parallel.

Keywords: Microbial fuel cell; Paper-based battery; Microfabrication; Series/parallel connection

Pollen Biotechnology

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Iran. Email: irian@tmu.ac.ir). A comparative study on the allergenicity, structure, and ultrastructure of two Acacia pollen grains in guinea pigs. *Aerobiologia*, Volume 299 (3) (2013): 333-339

Acacia saligna and Acacia victoria are native to Australia brought to and grown in Southern Iran. They have since had a widespread distribution and become native in Iran. As people of the region are exposed to the pollen grains from these plants during a 5 months period, this study aimed at investigating the allergenicity of their mature pollen grains. In addition, the structural and ultrastructural as well as the total protein content of the mature pollen grains were analyzed. Pollen grains of *A. saligna* and *A. victoria* were collected from the suburbs of Ahvaz, Iran. Pollen extracts (15 % concentration) were prepared in PBS (pH 7.2), and 4- to 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. Comparative analysis of the allergenicity of *A. saligna* and *A. victoria* pollen grains revealed a higher IgE level in the latter. Comparative analysis of pollen characteristics was performed using both light and electron microscopy for sporoderm structure and ultrastructure, respectively. Twelve percent PAGE analysis of the total protein content of mature pollen grains showed a greater number of bands in *A. victoria*, while four bands were common in both species. In conclusion, this study demonstrates that both *A. saligna* and *A. victoria* pollen grains are allergenic, with a greater allergenicity of *A. victoria* pollen grains. In addition, the structural and ultrastructural as well as the total protein content of the mature pollen grains are revealed, and a potential protein allergen is proposed.

Keywords: Pollen grain, Allergenicity, Acacia, Ontogeny

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Cupressaceae pollen allergy is an important cause of pollen allergy throughout the world. Prevalence of allergy to Cupressaceae pollen has increased significantly during the winter over the past 3 decades because of extensive planting of cypress trees for different purposes. *Thuja orientalis* (Cupressaceae) is a naturally grown plant in Iran and is widely cultivated as a common ornamental plant in this country and other ones. Allergenicity of its pollen has been established, but to this day no allergenic component has been detected. The aim of this research is to study allergenicity and evaluate the immunoglobulin E reactivity to *T. orientalis* pollen extracts. Pollen grains were directly collected from mature male cones of trees. Pollen proteins were extracted and were analyzed by SDS-PAGE. Total protein content of pollen extracts was measured by Bradford assay. Immunoblotting using the serum of sensitized rats showed a single immunogenic band at about 44KD in pollen extracts. Result of this research proved that pollen grains of *T. orientalis* are allergenic.

Keywords: Allergy, Cupressaceae, Immunoblotting, Pollen protein content, *Thuja orientalis*

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Parietaria pollen has never been considered as a significant cause of pollinosis in Chile; therefore, the sensitization to Parietaria study has never been included in the study of patients with clinical suspicion of pollinosis in this region. The objective of this study was to describe the clinical characteristics of pollinosis caused by Parietaria in the Valparaíso region, related to air concentrations of this kind of pollen. A cross-sectional study was performed in the city of Valparaíso. It consisted of two stages: In the first, pollen grains were counted between 1999 and 2001. In the second, a sensitization profile on a patient population diagnosed with ARC (allergic rhinoconjunctivitis) was evaluated. *Parietaria judaica* (*P. judaica*) presented pollination all year long, with aggravation in the spring and summer, and with values reaching 80 grains/m³ (weekly average). These findings determined the transience of the symptoms in this population, which is mainly perennial with seasonal aggravations. A total of 72 atopic subjects were obtained during the whole sample recollection period. *P. judaica* was the second most frequent cause of sensitization (60 %) after *Dermatophagoides* in the sample overall. Also, in monosensitized subjects, it was the first cause of pollen sensitization. *P. judaica* represents the second cause of allergy in Valparaíso and the first cause of pollinosis. These findings suggest the importance of quantifying *Parietaria* in Valparaíso and near cities, plus investigating the presence of sensitization and symptoms to allergies in a significant proportion of patients in this region.

Keywords: Allergenic pollen, Airborne pollen, Hypersensitivity, Valparaiso, Chile *Parietaria* pollen

Oliver¹, L. Bjermer², D. Quinn³, P. Saggu⁴, P. Thomas⁵, K. Yarnall^{1,†}, J. Lötval⁶. (1GlaxoSmithKline Respiratory and Immuno-Inflammation Medicines Development Centre, Stockley Park, UK, 2Department of Respiratory Medicine and Allergology, Skane University, Institute for Clinical Science, Lund, Sweden, 3P3 Research, Wellington, New Zealand, 4Betaplex, London, UK, 5Faculty of Medicine, The University of New South Wales, Sydney, NSW, Australia, 6Krefting Research Centre, University of Gothenburg, Gothenburg, Sweden, †Quintiles Clinical Research, Green Park, Reading, UK. Correspondence: Prof. Jan Lötval, Chairman, Krefting Research Centre, University of Gothenburg, BOX 424, SE 40530 Gothenburg, Sweden). Modulation of allergen-induced bronchoconstriction by fluticasone furoate and vilanterol alone or in combination. *Allergy*, Volume 68(9) (2013): 1136–1142

This placebo-controlled study assessed the effects of the once-daily inhaled corticosteroid (ICS) fluticasone furoate (FF) and long-acting beta₂-agonist (LABA) vilanterol (VI) on early and late asthmatic responses (EAR/LAR) and airway hyper-responsiveness (AHR).

Patients ($n = 27$) were randomized to FF (100 μg), VI (25 μg), FF/VI (100/25 μg), and placebo for 21 days (four periods). Allergen challenge was performed 1 h post-dose on day 21. AHR was assessed on day 22 using methacholine.

Allergen challenge caused an early change (0–2 h) in minimum forced expiratory volume in 1 s (FEV_1) of -1.091 l (95% CI: -1.344 ; -0.837) following placebo therapy; changes were -0.955 l (-1.209 ; -0.702), -0.826 l (-1.070 ; -0.581), and -0.614 l (-0.858 ; -0.370) following VI, FF, or FF/VI therapy, respectively. Treatment differences were significant for all comparisons between therapies. Mean changes in 0–2 h % FEV_1 were as follows: -28.05 (placebo), -23.10 (VI), -22.33 (FF), and -16.10 (FF/VI). Following placebo, the late change (4–10 h) in weighted mean FEV_1 was -0.466 l (-0.589 ; -0.343) and -0.298 l (-0.415 ; -0.181) after VI, and was $+0.018$ l with both FF/VI (-0.089 ; 0.124) and FF (-0.089 ; 0.125). Treatment differences were significant for all comparisons between therapies except FF/VI vs FF. Mean changes in 4–10 h % FEV_1 were as follows: -21.08 (placebo), -14.30 (VI), -5.02 (FF), and -5.83 (FF/VI). AHR 24 h after allergen challenge was significantly reduced with FF/VI and FF vs placebo, and FF/VI was superior to either component.

Combined treatment with FF/VI provides additive protection from the EAR relative to its components, significant protection over VI alone from the LAR, and confers sustained protection from hyper-responsiveness 24 h post-dose.

Keywords: allergen challenge; asthma; atopy; inhaled corticosteroid; long-acting beta₂-agonist

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The natural course of childhood asthma and allergy is complex and not fully understood. We aimed to identify phenotypes based upon the time course of respiratory/allergic symptoms throughout preschool years.

As part of the PARIS cohort, symptoms of wheezing, dry night cough, rhinitis and dermatitis were collected annually from birth to age 4 years. K-means clustering was used to group into phenotypes children with similar symptoms trajectories over the study period. Associations of phenotypes with IgE sensitization and risk factors were studied using multinomial logistic regression.

Besides a group with low prevalence of symptoms considered as reference ($n = 1236$, 49.0%), four distinct respiratory/allergic phenotypes were identified: two transient [transient rhinitis phenotype ($n = 295$, 11.7%), transient wheeze phenotype ($n = 399$, 15.8%)], without any relation with IgE sensitization, and two persistent [cough/rhinitis phenotype ($n = 284$, 11.3%), dermatitis phenotype ($n = 308$, 12.2%)], associated with IgE sensitization. Transient rhinitis phenotype was

only associated with tobacco smoke exposure, which could irritate the airways. Transient wheeze phenotype was related to male sex and contact with other children (older siblings, day care attendance). Lastly, risk factors for both IgE-associated phenotypes encompassed parental history of allergy, potential exposure to allergens and stress, known to be associated with the development of allergic diseases.

This study provides evidence for the existence of different respiratory/allergic phenotypes before school age. The fact that they differ in terms of sensitization and risk factors reinforces the plausibility of distinct phenotypes, potentially linked to irritation and infections for the transient phenotypes and to allergy for the persistent phenotypes.

Keywords: allergy; cluster analysis; phenotype; preschool children; symptom trajectory

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We report on the development of personalized pollen-related information services that include sensitivity categorization, threshold identification, and symptom forecasting, addressing patients with allergic rhinitis in Europe.

Biotechnology Policy Issue

Raphaël Billé¹, Ryan Kelly^{2, 3}, Arne Biastoch⁴, Ellycia Harrould-Kolieb⁵, Dorothée Herr⁶, Fortunat Joos⁷, Kristy Kroeker⁸, Dan Laffoley⁶, Andreas Oschlies⁴ and Jean-Pierre Gattuso^{9, 10}. (¹Institute for Sustainable Development and International Relations (IDDRI), Sciences Po, 27 Rue Saint Guillaume, 75337 Paris Cedex 7, France, ²Center for Ocean Solutions, Stanford University, Stanford, CA 94305, USA, ³School of Marine and Environmental Affairs, University of Washington, Seattle, WA, USA, ⁴GEOMAR, Helmholtz Centre for Ocean Research, Kiel, Germany, ⁵Melbourne School of Land and Environment, University of Melbourne, Parkville, VIC, Australia, ⁶International Union for the Conservation of Nature (IUCN), Gland, Switzerland, ⁷Physics Institute, University of Bern, 3012 Bern, Switzerland, ⁸Bodega Marine Lab, UC Davis, Bodega Bay, CA 94923, USA, ⁹Laboratoire d'Océanographie de Villefranche, CNRS-INSU, BP 28, 06234 Villefranche-sur-Mer Cedex, France, ¹⁰Observatoire Océanologique de Villefranche, Université Pierre et Marie Curie-Paris 6, 06230 Villefranche-sur-Mer, France. Email: raphael.bille@iddri.org). **Environmental Management, Volume 52(4)(2013): 761-779**

Ocean acidification has emerged over the last two decades as one of the largest threats to marine organisms and ecosystems. However, most research efforts on ocean acidification have so far neglected management and related policy issues to focus instead on understanding its ecological and biogeochemical implications. This shortfall is addressed here with a systematic, international

and critical review of management and policy options. In particular, we investigate the assumption that fighting acidification is mainly, but not only, about reducing CO₂ emissions, and explore the leeway that this emerging problem may open in old environmental issues. We review nine types of management responses, initially grouped under four categories: preventing ocean acidification; strengthening ecosystem resilience; adapting human activities; and repairing damages. Connecting and comparing options leads to classifying them, in a qualitative way, according to their potential and feasibility. While reducing CO₂ emissions is confirmed as the key action that must be taken against acidification, some of the other options appear to have the potential to buy time, e.g. by relieving the pressure of other stressors, and help marine life face unavoidable acidification. Although the existing legal basis to take action shows few gaps, policy challenges are significant: tackling them will mean succeeding in various areas of environmental management where we failed to a large extent so far.

Keywords: Ocean acidification, Marine ecosystems, Management, Policy Resilience, Adaptation

Agricultural Biotechnology

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Three pot experiments were carried out to evaluate the phytoextraction efficiency of cadmium (Cd) by an amaranth (*Amaranthus hypochondriacus* L.). To enhance phytoremediation potential, this study examined the effect of fertilization, repeated harvests, and growth time on the efficiency of Cd removal from soil. The result showed that fertilizing with NPK increased dry biomass by a factor of 4.2, resulting in a large increment of Cd accumulation. Repeated harvests had a significant effect on the plant biomass and thus on overall Cd removal and an optimal cutting position influenced the amount of Cd extracted from soils. Plant growth time was found to significantly affect the amount of Cd extracted by *A. hypochondriacus*. This study indicates that *A. hypochondriacus* has great phytoremediation potential in Cd-contaminated soil. For best practice, the recommendation is to maximize the phytoextraction efficiency of *A. hypochondriacus* by repeated harvests, harvesting at the squaring stage (soon after the flower begins to appear), and apply NPK compound fertilizer as base application.

Keywords: Amaranth, Cadmium Phytoremediation, Heavy metal, Repeated harvest Fertilizer

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Agricultural biotechnology, intellectual property rights and seed industry in India. Asian Biotechnology and Development Review, Volume 15(2) (2013): 61-79

It is contended that while Green Revolution was led by public sector, Gene Revolution in agriculture is led by private sector. While increased emphasis on intellectual property rights protection over seeds and germplasm by the private sector, the seed industry dominated by private sector would deliver more of inputs like seeds and improved varieties that bring in more revenue. In this article we discuss the growth of agri-biotechnology in India and the changing profile of seed industry in cereal crops. We find that favourable policy frameworks and liberalisation have resulted in more investment by private sector in R&D for developing new varieties and seeds and this mirrors the trends elsewhere. While incentives are necessary for private sector to invest in R&D corresponding measures like effective competition policy are also required so that benefits of biotechnology reach small and medium farmers for whom affordability and accessibility of seed a key input is important. The challenge lies in harmonising commitments under WTO Agreements and Convention on Biological Diversity with effective measures that would make biotechnology based inputs affordable and accessible. Otherwise this may be a barrier in diffusion of agricultural biotechnology.

Keywords: Agriculture; Biotechnology; Intellectual property rights; Research and development; Seed

Liu, W.^a, Yuan, J.S.^b, Stewart Jr., C.N.^{ac}. (^a Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996, United States , ^b Department of Plant Pathology and Microbiology, Institute of Plant Genomics and Microbiology, Texas A and M University, College Station, TX 77843, United States , ^c BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN 37831, United States). **Advanced genetic tools for plant biotechnology. Nature Reviews Genetics, Volume 14(11)(2013): 781-793**

Basic research has provided a much better understanding of the genetic networks and regulatory hierarchies in plants. To meet the challenges of agriculture, we must be able to rapidly translate this knowledge into generating improved plants. Therefore, in this Review, we discuss advanced tools that are currently available for use in plant biotechnology to produce new products in plants and to generate plants with new functions. These tools include synthetic promoters, 'tunable' transcription factors, genome-editing tools and site-specific recombinases. We also review some tools with the potential to enable crop improvement, such as methods for the assembly and synthesis of large DNA molecules, plant transformation with linked multigenes and plant artificial chromosomes. These genetic technologies should be integrated to realize their potential for applications to pressing agricultural and environmental problems.

Keywords: EMTREE drug terms: DNA; DNA fragment; genomic DNA; guide RNA; hybrid protein; protein; recombinant protein; repetitive DNA; restriction endonuclease; RNA precursor; transcription factor; type II site specific deoxyribonuclease; zinc finger protein

Bioenergy

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Guangxi, 530007, China. Email: hongguanglishibahao@yahoo.com). Predictions of Enzymatic Parameters: A Mini-Review with Focus on Enzymes for Biofuel. Applied Biochemistry and Biotechnology, Volume 171(3) (2013): 590-615

Enzymatic reactions are very basic processes in biological systems, and parameters related to enzymatic reactions always provide good indicators for understanding of mechanisms underlined in enzymatic reactions, for better controlling of enzymatic reactions, and for comparison of different enzymes. In this mini-review: first, parameters in enzymatic reactions were briefly reviewed from three different standpoints; second, predictions of parameters in enzymatic reactions without information on enzyme structure were shortly reviewed from viewpoints of geometric approach, graphic approach and compartmental approach; third, predictions of parameters in enzymatic reaction with information on enzyme structure were reviewed from the points of view of modeling, with 19 currently available databases, and 17 software packages and web servers; fourth, the current state of prediction on parameters in enzymatic reaction in biofuel industry with respect to cellulolytic enzymes were reviewed; fifth, the pros and cons for future development were discussed; and finally, a worked example was given in the Appendix to describe the whole procedures of prediction of enzymatic parameters in reactions.

Keywords: Biofuel, Enzyme Prediction Review

Zhen Wang¹, Xiaochen Ma¹, Wenguang Zhou¹, Min Min¹, Yanling Cheng¹, Paul Chen¹, Jian Shi¹, Qin Wang¹, Yuhuan Liu¹ and Roger Ruan¹ (¹Center for Biorefining, Bioproducts and Biosystems Engineering Department, University of Minnesota, 1390 Eckles Ave, Saint Paul, MN 55108, USA. Corresponding author: Email: zhouw@umn.edu, Email: ruanx001@umn.edu). Oil Crop Biomass Residue-Based Media for Enhanced Algal Lipid Production. Applied Biochemistry and Biotechnology, Volume 171(3) (2013): 689-703

The aim of this study was to evaluate the use of hydrolysates from acid hydrolysis of four different oil crop biomass residues (OCBR) as low cost culture media for algae growth. The one-factor-at-a-time method was used to design a series of experiments to optimize the acid hydrolysis conditions through examining the total nitrogen, total phosphorus, chemical oxygen demand, and ammonia nitrogen in the hydrolysates. The optimal conditions were found to be using 3 % sulfuric acid and hydrolyzing residues at 90 °C for 20 h. The hydrolysates (OCBR media) produced under the optimal conditions were used to cultivate the two algae strains, namely UM258 and UM268. The results from 5 days of cultivation showed that the OCBR media supported faster algae growth with maximal algal biomass yield of 2.7 and 3 g/L, respectively. Moreover, the total lipids for UM258 and UM268 were 54 and 35 %, respectively, after 5 days of cultivation, which suggested that the OCBR media allowed the algae strains to accumulate higher lipids probably due to high C/N ratio. Furthermore, over 3 % of omega-3 fatty acid (EPA) was produced for the two algae strains. In conclusion, OCBR media are excellent alternative for algae growth and have a great potential for large-scale production of algae-based ingredients for biodiesel as well as high-value food and pharmaceutical products.

Keywords: Oil crop biomass residue (OCBR), Acid hydrolysis, Facultative heterotrophic microalgae, Lipid Omega-3 fatty acid

Rajni Kumari¹ and Krishna Pramanik¹. (¹Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, Odisha, India. Email: rajni.nitrkl@gmail.com, Email: kpr@nitrkl.ac.in). Bioethanol Production from Ipomoea Carnea Biomass Using a Potential Hybrid Yeast Strain. Applied Biochemistry and Biotechnology, Volume 171(3) (2013):771-785

The paper deals with the exploitation of Ipomoea carnea as a feedstock for the production of bioethanol. Dilute acid pretreatment under optimum conditions (3 %H₂SO₄, 120 °C for 45 min) produced 17.68 g L⁻¹ sugars along with 1.02 g L⁻¹ phenolics and 1.13 g L⁻¹ furans. A combination of overliming and activated charcoal adsorption facilitated the removal of 91.9 % furans and 94.7 % phenolics from acid hydrolysate. The pretreated biomass was further treated with a mixture of sodium sulphite and sodium chlorite and, a maximum lignin removal of 81.6 % was achieved. The enzymatic saccharification of delignified biomass resulted in 79.4 % saccharification with a corresponding sugar yield of 753.21 mg g⁻¹. Equal volume of enzymatic hydrolysate and acid hydrolysate were mixed and used for fermentation with a hybrid yeast strain RPRT90. Fermentation of mixed detoxified hydrolysate at 30 °C for 28 h produced ethanol with a yield of 0.461 g g⁻¹. A comparable ethanol yield (0.414 g g⁻¹) was achieved using a mixture of enzymatic hydrolysate and undetoxified acid hydrolysate. Thus, I. carnea biomass has been demonstrated to be a potential feedstock for bioethanol production, and the use of hybrid yeast may pave the way to produce bioethanol from this biomass.

Keywords: Bioethanol, Ipomoea carnea, Hybrid yeast, Hydrolysate, Fermentation

E. I. Garcia-Peña¹, M. Canul-Chan¹, I. Chairez¹, E. Salgado-Manjarez² and J. Aranda-Barradas². (¹Bioprocesses Department, Unidad Profesional Interdisciplinaria de Biotecnología, Instituto Politécnico Nacional, Av Acueducto s/n, P.O. Box 07340, Mexico City, Mexico, ²Bioengineering Department, Unidad Profesional Interdisciplinaria de Biotecnología, Instituto Politécnico Nacional, Av Acueducto s/n, P.O. Box 07340 Mexico City, Mexico). Biohydrogen Production Based on the Evaluation of Kinetic Parameters of a Mixed Microbial Culture Using Glucose and Fruit–Vegetable Waste as Feedstocks. Applied Biochemistry and Biotechnology, Volume 171(2) (2013): 279-293

Hydrogen (H₂) production from the organic fraction of solid waste such as fruit and vegetable waste (FVW) is a novel and feasible energy technology. Continuous application of this process would allow for the simultaneous treatment of organic residues and energy production. In this study, batch experiments were conducted using glucose as substrate, and data of H₂ production obtained were successfully adjusted by a logistic model. The kinetic parameters ($\mu_{\max} = 0.101 \text{ h}^{-1}$, $K_s = 2.56 \text{ g/L}$) of an H₂-producing microbial culture determined by the Monod and Haldane–Andrews growth models were used to establish the continuous culture conditions. This strategy led to a productive steady state in continuous culture. Once the steady state was reached in the continuous reactor, a maximum H₂ production of 700 mL was attained. The feasibility of producing H₂ from the FVW obtained from a local market in Mexico City was also evaluated using batch conditions. The effect of the initial FVW concentration on the H₂ production and waste organic material degradation was determined. The highest H₂ production rate (1.7 mmol/day), the highest cumulative H₂ volume (310 mL), and 25 % chemical oxygen demand (COD) removal were obtained with an initial substrate (FVW) concentration of 37 g COD/L. The lowest H₂ production rates were obtained with relatively low initial substrate concentrations of 5 and 11 g COD/L. The H₂ production rates with FVW were also characterized

by the logistic model. Similar cumulative H₂ production was obtained when glucose and FWW were used as substrates.

Keywords: Continuous culture, Hydrogen production, Kinetic study, Mixed culture

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The aims of this study were to develop the kinetic model and determine kinetic parameters describing ethanol production from sweet sorghum juice using very high gravity technology in the batch fermentation of *Saccharomyces cerevisiae* NP01. The obtained experimental data were tested with four different types of model, based on the experimental data, accounting for the substrate limitation, substrate inhibition, product inhibition, and the combination of those three effects, respectively. The optimization technique to find kinetic parameters was non-linear regression using Marquardt method performed through numerical procedure. The chosen model with its kinetic parameters obtained in the batch mode was validated and tested against the other independent experimental data in the small batch-scale and large-scale fermenter, in order to investigate the applicability and scale-up effect of the model, respectively. Then, the obtained model with its parameters was applied in the simulations of the continuous and fed-batch operations to examine the concentration profiles of fermentation components with the variations in operating parameters such as the dilution rate, feed-flow rate, start-up time, and feed concentration. The results indicated that the kinetic model (the substrate limitation with substrate and product inhibition effects) was suitable to describe ethanol fermentation. In the continuous mode, using the dilution rate of 0.01 h⁻¹, the maximum ethanol concentration obtained was, approximately, 90 g/l whereas the simulated results from the fed-batch operation revealed that the maximum ethanol concentration at quasi-steady state condition was, approximately, 96 g/l. The start-up time of 21 h was the fastest time to reach the steady-state and quasi-steady state for both the continuous and fed-batch modes, respectively.

Keywords: Simulation, Kinetic model, Ethanol, Very high gravity technology, Sweet sorghum juice

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Exiguobacterium sp. VSG-1 was isolated from the soil sample and characterized for the production of lignocellulolytic enzymes. Production of these enzymes by the strain VSG-1 was carried out using steam-exploded sugarcane bagasse (SCB) and found to secrete cellulase, pectinase, mannanase, xylanase, and tannase. The growth and enzyme production were found to be optimum at pH 9.0 and 37 °C. Upon steam explosion of SCB, the cellulose increased by 42 %, whereas hemicelluloses and lignin decreased by 40 and 62 %, respectively. Enzymatic hydrolysis of steam-exploded SCB yielded 640 g/l of total sugars. Fermentation of sugars produced from pretreated SCB was carried out by using *Saccharomyces cerevisiae* at pH 5.0 and 30 °C. The alcohol produced was calculated and found to be 62.24 g/l corresponding to 78 % of the theoretical yield of ethanol. Hence, the strain VSG-1 has an industrial importance for the production of fermentable sugars for biofuels.

Keywords: *Exiguobacterium* sp. VSG-1 Lignocellulolytic enzymes, Sugarcane bagasse (SCB), Enzymatic hydrolysis, Bioethanol

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The concentration of ethanol produced from lignocellulosic biomass should be at least 40 g l⁻¹ [about 5 % (v/v)] to minimize the cost of distillation process. In this study, the conditions for the simultaneous saccharification and fermentation (SSF) at fed-batch mode for the production of ethanol from alkali-pretreated empty palm fruit bunch fibers (EFB) were investigated. Optimal conditions for the production of ethanol were identified as temperature, 30 °C; enzyme loading, 15 filter paper unit g⁻¹ biomass; and yeast (*Saccharomyces cerevisiae*) loading, 5 g l⁻¹ of dry cell weight. Under these conditions, an economical ethanol concentration was achieved within 17 h, which further increased up to 62.5 g l⁻¹ after 95 h with 70.6 % of the theoretical yield. To our knowledge, this is the first report to evaluate the economic ethanol production from alkali-pretreated EFB in fed-batch SSF using *S. cerevisiae*.

Keywords: Lignocellulosic Biomass, Empty Palm Fruit Bunch Fiber (EFB), SSF Ethanol Pretreatment

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The pretreatment of lignocellulosic biomass with white-rot fungi to produce bioethanol is an environmentally friendly alternative to the commonly used physico-chemical processes. After biological pretreatment, a solid substrate composed of cellulose, hemicellulose and lignin, the two latter with a composition lower than that of the initial substrate, is obtained. In this study, six microorganisms and four process configurations were utilised to ferment a hydrolysate obtained from wheat straw pretreated with the white-rot fungus *Irpex lacteus*. To enhance total sugars utilisation, five of these microorganisms are able to metabolise, in addition to glucose, most of the pentoses obtained after the hydrolysis of wheat straw by the application of a mixture of hemicellulolytic and cellulolytic enzymes. The highest overall ethanol yield was obtained with the yeast *Pachysolen tannophilus*. Its application in combination with the best process configuration yielded 163 mg ethanol per gram of raw wheat straw, which was between 23 and 35 % greater than the yields typically obtained with a conventional bioethanol process, in which wheat straw is pretreated using steam explosion and fermented with the yeast *Saccharomyces cerevisiae*.

Keywords: Bioethanol, Lignocellulosic biomass pretreatment, Alcoholic fermentation *Pachysolen tannophilus*, Wheat straw

Ming-Hsu Chen¹, Prabhjot Kaur¹, Bruce Dien², Frederick Below³, Michael L. Vincent³ and Vijay Singh¹ (¹Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA, ²BioEnergy Research Unit, Agricultural Research Service, United States Department of Agriculture, National Center for Agricultural Utilization Research, Peoria, IL, USA, ³Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA. Email: vsingh@illinois.edu). Use of tropical maize for bioethanol production. *World Journal of Microbiology and Biotechnology*, Volume 29(8) (2013): 1509-1515

Tropical maize is an alternative energy crop being considered as a feedstock for bioethanol production in the North Central and Midwest United States. Tropical maize is advantageous because it produces large amounts of soluble sugars in its stalks, creates a large amount of biomass, and requires lower inputs (e.g. nitrogen) than grain corn. Soluble sugars, including sucrose, glucose and fructose were extracted by pressing the stalks at dough stage (R4). The initial extracted syrup fermented faster than the control culture grown on a yeast extract/phosphate/sucrose medium. The syrup was subsequently concentrated 1.25–2.25 times, supplemented with urea, and fermented using *Saccharomyces cerevisiae* for up to 96 h. The final ethanol concentrations obtained were 8.1 % (v/v) to 15.6 % (v/v), equivalent to 90.3–92.2 % of the theoretical yields. However, fermentation productivity decreased with sugar concentration, suggesting that the yeast might be osmotically stressed at the increased sugar concentrations. These results provide in-depth information for utilizing tropical maize syrup for bioethanol production that will help in tropical maize breeding and development for use as another feedstock for the biofuel industry.

Keywords: Tropical maize, Fermentation Ethanol

K. Ponnusamy^{1, 4}, S. Kappachery¹, M. Thekeettle², J. H. Song³ and J. H. Kweon¹. (¹Department of Environmental Engineering, Konkuk University, 1 Hwayang Dong, Gwangjin Gu, Seoul, 143-701, Republic of Korea, ²Department of Biotechnology, School of

Science, Engineering and Technology, Indus International University, Una, 174301, Himachal Pradesh, India, ³Department of Civil and Environmental Engineering, Sejong University, 98 Gunja Dong, Gwangjin Gu, Seoul, 143-747, Republic of Korea, ⁴Present address: Department Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarland University, 66123 Saarbrücken, Germany. Email: kannan_microl@yahoo.com, Email: jhkweon@konkuk.ac.kr). Anti-biofouling property of vanillin on *Aeromonas hydrophila* initial biofilm on various membrane surfaces. *World Journal of Microbiology and Biotechnology*, Volume 29(9) (2013): 1695-1703

Biofouling is a serious problem on filter membranes of water purification systems due to formation of bacterial biofilms, which can be detrimental to the membrane performance. Biofouling occurs on membrane surface and therefore greatly influences the physical and chemical aspects of the surface. Several membranes including microfiltration, ultrafiltration, and reverse osmosis (RO) membranes were used to learn about the anti-biofouling properties of vanillin affecting the membrane performances. Vanillin has been recognized as a potential quorum quenching compound for *Aeromonas hydrophila* biofilms. The initial attachment and dynamics of biofilm growth were monitored using scanning electron microscopy and confocal laser scanning microscopy. Biofilm quantities were measured using a plate count method and total protein determinations. Vanillin addition was effective in the prevention of biofilm formation on the tested membrane surfaces. Among the membranes, RO membranes made with cellulose acetate showed the most substantial reduction of biofilm formation by addition of vanillin. The biofilm reduction was confirmed by the results of surface coverage, biomass and protein accumulation. The HPLC spectrum of the spent culture with vanillin addition showed that vanillin may interfere with quorum sensing molecules and thus prevent the formation of the biofilms.

Keywords: Anti-biofouling, *Aeromonas hydrophila*, RO membrane, Vanillin Quorum sensing

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Apart from being applied as an energy carrier, hydrogen is in increasing demand as a commodity. Currently, the majority of hydrogen (H₂) is produced from fossil fuels, but from an environmental perspective, sustainable H₂ production should be considered. One of the possible ways of hydrogen production is through fermentation, in particular, at elevated temperature, i.e. thermophilic biohydrogen production. This short review recapitulates the current status in thermophilic biohydrogen production through fermentation of commercially viable substrates produced from readily available renewable resources, such as agricultural residues. The route to commercially viable biohydrogen production is a multidisciplinary enterprise. Microbiological studies have pointed out certain desirable physiological characteristics in H₂-producing microorganisms. More process-oriented research has identified best applicable reactor types and cultivation conditions. Techno-economic and life cycle analyses have identified key process bottlenecks with respect to economic feasibility and its environmental impact. The review has further identified current limitations and gaps in the knowledge, and also deliberates directions for future research and development of thermophilic biohydrogen production.

Keywords: Thermophilic, Biohydrogen, Agricultural residues, Techno-economic analysis LCA

Claudia Sheinbaum-Pardo, Andrea Calderón-Irazoque, Mariana Ramírez-Suárez. (Instituto de Ingeniería, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, 04510 México D.F., Mexico). Potential of biodiesel from waste cooking oil in Mexico. Biomass and Bioenergy, Volume 56(2013): 230–238

The aim of this study is to evaluate the potential use of biodiesel produced from waste cooking oil (WCO) in Mexico and its CO₂ emission reduction potential for the Mexican transport sector and associated costs. The results show, based on 2010 data, that the potential of biodiesel from WCO is between 7.8 PJ and 17.7 PJ that represent between 1.5% and 3.3% of petro-diesel consumption for the road transport sector and can reduce between 0.51 and 1.02 Mt of CO₂, (1.0%–2.7% of CO₂-associated emissions), depending on the recovery ratio of WCO from vegetable oil consumption for cooking and considering CO₂ emissions for biodiesel production and methanol emissions during production and combustion in the blend. Primary energy used to produce 1 MJ of WCO-biodiesel is 0.8727 MJ, while literature reports 1.2007 MJ to produce 1 MJ of petro-diesel. Biodiesel costs are similar to petro-diesel costs if WCO is free. The paper offers suggestions for policies that promote increased recollection of WCO for biodiesel production and reduced illegal marketing of WCO, which is the main barrier to increase biodiesel production from WCO. The data used for the analysis is based on a case study of a WCO biodiesel plant that operates in Mexico City.

Keywords: Waste cooking oil; Biodiesel; Mexico; Waste cooking oil; Biodiesel; CO₂ emissions

Amy Thomas, Alan Bond, Kevin Hiscock. (School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK). A GIS based assessment of bioenergy potential in England within existing energy systems. Biomass and Bioenergy, Volume 55(2013): 107–121

This paper presents an analysis of the spatial supply and demand relationships for biomass energy potential for England, using Geographical Information System (GIS) mapping techniques. Due to energy use and cost of biomass feedstock transportation, the spatial relationship between potential supply and demand is crucial to efficient usage of this distributed feedstock. Previous studies have identified potential for biomass generation at individual sites, according to local factors dictating viable transport distances and costs. The research presented here necessarily takes a more generalised approach, to allow national scale assessment of capability to meet fixed location demands, and quantify theoretical potential generation under relevant scenarios. The approach is illustrated for England, although techniques are applicable elsewhere when suitable data are available.

Mapping for England indicates that of the 2,521,996 ha viable for cultivation of *Miscanthus*, 1,998,435 ha are within 25 km of the identified potential end uses of feedstock, and 2,409,541 ha are within 40 km. Potential generation exceeds the 2020 UK biomass generation target of 259 PJ, whichever radius is applied. However, predictions assume *Miscanthus* cultivation at all appropriate sites, and no policy interventions to limit transport distance.

Results from national scale analysis may be useful in informing government decisions, for example to identify impacts on total generation potential of incentives affecting decisions on

allocation of overlap feedstock. Variation in GHG balance and environmental impacts between cultivation sites creates spatial variation in benefits of bioenergy, which should be taken into account in addition to the spatial relationship between supply and demand.

Keywords: Biomass supply and demand; *Miscanthus*; Co-firing; Combined heat and power (CHP); District heating; Bioenergy

Bradley E. Skidmore, Ryan A. Baker, Dila R. Banjade, Jason M. Bray, Douglas R. Tree, Randy S. Lewis. (Department of Chemical Engineering, 350 CB, Brigham Young University, Provo, UT 84602, United States). Syngas fermentation to biofuels: Effects of hydrogen partial pressure on hydrogenase efficiency. Biomass and Bioenergy, Volume 55(2013): 156–162

Producing biofuels from gasified biomass (synthesis gas) via microbial fermentation is currently being pursued as one alternative in biofuels development. In synthesis gas fermentation, reducing equivalents from H₂ oxidation via hydrogenase is important towards directing more carbon towards product formation. In this work, kinetic studies of H₂ utilization via the *Clostridium P11* hydrogenase enzyme were performed to determine the most appropriate model to predict hydrogenase activity as a function of H₂ partial pressure. An important aspect of this work included the proper analysis of electron acceptors used in the kinetic studies. The K_{H_2} model parameter governing the effect of H₂ partial pressure on activity was ≈ 30 kPa (absolute), independent of the type and concentration of electron acceptor. The K_{H_2} value indicates that H₂ partial pressures typically associated with syngas fermentation will result in compromised efficiency of the hydrogenase activity.

Keywords: Syngas; Fermentation; Biofuels; Hydrogenase; Ethanol; *Clostridium*

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We compare different cogeneration system scenarios for efficient energy production from bagasse fuel in an Indonesian sugar and ethanol factory. These scenarios include the use of condensing-extraction steam turbines, variable speed electric drives for process equipment, measures to reduce low pressure steam demand for process needs, and two advanced cogeneration systems. One advanced system includes an 80 bar high pressure direct combustion steam Rankine cycle (advanced SRC), while the other uses a biomass integrated gasifier combined cycle (BIGCC); both utilize fuel dryers. Using steady-state thermodynamic models, we estimate that the net electricity generation potentials of the BIGCC and advanced SRC systems are approximately seven and five times the potential of the existing factory, respectively. The maximum net electricity generation potentials for the respective systems are 170 kWh/tc (BIGCC) and 140 kWh/tc (advanced SRC). However, the BIGCC system needs a bagasse feed rate that is 50 percent higher than the advanced SRC system to satisfy the factory low pressure steam demand for sugar and ethanol processing, which may affect its ability to provide steam and electricity during the off-season. For the Indonesian sugar factory, the annual revenue potential of the BIGCC system is US\$14 million per year, approximately 50 percent higher than that of the advanced SRC system (electricity sale rate: US\$45/MsWh; carbon credit

price: US\$13.60). BIGCC technology is still in an early stage of development and there are no commercial systems in sugar factories, so an advanced SRC system may be a more suitable option in the near future.

Keywords: Gasification; Cogeneration; Sugarcane; Bagasse; Renewable energy

Ashish Pratap Singh Chouhan, Anil Kumar Sarma. (Sardar Swaran Singh National Institute of Renewable Energy, Jalandhar-Kapurthala Road, Kapurthala, Punjab 144601, India). Biodiesel production from *Jatropha curcas* L. oil using *Lemna perpusilla* Torrey ash as heterogeneous catalyst. *Biomass and Bioenergy*, Volume 55(2013): 386–389

Refined *Jatropha curcas* L. oil (JCO) and methanol were used as the reactants for the transesterification reactions in a Radleys reactor in the presence of a heterogeneous ash catalyst derived from the waste aquatic plant *Lemna perpusilla* Torrey. Physical characterization of the catalyst showed partly crystalline behaviour and a moderate surface area $9.622 \text{ m}^2 \text{ g}^{-1}$. The *L. perpusilla* Torrey ashes obtained from traditional combustion method were further calcined at $550 \pm 5 \text{ }^\circ\text{C}$ before use. In addition to other non-metal and metallic constituents the ash contains 11.3% potassium which attributed to its catalytic behaviour. The cumulative mass fraction of 89.43% of the oil was converted to biodiesel at $65 \pm 5 \text{ }^\circ\text{C}$ in 5 h at 1:9 M ratio of oil to alcohol with 5% of the ash as catalyst. The biodiesel (FAME) so obtained were characterized using appropriate ASTM methods and found within the defined standard limits. The catalyst could be reused up to 3-times but there is a reduction of efficacy by about 25% for 3rd consecutive batch reaction. The activation energy was calculated for FAME and found to be $29.49 \text{ kJ mol}^{-1}$.

Keywords: *Jatropha curcas* L. oil (JCO); *Lemna perpusilla* Torrey ash; Heterogeneous catalyst; FAME; Gas chromatograph analysis

Sara Alongi Skenhall^{a, b}, Göran Berndes^a, Jeremy Woods^b. (^a Department of Energy and Environment, Division of Physical Resource Theory, Chalmers University of Technology, SE-412 96 Göteborg, Sweden, ^b Centre for Environmental Policy, Imperial College, London SW7 2AZ, UK). Integration of bioenergy systems into UK agriculture—New options for management of nitrogen flows. *Biomass and Bioenergy*, Volume 54(2013): 219–226

The large flow of reactive nitrogen (N) through agriculture causes negative environmental impacts, pointing to a need for changes in agricultural practices. At the same time, agriculture is expected to provide biomass to support the increasing demand from the UK bioenergy sector. A high-level aggregated model of the agricultural system in the UK was developed, which maintains the existing level of food and livestock production and at the same time increases N recirculation. Integrating three different bioenergy sub-systems into the agricultural system was an essential component of the model development. Cellulosic bioenergy crops were located in the landscape as vegetation filters to intercept and capture N and thereby reduce N leaching. Efficient collection and digestion of manure produced organic N fertiliser and biogas. Efficient forage production for cattle allowed further cultivation of bioenergy plants. Five implementation scenarios were developed to clarify the contribution of these bioenergy sub-systems to improved N management. The results point to a significant potential for improving the productive use of reactive N and for decreasing N losses to water and air. The interception and recirculation of N

presently leaching from arable fields is assessed as the most important option. It is also important to increase recirculation of N in manure and in bioenergy system by-flows. Besides mitigating the environmental impacts of agriculture these measures reduce the requirements for newly synthesised N fertilisers. A systems perspective on N, agriculture, and bioenergy systems facilitates N recirculation and promotes effective N use, reducing the need for additional N inputs.

Keywords: Agriculture; Nitrogen; Bioenergy; Environment; Land use; Recirculation

Loan T. Le^a, Ekko C. van Ierland^a, Xueqin Zhu^a, Justus Wesseler^b, Giang Ngo^c. (^a Environmental Economics and Natural Resources Group, Wageningen University, Hollandseweg 1, 6706 KN Wageningen, The Netherlands, ^b Agricultural and Food Economics Group, Technische Universität München, Weihenstephaner Steig 22, 85354 Freising, Germany, ^c Research Institute for Oil and Oil Plants, Ministry of Industry and Trade, 171 Ham Nghi Street, District 1, Ho Chi Minh City, Viet Nam). Comparing the social costs of biofuels and fossil fuels: A case study of Vietnam. *Biomass and Bioenergy*, Volume 54(2013): 227–238

Biofuel substitution for fossil fuels has been recommended in the literature and promoted in many countries; however, there are concerns about its economic viability. In this paper we focus on the cost-effectiveness of fuels, i.e., we compare the social costs of biofuels and fossil fuels for a functional unit defined as 1 km of vehicle transportation. We base our empirical results on a case study in Vietnam and compare two biofuels and their alternative fossil fuels: ethanol and gasoline, and biodiesel and diesel with a focus on the blends of E5 and E10 for ethanol, and B5 and B10 for biodiesel. At the discount rate of 4%, ethanol substitution for gasoline in form of E5 or E10 saves 33% of the social cost of gasoline if the fuel consumption of E5 and E10 is the same as gasoline. The ethanol substitution will be cost-effective if the fuel consumption of E5 and E10, in terms of L km⁻¹, is not exceeding the consumption of gasoline by more than 1.7% and 3.5% for E5 and E10 respectively. The biodiesel substitution would be cost-effective if the fuel consumption of B5 and B10, in terms of L km⁻¹ compared to diesel, would decrease by more than 1.4% and 2.8% for B5 and B10 respectively at the discount rate of 4%.

Keywords: Cassava-based ethanol; Jatropha-based biodiesel; Fossil fuels; Social cost; Cost-effectiveness; Vietnam

Oparaku, N. F., Ofomatah, A. C* and Okoroigwe, E. C. (National Center for Energy Research and Development, University of Nigeria, Nsukka, Enugu State, Nigeria. Email: ofomatony@yahoo.co.uk). Biodigestion of cassava peels blended with pig dung for methane generation. *African Journal of Biotechnology*, Volume 12(40) (2013): 5956-5961

Biogas production from cassava (*Manihot esculentus*) peels and pig dung under a mesophilic temperature condition was investigated. Three blends of the wastes and a control labeled as B1, B2, B3 and C representing blend 1 (50:50 peel/dung), blend 2 (30:70 peel/dung), blend 3 (10:90 peel/dung) and control (pig dung alone) were used, respectively. Biodigestion of the wastes blends and control was carried out simultaneously under the same environmental and operational conditions of 30 days retention period using four metallic biodigesters of 32 L capacity each. The biogas yield result shows that blend 2 yielded the highest cumulative biogas of 78.5 L, while the least yield of 61.7 L was obtained by blend 3. When compared with the control set up and biodigestion of cassava waste alone from literature, there was blending effect resulting in

increase in yield of biogas over the sole digestion of cassava peel or pig dung. Methane production leading to the combustibility of the biogas started at 6th, 5th, 5th and 4th days for B1, B2, B3 and C, respectively. This, in agreement with earlier studies show that better handling of cassava peels for energy production would be achieved by blending it with animal wastes in the right proportion.

Keywords: Cassava peel, biogas, co-digestion, anaerobic digestion, wastes blends, lag days.

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A novel combined system of sludge microbial fuel cell (S-MFC) stack and membrane bioreactor (MBR) was proposed in this study. The non-consumed sludge in the MBR sludge-fed S-MFC was recycled to the MBR. In the combined system, the COD and ammonia treatment efficiencies were more than 90% and the sludge reduction was 5.1% higher than that of the conventional MBR. It's worth noting that the energy recovery and fouling mitigation were observed in the combined system. In the single S-MFC, about 75 mg L⁻¹ COD could be translated to electricity during one cycle. The average voltage and maximum power production of the single S-MFC were 430 mV and 51 mW m⁻², respectively. Additionally, the combined system was able to mitigate membrane fouling by the sludge modification. Except for the content decrease (22%), S-MFC destroyed simple aromatic proteins and tryptophan protein-like substances in loosely bound extracellular polymeric substances (LB-EPS). These results indicated that effective wastewater treatment, sludge reduction, energy recovery and membrane fouling mitigation could be obtained in the combined system.

Keywords: Membrane bioreactor (MBR); Microbial fuel cell (MFC); Membrane fouling; Loosely bound EPS (LB-EPS); Energy recovery

Nano Biotechnology

Sung Hee Joo¹. (¹Institute of Environmental Technology, Coway Co., Ltd., 4F, Woongjin R&D Center, San 4-1, Nakseongdae-dong, Gwanak-guSeoul, South Korea). **The Ecotoxicological Impact of Metal Oxide Nanoparticles on Pool Algae in the Presence and Absence of Disinfection Byproducts: a New Research Direction for the Public Health and Safety of Engineered Nanoparticles used in Consumer Products. Water, Air, & Soil Pollution, 224(2013): 1681**

Recent research on potential carcinogens in recreational waters has spawned public concerns about the long-term public health impacts of disinfectants used in pools. However, no attention has been given to the ecological and public health impacts of metal oxides in cosmetics and

sunscreens within swimming pools where leisure activities occur. The discussion in this perspective focuses on the interaction between metal oxide nanoparticles released from swimmers into pools where algae is present, and the synergistic toxicological effects of pool algae adsorbed by metal oxide nanoparticles in the presence of disinfection byproducts in comparison to the absence of contaminants. This perspective will address research approaches to evaluating metal oxide nanoparticle impacts on pool algae, and the challenge of identifying the potential mechanisms leading to transformed algae.

Keywords: Metal Oxide, Nanoparticles, Ecotoxicity, Disinfection, Byproducts, Consumer Products Pool, Algae Public Health

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A study was carried out under in vitro conditions to characterize the growth of blue green alga, *Spirulina platensis*, in standard CFTRI medium containing different nanoparticles of copper oxide (CuO) (50 nm, 10 ppm), zinc oxide (ZnO) (50 nm, 10 ppm), tricalcium phosphate (TCP) (<100 nm, 90 ppm), and hydroxy apatite (HA) (<200 nm, 90 ppm). *S. platensis* exhibited significant higher growth in standard CFTRI medium containing 90 ppm phosphorus as nanoparticles of TCP and HA. On the other hand, calcium phosphate nanoparticles caused significant reduction in nitrate reductase activity as well as in protein content of the alga. Marked change in chlorophyll-a/b ratio was also noted when phosphorus was supplied through nano tricalcium phosphate and nano hydroxy apatite particles as compared to ionic form (K₂HPO₄). The study revealed that the growth of *Spirulina* in the presence of ZnO nanoparticles was retarded, while no growth was observed with CuO nanoparticles. It was concluded that alga *Spirulina* showed much sensitivity to nanoparticles of zinc and copper (<50 nm) and was able to tolerate the toxicity of nanophosphate (tricalcium phosphate <100 nm; hydroxy apatite <200 nm).

Keywords: *Spirulina* Growth, Nanoparticles, Toxicity Sensitivity

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Nanotechnology, the area of science focused on the control of matter in the nanometer scale, allows ground-breaking changes of the fundamental properties of matter that are often radically

different compared to those exhibited by the bulk counterparts. In view of the fact that dimensionality plays a key role in determining the qualities of matter, the realization of the great potential of nanotechnology has opened the door to other disciplines such as life sciences and medicine, where the merging between them offers exciting new applications, along with basic science research. The application of nanotechnology in life sciences, nanobiotechnology, is now having a profound impact on biological circuit design, bioproduction systems, synthetic biology, medical diagnostics, disease therapy and drug delivery. This special issue is dedicated to the overview of how we are learning to control biopolymers and biological machines at the molecular- and nanoscale. In addition, it covers far-reaching progress in the design and synthesis of nanoscale materials, thus enabling the construction of integrated systems in which the component blocks are comparable in size to the chemical and biological entities under investigation.

Veikko Linko, Hendrik Dietz . (Walter Schottky Institute, Physik Department, Technische Universität München, Garching near Munich, Germany). The enabled state of DNA nanotechnology. Current Opinion in Biotechnology, Volume 24(4) (2013): 555–561

It is notoriously difficult to observe, let alone control, the position and orientation of molecules due to their small size and the constant thermal fluctuations that they experience in solution. Molecular self-assembly with DNA enables building custom-shaped nanometer-scale objects with molecular weights up to the megadalton regime. It provides a viable route for placing molecules and constraining their fluctuations in user-defined ways, thereby opening up completely new avenues for scientific and technological exploration. Here, we review progress that has been made in recent years toward the state of an enabled DNA nanotechnology.

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Biological systems have developed unique capabilities to generate, harness and store energy in efficient ways. In recent years, biologically inspired approaches have been introduced as a new approach to find breakthroughs for next generation energy devices and storage. Of particular interest are efforts to translate biological principles directly into synthetic energy systems. In this review, we focus on the use of proteins and protein mimicry for energy applications. We highlight the major advances and results achieved with proteins as new concept energy devices.

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Silver nanoparticles (AgNPs) are widely believed to be retained in the sewage sludge during sewage treatment. The AgNPs and their derivatives, however, re-enter the environment with the sludge and via the effluent. AgNP were shown to occur in surface water, while evidence of a potential toxicity of AgNPs in aquatic organisms is growing. This study aims to examine the toxicity of AgNPs to the embryos of the aquatic vertebrate model zebrafish (*Danio rerio*) before and after sewage treatment plants (STPs) processes. Embryos were treated with AgNP (particle size: >90 % <20 nm) and AgNO₃ in ISO water for 48 h and consequently displayed effects such as delayed development, tail malformations and edema. For AgNP, the embryos were smaller than the controls with conspicuously smaller yolk sacs. The corresponding EC₅₀ values of 48 hours post fertilization (hpf) were determined as 73 µg/l for AgNO₃ and 1.1 mg/l for AgNP. Whole-mount immunostainings of primary and secondary motor neurons also revealed secondary neurotoxic effects. A TEM analysis confirmed uptake of the AgNPs, and the distribution within the embryo suggested absorption across the skin. Embryos were also exposed (for 48 h) to effluents of AgNP-spiked model STP with AgNP influent concentrations of 4 and 16 mg/l. These embryos exhibited the same malformations than for AgNO₃ and AgNPs, but the embryo toxicity of the sewage treatment effluent was higher (EC₅₀ = 142 µg/l; 48 hpf). On the other hand, control STP effluent spiked with AgNPs afterwards was less toxic (EC₅₀ = 2.9 mg/l; 48 hpf) than AgNPs in ISO water. This observation of an increased fish embryo toxicity of STP effluents with increasing AgNP influent concentrations identifies the accumulation of AgNP in the STP as a potential source of effluent toxicity.

Keywords: Silver nanoparticles, Silver Zebrafish embryo, Sewage treatment processes, Toxicity

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Scientific consensus predicts that the worldwide use of engineered nanomaterials (ENM) leads to their release into the environment. We reviewed the available literature concerning environmental concentrations of six ENMs (TiO₂, ZnO, Ag, fullerenes, CNT and CeO₂) in surface waters, wastewater treatment plant effluents, biosolids, sediments, soils and air. Presently, a dozen modeling studies provide environmental concentrations for ENM and a handful of analytical works can be used as basis for a preliminary validation. There are still major knowledge gaps (e.g. on ENM production, application and release) that affect the modeled values, but over all an agreement on the order of magnitude of the environmental concentrations can be reached. True validation of the modeled values is difficult because trace analytical methods that are specific for ENM detection and quantification are not available. The modeled and measured results are not always comparable due to the different forms and sizes of particles that these two approaches target.

Keywords: Engineered nanomaterials; Predicted environmental concentrations (PEC); Measured environmental concentrations

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Street, Griffin, GA 30223, United States, ^b Material Measurement Laboratory, NIST, Gaithersburg, MD 20899, United States, ^c State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210093, PR China). Degradation of multiwall carbon nanotubes by bacteria. *Environmental Pollution*, Volume 181(2013) : 335–339

Understanding the environmental transformation of multiwall carbon nanotubes (MWCNTs) is important to their life cycle assessment and potential environmental impacts. We report that a bacterial community is capable of degrading ¹⁴C-labeled MWCNTs into ¹⁴CO₂ in the presence of an external carbon source via co-metabolism. Multiple intermediate products were detected, and genotypic characterization revealed three possible microbial degraders: *Burkholderia kururiensis*, *Delftia acidovorans*, and *Stenotrophomonas maltophilia*. This result suggests that microbe/MWCNTs interaction may impact the long-term fate of MWCNTs.

Keywords: Carbon nanotubes; Nanotoxicology; Microbial degradation; Aerobic biotransformation

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Silver nanoparticles (AgNPs) are an important model system for studying potential environmental risks posed by the use of nanomaterials. So far there is no consensus as to whether toxicity is due to AgNPs themselves or Ag⁺ ions leaching from their surfaces. In sea urchin *Paracentrotus lividus*, AgNPs cause dose dependent developmental defects such as delayed development, bodily asymmetry and shortened or irregular arms, as well as behavioural changes, particularly in swimming patterns, at concentration \square 0.3 mg/L AgNPs. It has been observed that AgNPs are more toxic than their equivalent Ag⁺ ion dose.

Keywords: Silver nanoparticles; Toxicity; Sea urchins

Asheesh Gupta^{a, b, c}, Pinar Avci^a, Magesh Sadasivam^{a, d}, Rakkiyappan Chandran^{a, d}, Nivaldo Parizotto^{a, e}, Daniela Vecchio^{a, b}, Wanessa C.M.A. de Melo^{a, f}, Tianhong Dai^{a, b}, Long Y. Chiang^g, Michael R. Hamblin^{a, b, h}. (^a Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, USA, ^b Department of Dermatology, Harvard Medical School, Boston, MA, USA, ^c Defence Institute of Physiology & Allied Sciences, Delhi, India, ^d Amity Institute of Nanotechnology, Amity University Uttar Pradesh, Noida, India, ^e Federal University of Sao Carlos, Sao Carlos, Brazil, ^f University of Sao Paulo, Sao Carlos-SP, Brazil, ^g Department of Chemistry, University of Massachusetts, Lowell, MA, USA, ^h Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA, USA). Shining light on nanotechnology to help repair and regeneration. *Biotechnology Advances*, Volume 31(5) (2013): 607–631

Phototherapy can be used in two completely different but complementary therapeutic applications. While low level laser (or light) therapy (LLLT) uses red or near-infrared light alone to reduce inflammation, pain and stimulate tissue repair and regeneration, photodynamic therapy (PDT) uses the combination of light plus non-toxic dyes (called photosensitizers) to produce reactive oxygen species that can kill infectious microorganisms and cancer cells or destroy unwanted tissue (neo-vascularization in the choroid, atherosclerotic plaques in the arteries). The recent development of nanotechnology applied to medicine (nanomedicine) has opened a new front of advancement in the field of phototherapy and has provided hope for the development of nanoscale drug delivery platforms for effective killing of pathological cells and to promote repair and regeneration. Despite the well-known beneficial effects of phototherapy and nanomaterials in producing the killing of unwanted cells and promoting repair and regeneration, there are few reports that combine all three elements i.e. phototherapy, nanotechnology and, tissue repair and regeneration. However, these areas in all possible binary combinations have been addressed by many workers. The present review aims at highlighting the combined multi-model applications of phototherapy, nanotechnology and, reparative and regeneration medicine and outlines current strategies, future applications and limitations of nanoscale-assisted phototherapy for the management of cancers, microbial infections and other diseases, and to promote tissue repair and regeneration.

Keywords: Liposomes; Low level laser (or light) therapy; Nanomaterials; Nanoparticles; Photodynamic therapy; Phototherapy; Repair and regeneration; Stem cells

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Nanomaterials with superior physiochemical properties have been rapidly developed and integrated in every aspect of cell engineering and therapy for translating their great promise to clinical success. Here we demonstrate the multifaceted roles played by innovatively-designed nanomaterials in addressing key challenges in cell engineering and therapy such as cell isolation from heterogeneous cell population, cell instruction *in vitro* to enable desired functionalities, and targeted cell delivery to therapeutic sites for prompting tissue repair. The emerging trends in this interdisciplinary and dynamic field are also highlighted, where the nanomaterial-engineered cells constitute the basis for establishing *in vitro* disease model; and nanomaterial-based *in situ* cell engineering are accomplished directly within the native tissue *in vivo*. We will witness the increasing importance of nanomaterials in revolutionizing the concept and toolset of cell engineering and therapy which will enrich our scientific understanding of diseases and ultimately fulfill the therapeutic demand in clinical medicine.

Keywords: Nanomaterials; Cell engineering; Cell therapy; Regenerative medicine

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Chemistry Laboratory, Institute of Chemistry, Universidade Estadual de Campinas, C. P. 6154, Campinas, São Paulo, 13083-970, Brazil, ⁵Center of Natural and Human Sciences, Universidade Federal do ABC, São Paulo, Brazil). Biogenic nanoparticles: copper, copper oxides, copper sulphides, complex copper nanostructures and their applications. *Biotechnology Letters*, Volume 35(9) (2013):1365-1375

Copper nanoparticles have been the focus of intensive study due to their potential applications in diverse fields including biomedicine, electronics, and optics. Copper-based nanostructured materials have been used in conductive films, lubrication, nanofluids, catalysis, and also as potent antimicrobial agent. The biogenic synthesis of metallic nanostructured nanoparticles is considered to be a green and eco-friendly technology since neither harmful chemicals nor high temperatures are involved in the process. The present review discusses the synthesis of copper nanostructured nanoparticles by bacteria, fungi, and plant extracts, showing that biogenic synthesis is an economically feasible, simple and non-polluting process. Applications for biogenic copper nanoparticles are also discussed.

Keywords: Biogenic Copper, Copper oxides, Copper sulphides, Nanoparticles

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Besides fundamental role in protein synthesis, leucine has metabolic roles as energy substrates, precursors for synthesis of other amino acids and as a modulator of muscle protein synthesis via the insulin-signaling pathway. Leucine concentration in cell and tissue is temporally dynamic as the metabolism of leucine is regulated through multiple enzymes and transporters. Assessment of cell-type specific activities of transporters and enzymes by physical fractionation is extremely challenging. Therefore, a method of reporting leucine dynamics at the cellular level is highly desirable. Given this, we developed a series of genetically encoded nanosensors for real-time *in vivo* measurement of leucine at cellular level. A leucine binding periplasmic binding protein (LivK) of *Escherichia coli* K12 was flanked with CFP (cyan fluorescent protein) and YFP (yellow fluorescent protein) at N-terminus and C-terminus, respectively. The constructed nanosensors allowed *in vitro* determination of fluorescence resonance energy transfer (FRET) changes in a concentration-dependent manner. These sensors were found to be specific to leucine, and stable to pH-changes within a physiological range. Genetically encoded sensors can be targeted to a specific cell type, and allow dynamic measurement of leucine concentration in bacterial and yeast cells.

Keywords: Fluorescent protein; FRET; Leucine; Genetically encoded nanosensor; Periplasmic binding protein

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silver–palladium alloy nanoparticle-based electrochemical biosensor for simultaneous detection of ractopamine, clenbuterol and salbutamol. Biosensors and Bioelectronics, Volume 49(2013): 14–19

A multiplexed electrochemical biosensor has been developed for fast and sensitive detection of ractopamine (RAC), salbutamol (SAL) and clenbuterol (CLB) based on reduced graphene oxide (rGO) and silver–palladium alloy nanoparticles (AgPd NPs). In this paper, rGO with high conductivity was used as an electrode material to immobilize artificial antigens and amplify electrochemical signal. AgPd NPs are used to label antibodies and generate a strong electrochemical signal in phosphate buffered saline (PBS) without any other substrates. Screen-printed carbon electrode (SPCE) and competition strategy were adopted to achieve simultaneous detection of RAC, SAL and CLB without cross-talk between adjacent electrodes. This method can simultaneously detect RAC, SAL and CLB ranging from 0.01 to 100 ng mL⁻¹ with detection limits of 1.52 pg mL⁻¹, 1.44 pg mL⁻¹ and 1.38 pg mL⁻¹, respectively. Satisfactory results are achieved in pork sample analysis. The designed strategy provides a promising potential in determination of other biological samples.

Keywords: Multiplexed electrochemical biosensor; Reduced graphene oxide; Silver–palladium alloy nanoparticles; Competition strategy; β -adrenergic agonists

Shenguang Ge^a, Weiyan Liu^a, Lei Ge^b, Mei Yan^a, Jixian Yan^a, Jiadong Huang^a, Jinghua Yu^a (^a Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong (University of Jinan), School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, China, ^b Shandong Provincial Key Laboratory of Preparation and Measurement of Building Materials, University of Jinan, 250022 Jinan, China). In situ assembly of porous Au-paper electrode and functionalization of magnetic silica nanoparticles with HRP via click chemistry for Microcystin-LR immunoassay. Biosensors and Bioelectronics, Volume 49(2013): 111–117

A simple, low-cost and sensitive origami electrochemical immunoassay-device was developed based on a novel gold nanoparticle modified porous paper working electrode (Au-PWE) for point-of-care testing. Azide-functionalized Au-PWE was prepared by the functionalization of Au-PWE with 1-azidoundecan-11-thiol. Alkyne end-terminated antibody was prepared with 4-pentynoic acid and antibody by the 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide hydrochloride and N-hydroxysuccinimide activation reaction. Alkyne-antibody was coupled to azido-Au-PWE by click reaction as a recognition element. Nearly monodispersed sphere-like silica-coated ferromagnetic oxide (Fe₃O₄@SiO₂) nanoparticles were prepared via the reverse microemulsion method. Azide-functionalized Fe₃O₄@SiO₂ was prepared by the functionalization of silica shell with 3-bromopropyltrichlorosilane followed by substitution with sodium azide. Alkyne-functionalized antibody and horse radish peroxidase were coupled to azide-functionalized Fe₃O₄@SiO₂ by click reaction as signal label. Horse radish peroxidase and ferromagnetic oxide could catalyze the oxidation of thionine in the presence of hydrogen peroxide. After the sandwich immunoreaction, the current was proportional to the logarithm of the Microcystin-LR. The linear regression equation was $i(\mu\text{A})=119.89+46.27 \log c_{\text{MC-LR}}(\mu\text{g/mL})$ in the range from 0.01 to 200 $\mu\text{g/mL}$. The limit of detection was 0.004 $\mu\text{g/mL}$. This immunoassay would provide a universal immunoassay method in environmental monitoring and public health.

Keywords: Microcystin-LR; Click chemistry; Electrochemistry; Immunoassay

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For understanding cells functionalities and their communications, there is a need for highly sensitive cell analysis platforms capable of assessing non-specific chemicals on the surface and in the vicinity of cells. We report a microfluidic system integrating dielectrophoresis and surface enhanced Raman scattering (SERS) for the trapping and real time monitoring of cell functions in isolated and grouped cell clusters. Yeast cells are coated with silver nanoparticles to enable highly sensitive SERS analysis. The SERS responses of cells are examined under various conditions: live vs. dead and isolated vs. grouped. This work illustrates the feasibility of the system for *in situ* cell monitoring and analysis of secreted chemicals during their growth, metabolism, proliferation and apoptosis.

Keywords: Microfluidics; Yeast cell; SERS; Principal component analysis; Dielectrophoresis

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A novel gold nanoprobe was prepared for the signal tracing of ultrasensitive nonenzymatic electrochemical immunoassay at a carbon nanotubes (CNTs)-based disposable immunosensor. The gold nanoprobe was prepared via *in situ* deposition of gold nanoparticles (Au NPs) on the polydopamine functionalized silica nanosphere followed by the labeling of signal antibodies. The immunosensor was prepared through the covalent immobilization of capturing antibodies on the CNTs modified screen-printed carbon electrode. After a sandwich-type immunoreaction on the immunosensor surface, the gold nanoprobe composites were then measured by electrochemical stripping analysis to obtain signal response. This method provided a simple and controllable way to prepare a novel gold nanoprobe which greatly amplified the signal response of every single immuno-recognition event. The modification of electrode surface with CNTs also facilitated the stripping current enhancement of Au NPs resulting in the ultrahigh sensitivity of this immunoassay method. Using human IgG as a model analyte, the proposed method showed a wide linear range over three orders of magnitude with the detection limit down to 6.9 pg/mL. Besides, this method showed excellent analytical performance with low cost, good portability, and acceptable reproducibility, stability and accuracy, thus providing great potentials for clinical applications.

Keywords: Biosensor; Immunoassay; Signal amplification; Gold nanoparticles; Polydopamine

Adaris M. López-Marzo^{a,b}, Josefina Pons^b, Diane A. Blake^c, Arben Merkoçi^{a,d}. (^a Nanobioelectronics & Biosensors Group, Catalan Institute of Nanoscience and Nanotechnology, Campus de la UAB, 08193 Bellaterra, Barcelona, Spain, ^b Department of Chemistry, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain, ^c Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, New Orleans, LA 70112, USA, ^d ICREA, Barcelona, Spain). **High sensitive gold-nanoparticle based lateral flow Immunodevice for Cd²⁺ detection in drinking waters. Biosensors and Bioelectronics, Volume 47(2013): 190–198**

In this work for first time a lateral flow immunosensor device (LFID) for Cd²⁺ determination in drinking and tap waters using the Cd–EDTA–BSA–AuNP conjugate as signal producer tool is introduced. The principle of working is based on competitive reaction between the Cd–EDTA–BSA–AuNP conjugate deposited on the conjugation pad strip and the Cd–EDTA complex formed in the analysis sample for the same binding sites of the 2A81G5 monoclonal antibody, specific to Cd–EDTA but not Cd²⁺ free, which is immobilized onto the test line. The device has a large response range within 0.4–2000 ppb, being the linear response between 0.4 and 10 ppb. The quantification and detection limits of 0.4 and 0.1 ppb, respectively, represent the lowest ones reported so far for paper based metal sensors. The obtained detection limit is 50 times lower than the maximum contamination level required for drinking water. Here we also show a new option for increasing the sensibility in the LFDs with competitive format, through the decreasing in concentrations of the Cd–EDTA–BSA–AuNP conjugate deposited in the conjugation strip and the mAbs deposited in the test and control zones until to reach optimized concentrations. It is an important result take into account that the increase in sensibility is one of the challenges in the field of LFD sensors, where are focused many of the ongoing researches. In addition, a specificity study of the device for several metal interferences, where potential metal interferences are masked with the use of the EDTA and OVA optimized concentrations, is presented too.

Keywords: Gold nanoparticle; Immunodevice; Cadmium; Detection; Water

Jiang Yang^a, Ji-Hyuk Yu^b, J. Rudi Strickler^c, Woo-Jin Chang^{b,d}, Sundaram Gunasekaran^a. (^a Department of Biological Systems Engineering, University of Wisconsin-Madison, 460 Henry Mall, Madison, WI 53706, USA, ^b Department of Mechanical Engineering, University of Wisconsin-Milwaukee, 3200 N. Cramer Street, Milwaukee, WI 53211, USA, ^c Great Lakes Water Institute, University of Wisconsin-Milwaukee, 600 E. Greenfield, Milwaukee, WI 53204, USA, ^d School of Freshwater Sciences, University of Wisconsin-Milwaukee, 600 E. Greenfield, Milwaukee, WI 53204, USA). **Nickel nanoparticle–chitosan-reduced graphene oxide-modified screen-printed electrodes for enzyme-free glucose sensing in portable microfluidic devices. Biosensors and Bioelectronics, Volume 47(2013): 530–538**

A facile one-step strategy is reported to synthesize nanocomposites of chitosan-reduced graphene oxide–nickel nanoparticles (CS-RGO–NiNPs) onto a screen-printed electrode (SPE). The synthesis is initiated by electrostatic and hydrophobic interactions and formation of self-assembled nanocomposite precursors of negatively charged graphene oxide (GO) and positively charged CS and nickel cations (Ni²⁺). The intrinsic mechanism of co-depositions from the nanocomposite precursor solution under cathodic potentials is based on simultaneous depositions

of CS at high localized pH and *in situ* reduced hydrophobic RGO from GO as well as cathodically reduced metal precursors into nanoparticles. There is no need for any pre- or post-reduction of GO due to the *in situ* electrochemical reduction and the removal of oxygenated functionalities, which lead to an increase in hydrophobicity of RGO and successive deposition on the electrode surface. The as-prepared CS-RGO–NiNPs-modified SPE sensor exhibited outstanding performance for enzymeless glucose (Glc) sensing in alkaline media with high sensitivity ($318.4 \mu\text{A mM}^{-1} \text{cm}^{-2}$), wide linear range (up to 9 mM), low detection limit (4.1 μM), acceptable selectivity against common interferents in physiological fluids, and excellent stability. A microfluidic device was fabricated incorporating the SPE sensor for real-time Glc detection in human urine samples; the results obtained were comparable to those obtained using a high-performance liquid chromatography (HPLC) coupled with an electrochemical detector. The excellent sensing performance, operational characteristics, ease of fabrication, and low cost bode well for this electrochemical microfluidic device to be developed as a point-of-care healthcare monitoring unit.

Keywords: Chitosan; Enzyme-free glucose sensing; Graphene; Nickel nanoparticles; Microfluidic

Biomimicry

John G Hardy^{1,4}, Jae Y Lee^{2,4}, Christine E Schmidt^{1,3}. (¹ Department of Biomedical Engineering, 107 West Dean Keeton Street, University of Texas at Austin, Austin, TX 78712, United States, ² School of Materials Science and Engineering, Gwangju Institute of Science and Technology, Gwangju 500-712, Republic of Korea, ³ J. Crayton Pruitt Family Department of Biomedical Engineering, Biomedical Sciences Building JG-56, P.O. Box 116131, Gainesville, FL 32611-6131, United States). **Biomimetic conducting polymer-based tissue scaffolds. Current Opinion in Biotechnology, Volume 24(5) (2013): 847–854**

Conducting polymer-based materials are promising for application as tissue scaffolds for the replacement or restoration of damaged or malfunctioning tissues, because a variety of tissues respond to electrical stimulation. This review focuses on conducting polymer-based materials with biomimetic chemical, mechanical and topological properties, and recent progress toward the fabrication of clinically relevant tissue scaffolds is highlighted.

Jayasundara, D.R., Duff, T., Angione, M.D., Bourke, J., Murphy, D.M., Scanlan, E.M., Colavita, P.E. (School of Chemistry, University of Dublin Trinity College, College Green, Dublin, Dublin D2, Ireland, Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), University of Dublin Trinity College, Dublin, Dublin D2, Ireland). **Carbohydrate coatings via aryldiazonium chemistry for surface biomimicry. Chemistry of Materials, Volume 25(20) (2013): 4122-4128**

Carbohydrates are extremely important biomolecules and their immobilization onto solid surfaces is of interest for the development of new biomimetic materials and of new methods for understanding processes in glycobiology. We have developed an efficient surface modification methodology for the functionalization of a range of materials with biologically active

carbohydrates based on aryldiazonium chemistry. We describe the synthesis and characterization of carbohydrate reagents, which were subsequently employed for the one-step, solution-based modification of carbon, metals, and alloys with monosaccharides. We used a combination of spectroscopic and nanogravimetric methods to characterize the structure of the carbohydrate layers; we report an average surface coverage of 7.8×10^{-10} mol cm⁻² under our experimental conditions. Concanavalin A, a mannose-binding lectin, and Peanut Agglutinin, a galactose-binding lectin, were found to bind from solution to their respective monosaccharide binding partners immobilized at the surface. This result suggests that the spontaneous chemisorption of aryldiazonium monosaccharide precursors leads to the formation of monosaccharide layers that retain the biological recognition specificity of the parent carbohydrate molecule. Finally, we carried out measurements using fluorescently labeled Bovine Serum Albumin (BSA) and found that these carbohydrate coatings reduce unspecific adsorption of this protein at carbon surfaces. These results suggest that aryldiazonium-derived carbohydrate coatings may offer a promising strategy for preventing undesirable protein accumulation onto surfaces.

Keywords: carbohydrate; carbon; coatings; diazonium; saccharide

Demange, E.^a, Kassim, Y.^a, Petit, C.^a, Buquet, C.^a, Dulong, V.^b, Cerf, D.L.^b, Buchonnet, G.^c, Vannier, J.-P.^a. (^a Laboratory MERCI EA3829, University of Rouen, France, ^b Laboratory of Polymers, Biopolymers, Surfaces, University of Rouen, Mont Saint-Aignan, France, ^c University of Rouen Hospital, France). **Survival of cord blood haematopoietic stem cells in a hyaluronan hydrogel for ex vivo biomimicry. *Journal of Tissue Engineering and Regenerative Medicine*, Volume 7(11) (2013): 901-910**

Haematopoietic stem cells (HSCs) and haematopoietic progenitor cells (HPCs) grow in a specified niche in close association with the microenvironment, the so-called 'haematopoietic niche'. Scaffolds have been introduced to overcome the liquid culture limitations, mimicking the presence of the extracellular matrix (ECM). In the present study the hyaluronic acid scaffold, already developed in the laboratory, has been used for the first time to maintain long-term cultures of CD34⁺ haematopoietic cells obtained from human cord blood. One parameter investigated was the impact on ex vivo survival of CD34⁺ cord blood cells (CBCs) on the hyaluronic acid surface, immobilized with peptides containing the RGD motif. This peptide was conjugated by coating the hyaluronan hydrogel and cultured in serum-free liquid phase complemented with stem cell factor (SCF), a commonly indispensable cytokine for haematopoiesis. Our work demonstrated that these hyaluronan hydrogels were superior to traditional liquid cultures by maintaining and expanding the HPCs without the need for additional cytokines, and a colonization of 280-fold increment in the hydrogel compared with liquid culture after 28 days of ex vivo expansion.

Keywords: Haematopoietic stem cells; Hyaluronan; Hydrogel; Migration; RGD; Scaffold

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4. Annual Review- Ecology and Systematics
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