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on
ENVIRONMENTAL BIOTECHNOLOGY

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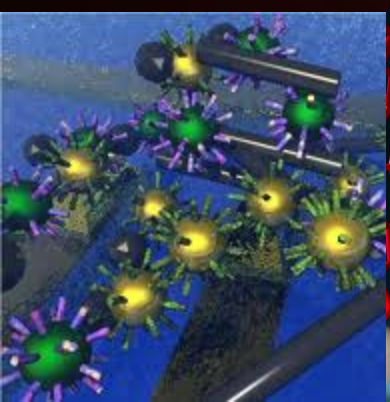
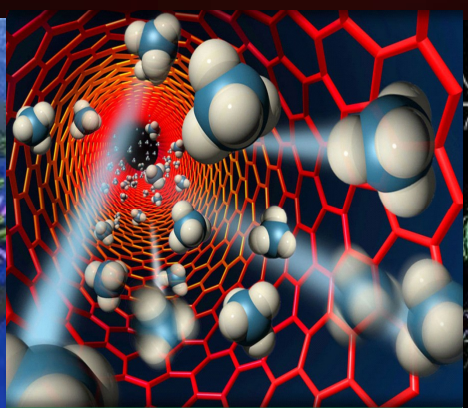
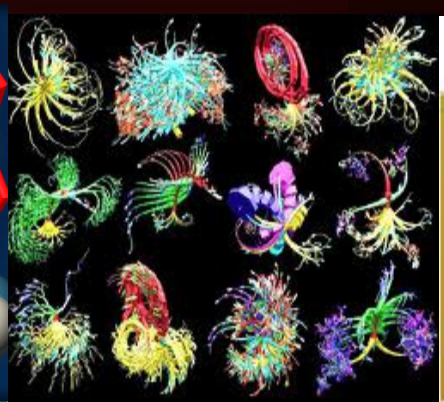


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ENVIS CENTRE

on

ENVIRONMENTAL BIOTECHBNOLOGY

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

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C O N T E N T S

BACKGROUND

Environmental Information System (ENVIS) is established in the year 1984 as a network of Information Centres. It is planned by the Ministry of Environment and Forest. Aim of this centre is to provide descriptive and environmental subject related numerical data. Now 78 centres are working under this network on various subject areas in the country. The focal point of this network is situated at the Ministry of Environment and Forest, Government of India, New Delhi.

This ENVIS Centre is established for studies on Environmental Biotechnology at the Department of Environmental Science, University of Kalyani, Nadia-741235, West Bengal.

The objective of this centre is to collect data related to the above mentioned subject, from different major libraries mainly in West Bengal and also from other states in India, through consultation with different journals, Annual Reviews, Internet and to generate a database and create a website uploaded with these information. Besides, we publish biannualy Abstract Volume on our thematic area Environmental Biotechnology under fourteen subheads. The volume contains abstracts of scientific articles from relavent 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 20th 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 June, 2012. In this issue, various topics like Bioenergy, Bioengineering, Bio-degradation, Bio-remediation, Bio-transformation etc. have been covered. We are grateful to the various libraries and their staff for their cooperation extended to us during the collection of the articles.

Abstract Format

The format of the abstract is as follows:

Abstract : The abstracts are arranged in different subheads.

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

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

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

GENERAL INFORMATION

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

Abstract are broadly classified and arranged under the following 14 heads:

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

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

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

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

Bio-Transformation: This is a process of Biological changes of complex compounds to simpler one or toxic to non-toxic and vice-versa. Several microorganisms are capable of transforming a varity 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 warm. 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 varity of environments. These specific substances may include sugars, proteins, or humas and varity 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 allerginicity 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 ecofreindly 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

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	Corpn	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	Biotechnique(s)	Endocrinol	Endocrinological
Biotechno	Biotechnology	Engg	Engineering
Biotechnol	Bitechnological	Engrs	Engineers
Bldg	Building	Env	Environment
Bot	Botany	Environ	Environmental
Botl	Botanical	Epidemic	Epidemiology
Br	Branch	Epidemiol	Epidemiological
Bull	Bulletin	Estd	Establishment
Cent	Centre	Ethnopharmaco	Ethnopharmacology
Centl	Central	Expt	Experiment

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
Geogrl	Geographical	Occ	Occassional
Geol	Geological	Occupl	Occupational
Geosci	Geoscience	Oceanogr	Oceanogoraphy
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	Immunlogical	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

Xi-Xiang Yin, L. H. Wang, R. Bai, H. Huang and Guo-Xin Sun. Accumulation and Transformation of Arsenic in the Blue-Green Alga *Synechocysis* sp. PCC6803. Water, Air, & Soil Pollution, Vol. 223(3) (2012) : 1183-1190

Synechocysis sp. PCC6803 is a unicellular blue alga which ubiquitously exists in aquatic system and is considered to play a role in arsenic cycling. Our results showed that *Synechocysis* can accumulate arsenic as much as 1.0 and 0.9 g□kg^{-1} DW when exposed to 0.5 mM arsenate and arsenite for 14 days, respectively. In addition, arsenic species in cells were assayed under different exposure conditions and it was found that inorganic arsenic, including arsenate and arsenite, is the dominant species. Organic methylated arsenicals can only be detected exposed to higher arsenic concentration range (100–500 μM). Arsenate is the dominant arsenic species and presents more than 80% of the total arsenic in cells. Efflux of both arsenate and arsenite was observed. When treated with 2.67 μM arsenite, *Synechocysis* can rapidly oxidize arsenite to arsenate and accumulate As rapidly. The observed arsenic oxidation in solute is solely caused by cellular oxidation. Given the robust ability of As accumulation, it can serve as a phytoremediation organism to efficiently remove arsenic from aquatic environments.

Keywords: Arsenic – Tolerance – Accumulation – Metabolism – Remediation – *Synechocysis* sp. PCC6803

Sara García-Salgado, David García-Casillas, Ma. Angeles Quijano-Nieto and Ma. Milagros Bonilla-Simón. Arsenic and Heavy Metal Uptake and Accumulation in Native Plant Species from Soils Polluted by Mining Activities. Water, Air, & Soil Pollution, Vol. 223(2) (2012): 559-572

Arsenic and heavy metal (specifically Cd, Cr, Cu, Ni, Pb, and Zn) uptake, translocation, and accumulation in ten native plant species spontaneously growing in soils polluted by mining activities were studied, with a focus on future phytoremediation work in polluted soils. Plant and soil samples were collected in the vicinity of the Mónica mine (NW Madrid, Spain). Soil analysis showed the ability of native plants for growing in soils with high concentration levels of Cd, Cu, Pb, Zn, and especially As. From these elements, the highest percentage of extractable elements was found for Cd and the lowest for Pb. A highly significant correlation was observed between total and extractable element concentrations in soils, except for Cu, indicating that total concentration is the most relevant factor for element mobility in these soils. Extractable elements in soils were better correlated with concentrations in plants than total elements in soils; thus, extraction methods applied are suitable to estimate the element phytoavailable fraction in soils, which depends on the plant species and not only on the element mobility in soils. High element concentrations were found in the aboveground parts of *Corrigiola telephiifolia* (As and Pb), *Jasione montana* (Cd and Zn), and *Digitalis thapsi* (As, Cd, Cu, Pb and Zn). However, considering the translocation and accumulation factors, together with the concentration levels found in roots and aboveground parts, only *C. telephiifolia* could be considered a Pb accumulator and an As hyperaccumulator plant, which could be used for future phytoremediation work in soils polluted with As.

Keywords: Arsenic – Heavy metals – Mining soils – Native plants – Accumulator plants – *Corrigiola telephiifolia*

Patricia G. Cardoso, Eduarda Pereira, Tiago F. Grilo, Armando C. Duarte and Miguel A. Pardal. Kinetics of Mercury Bioaccumulation in the Polychaete *Hediste diversicolor* and in the Bivalve *Scrobicularia plana*, Through a Dietary Exposure Pathway. Water, Air, & Soil Pollution, Vol. 223(1) (2012): 421-428

Mercury bioaccumulation kinetics of two important macrobenthic species, the polychaete *Hediste diversicolor* and the bivalve *Scrobicularia plana*, were evaluated following a dietary pathway (i.e. contaminated algae), through a mesocosm laboratory experiment. Both studied species presented a similar model of Hg bioaccumulation kinetics, a linear pattern of accumulation through time being the mercury accumulation in the organisms proportional to the mercury concentration in the food. Mercury bioaccumulation rates were higher in the polychaete *H. diversicolor* (reaching approximately 0.15 $\mu g\ g^{-1}$ at the end of the experiment) than in the bivalve *S. plana* ($\approx 0.07\ \mu g\ g^{-1}$), which could be related to their feeding strategies, ingestion rates and assimilation efficiencies. Moreover, the mercury bioaccumulation revealed to be quite a fast process especially for the polychaete, and despite the fact that this species is not an edible organism, it is an important prey item, which can greatly contribute to the transport of contaminants to higher trophic levels. Therefore, the bioaccumulation of mercury by these important macrobenthic species, especially the bivalves, represents a non-negligible risk for humans.

Keywords: Mercury – Dietary pathway – Bioaccumulation – Kinetics – Bivalves – Polychaetes

Xing Wu[a, b], Yongfeng Jia[a], Huijie Zhu[a]. ([a] Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China, [b] Central Laboratory, Shandong Academy of Agriculture Science (Shandong Key Laboratory of Test Technique on Food Quality and Safety), Jinan 250100, China). Bioaccumulation of cadmium bound to ferric hydroxide and particulate organic matter by the bivalve *M. meretrix*. Environmental Pollution, Vol. 165(2012) : 133–139

Ferric hydroxide and particulate organic matter are important pools of trace metals in sediments and control their accumulation by benthic animals. We investigated bioaccumulation of cadmium in bivalve *Meretrix meretrix* by using a simplified system of laboratory synthesized iron oxides and commercially obtained humic acids to represent the inorganic and organic matrix found in nature. The results showed that bioaccumulation characteristics were distinctly different for these two substrates. Bioaccumulation from ferric hydroxide was not observed at 70 and 140 mg/kg, while the clams started to absorb Cd at 140 mg/kg from organic matter and the bioaccumulation rate was faster than that from ferric hydroxide. Within 28 d, accumulation of Cd from organic matter appeared to reach a steady state after rising to a certain level, while absorption from ferric hydroxide appeared to follow a linear profile. The findings have implications about the assimilation of trace metals from sediments by benthic animals.

Keywords: Bioaccumulation; Cadmium; Ferric hydroxide; Particulate organic matter; Meretrix meretrix Linnaeus

Ángel A. Carbonell-Barrachina[a], Xiangchun Wu[b], Amanda Ramírez-Gandolfo[a], Gareth J. Norton[b], Francisco Burló[a], Claire Deacon[b], Andrew A. Meharg[b]. ([a] Universidad Miguel

Hernandez, Departamento Tecnología Agroalimentaria, Grupo Calidad y Seguridad Alimentaria, Carretera de Beniel, km 3.2, 03312 Orihuela, Alicante, Spain, [b] Institute of Biological and Environmental Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU, UK). Inorganic arsenic contents in rice-based infant foods from Spain, UK, China and USA. Environmental Pollution, Vol. 163(2012): 77–83

Spanish gluten-free rice, cereals with gluten, and pureed baby foods were analysed for total (t-As) and inorganic As (i-As) using ICP-MS and HPLC–ICP-MS, respectively. Besides, pure infant rice from China, USA, UK and Spain were also analysed. The i-As contents were significantly higher in gluten-free rice than in cereals mixtures with gluten, placing infants with celiac disease at high risk. All rice-based products displayed a high i-As content, with values being above 60% of the t-As content and the remainder being dimethylarsinic acid (DMA). Approximately 77% of the pure infant rice samples showed contents below 150 μg kg^{-1} (Chinese limit). When daily intake of i-As by infants (4–12 months) was estimated and expressed on a bodyweight basis (μg d^{-1} kg^{-1}), it was higher in all infants aged 8–12 months than drinking water maximum exposures predicted for adults (assuming 1 L consumption per day for a 10 μg L^{-1} standard).

Graphical abstract

Keywords: Arsenic; Baby foods; Dietary exposure; Gluten; Rice

Xiaoyan Peng[a], Fengjie Liu[b], Wen-Xiong Wang[b], Zhihong Ye[a]. ([a] State Key Laboratory for Bio-control and Guangdong Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, 135 Xin Gang West Road, Guangzhou 510006, PR China. [b] Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong, PR China). Reducing total mercury and methylmercury accumulation in rice grains through water management and deliberate selection of rice cultivars. Environmental Pollution, Vol. 162(2012) : 202–208

Rice consumption has been identified as a major route of methylmercury (MeHg) exposure in some areas of inland China. We investigated two potential mitigation methods (water management and deliberate selection of rice cultivars) to reduce the amount of total mercury (Hg) and MeHg within the grain. Rice grown aerobically had markedly reduced total Hg and MeHg concentrations as well as a much lower proportion of MeHg in the grain. Remarkably, there were considerable variations in the total Hg and MeHg concentrations as well as the proportion of MeHg in the grain among the 24 cultivars grown in the same paddy soil. The Hg

tolerance index (expressed as % mean of control root growth) also varied substantially among the different cultivars. Furthermore, negative correlations were found between the total Hg and MeHg concentrations ($P < 0.05$) of grain and the proportion of MeHg in the grain ($P < 0.01$).

Keywords: *Oryza sativa* L.; Mercury; Methylmercury; Soil redox potential; Genetic variation

Bioremediation

Manal Ahmed Fawzy, Nadia El-sayed Badr, Ahmed El-Khatib and Amany Abo-El-Kassem. Heavy metal biomonitoring and phytoremediation potentialities of aquatic macrophytes in River Nile. Environmental Monitoring and Assessment, Vol. 184(3) (2012): 1753-1771

The concentrations of Cd, Cu, Pb, and Zn in sediments, water, and different plant organs of six aquatic vascular plant species, *Ceratophyllum demersum* L. *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase; *Eichhornia crassipes* (Mart.) Solms-Laub; *Myriophyllum spicatum* L.; *Phragmites australis* (Cav.) Trin. ex Steud; and *Typha domingensis* (Pers.) Poir. ex Steud, growing naturally in the Nile system (Sohag Governorate), were investigated. The aim was to define which species and which plant organs exhibit the greatest accumulation and evaluate whether these species could be usefully employed in biomonitoring and phytoremediation programs. The recorded metals in water samples were above the standard levels of both US Environmental Protection Agency and Egyptian Environmental Affairs Agency except for Pb. The concentrations of heavy metals in water, sediments, and plants possess the same trend: Zn > Cu > Pb > Cd which reflects the biomonitoring potentialities of the investigated plant species. Generally, the variation of heavy element concentrations in water and sediments in relation to site and season, as assessed by two-way repeated measured ANOVA, was significant ($p< 0.05$). However, insignificant variations were observed in the concentrations of Pb and Cd in sediments in relation to season and of Cu and Zn in relation to site. Results also showed that the selectivity of the heavy elements for the investigated plants varied significantly ($p< 0.05$) with species variation. The accumulation capability of the investigated species could be arranged according to this pattern: *C. demersum* > *E. crassipes* > *M. spicatum* > *E. pyramidalis* > *T. domingensis* > *P. australis*. On the basis of the element concentrations, roots of all the studied species contain higher concentrations of Cu and Zn than shoots while leaves usually acquire the highest concentrations of Pb. Cd concentrations among different plant organs are comparable except in *M. spicatum* where the highest Cd concentrations were recorded in the leaves. Our results also demonstrated that all the studied species can accumulate more than 1,450-fold the concentration of the investigated heavy elements in water rendering them of interest for use in phytoremediation studies of polluted waters. Given the absence of systematic water quality monitoring, heavy elements in plants, rather than sediments, provide a cost-effective means for assessing heavy element accumulation in aquatic systems during plant organ lifespan.

Keywords: Bioaccumulation – Heavy metals – *Ceratophyllum demersum* – *Eichhornia crassipes* – *Echinochloa pyramidal* – *Myriophyllum spicatum* – *Phragmites australis* – *Typha domingensis*

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Mining Engineering, Mineral Processing Division (Mineral–Metal Recovery and Recycling Research Group), Suleyman Demirel University, TR32260, Isparta, Turkey). Biohydrometallurgy techniques of low grade ores: A review on black shale. Hydrometallurgy, Vol. 117–118(2012): 1–12

The demand for metals is ever increasing with the advancement of the industrialized world. On the other hand, worldwide reserves of high grade ores are close to depletion. However, there exists a large reserve of metals in low and lean grade ores and other secondary sources. Metal recovery from low and lean grade ores using conventional techniques such as pyrometallurgy, etc. requires high energy and capital inputs which often result in the secondary environmental pollution. Thus, there is a need to utilize more efficient technologies to recover metals. Biohydrometallurgy, which exploits microbiological processes to recover metal ions, is regarded as one of the most promising and revolutionary biotechnologies. The products of such processes are dissolved in aqueous solution, thereby rendering them more amenable to containment, treatment and recovery. On top of this, biohydrometallurgy can be conducted under mild conditions, usually without the use of any toxic chemicals. Consequently, the application of biohydrometallurgy in the recovery of metals from lean grade ores and wastes has made it an eco-friendly technology for enhanced metal production. This paper reviews the current status of biohydrometallurgy of low grade ores around the world. Particular attention is focused on the bioleaching of black shale ore and its metallogenic diversity in the world. The review assesses the status of bioprocesssing of metals to evaluate promising developments. Bioleaching of metals is comprehensively reviewed with the emphasis on the contribution of microbial community, especially fungal bioleaching coupled with ultrasound treatment. In this manuscript, the principles of bioleaching, their mechanisms, and commercial applications are presented. The case studies and future technology directions are also reviewed.

Keywords: Bioleaching; Sonobioleaching; Black shale; Microorganism; Minerals dissolution

Eduardo V. Soares and Helena M. V. M. Soares. Bioremediation of industrial effluents containing heavy metals using brewing cells of *Saccharomyces cerevisiae* as a green technology: a review. Environmental Science and Pollution Research, Vol. 19(4) (2012):1066-1083

The release of heavy metals into the environment, mainly as a consequence of anthropogenic activities, constitutes a worldwide environmental pollution problem. Unlike organic pollutants, heavy metals are not degraded and remain indefinitely in the ecosystem, which poses a different kind of challenge for remediation. It seems that the “best treatment technologies” available may not be completely effective for metal removal or can be expensive; therefore, new methodologies have been proposed for the detoxification of metal-bearing wastewaters. The present work reviews and discusses the advantages of using brewing yeast cells of *Saccharomyces cerevisiae* in the detoxification of effluents containing heavy metals. The current knowledge of the mechanisms of metal removal by yeast biomass is presented. The use of live or dead biomass and the influence of biomass inactivation on the metal accumulation characteristics are outlined. The role of chemical speciation for predicting and optimising the efficiency of metal removal is highlighted. The problem of biomass separation, after treatment of the effluents, and the use of flocculent characteristics, as an alternative process of cell–liquid separation, are also discussed. The use of yeast cells in the treatment of real effluents to bridge the gap between fundamental

and applied studies is presented and updated. The convenient management of the contaminated biomass and the advantages of the selective recovery of heavy metals in the development of a closed cycle without residues (green technology) are critically reviewed.

Keywords: Chemical speciation – Electroplating wastewater bioremediation – Heavy metal biosorption – Incineration – Metal selective recovery – Yeast flocculation

Yunhai Wu, Li Jiang, YaJun Wen, JianXin Zhou and Shixun Feng. Biosorption of Basic Violet 5BN and Basic Green by waste brewery's yeast from single and multicomponent systems. Environmental Science and Pollution Research, Vol.19(2) (2012): 510-521

The biosorption of Basic Violet 5BN (BV) and Basic Green (BG) by waste brewery's yeast (WBY) from single and binary systems was investigated.

For the single system, the adsorption of both dyes is pH-dependent and the optimum value is 5.0. At a lower initial concentration, the kinetic data agree well with both pseudo-first-order and pseudo-second-order models, while at a higher initial concentration the data fit better with the pseudo-second-order model. External diffusion is the rate-controlling step at initial fast adsorption, and then the intraparticle diffusion dominated the mass transfer process. Equilibrium data for BV and BG fit better with the Langmuir model. The maximum biosorption capacities of WBY onto BV and BG obtained at 303 K are 114.65 and 141.89 mg/g, respectively. Thermodynamic analysis reveals that the adsorption process for the two dyes is spontaneous and exothermic.

The hydroxyl, amino, amide, carboxyl, and phosphate groups are responsible for the biosorption based on Fourier transform infrared analysis. The presence of BV significantly affects the biosorption of BG, but not vice versa. The *P*-factor model and Sheindrof–Rebhun–Sheintuch equation gave a good description of the equilibrium adsorption data at the multicomponent system.

Keywords: Biosorption – Waste brewery's yeast – Dyes – Single-component system – Multicomponent system – Competitive adsorption

K. Sarayu and S. Sandhya. Current Technologies for Biological Treatment of Textile Wastewater–A Review. Applied Biochemistry and Biotechnology, Vol. 167(3) (2012): 645-661

The release of colored wastewater represents a serious environmental problem and public health concern. Color removal from textile wastewater has become a big challenge over the last decades, and up to now, there is no single and economically attractive treatment method that can effectively decolorize the wastewater. Effluents from textile manufacturing, dyeing, and finishing processes contain high concentrations of biologically difficult-to-degrade or even inert auxiliaries, chemicals like acids, waxes, fats, salts, binders, thickeners, urea, surfactants, reducing agents, etc. The various chemicals such as biocides and stain repellents used for brightening, sequestering, anticreasing, sizing, softening, and wetting of the yarn or fabric are also present in wastewater. Therefore, the textile wastewater needs environmental friendly, effective treatment process. This paper provides a critical review on the current technology available for decolorization and degradation of textile wastewater and also suggests effective and economically attractive alternatives.

Keywords: Textile wastewater – Toxicity – Biodegradation – Treatment – Problems

Ismat Bibi and Haq Nawaz Bhatti. Enhanced Biodecolorization of Reactive Dyes by Basidiomycetes Under Static Conditions. Applied Biochemistry and Biotechnology, Vol. 166(8) (2012): 2078-2090

This study presents the biodecolorization potential of basidiomycete fungi *Trametes hirsuta*, *Pycnoporus* sp., and *Irpex* sp. for different reactive dyes viz. Reactive Red 120, Remazol Brilliant Blue R (RBBR), Reactive Orange G, and Reactive Orange 16 under static and shaking conditions. The screening trials revealed that *T. hirsuta* exhibited maximum potential (83.75 %) for biodecolorization of RBBR dye under static conditions after the fifth day of incubation. However, the rate of biodecolorization of RBBR dye by *Pycnoporus* sp. was much slow and reached maximum (81.25 %) after 15 days of incubation under shaking conditions. By process optimization, enhanced decolorization (91.2 %) of RBBR by *T. hirsuta* was achieved at pH 5.5 within 24 h using a defined salt medium amended with *p*-coumaric acid under static conditions. pH was found to be an important parameter for the enzymatic system involved in RBBR dye decolorization by *T. hirsuta* and *Pycnoporus* sp. Biodecolorization of RBBR dye was determined by a reduction in optical density at the wavelength of maximum absorbance (λ, 578 nm) by UV–vis spectrophotometer. The shift in maximum wavelength toward shorter/longer wavelength in UV–vis scanning spectrum revealed the degradation of RBBR dye into different transformation products.

Keywords: White rot fungi – Reactive dyes – Biodegradation – Peroxidases

Wen-Jie Xia, Zhi-bin Luo, Han-Ping Dong, Li Yu and Qing-Feng Cui, et al. Synthesis, Characterization, and Oil Recovery Application of Biosurfactant Produced by Indigenous *Pseudomonas aeruginosa* WJ-1 Using Waste Vegetable Oils. Applied Biochemistry and Biotechnology, Vol. 166(5) (2012): 1148-1166

A bacterial strain was isolated and cultured from the oil excavation areas in tropical zone in northern China. The biochemical characteristics and partial sequenced 16S rRNA gene of isolate, WJ-1, was identical to those of cultured representatives of the species *Pseudomonas aeruginosa*. This bacterium was able to produce a type of biosurfactant. Compositional analysis revealed that the extracted biosurfactant was composed of high percentage lipid (74%, *w*/*w*) and carbohydrate (20%, *w*/*w*) in addition to a minor fraction of protein (6%, *w*/*w*). The best production of 50.2 g/l was obtained when the cells were grown on minimal salt medium containing 6.0% (*w*/*v*) glucose and 0.75% (*w*/*v*) sodium nitrate supplemented with 0.1% (*v*/*v*) element solution at 37 °C and 180 rpm after 96 h. The optimum biosurfactant production pH value was found to be 6.0–8.0. The biosurfactant of WJ-1, with the critical micelle concentration of 0.014 g/L, could reduce surface tension to 24.5 mN/m and emulsified kerosene up to EI_{24} ≈95. The results obtained from time course study indicated that the surface tension reduction and emulsification potential was increased in the same way to cell growth. However, maximum biosurfactant production occurred and established in the stationary growth phase (after 90 h). Thin layer chromatography, Fourier transform infrared spectrum, and mass spectrum analysis indicate the extracted biosurfactant was affiliated with rhamnolipid. The core holder flooding experiments demonstrated that the oil recovery efficiency of strain and its biosurfactant was 23.02% residual oil.

Keywords: *Pseudomonas aeruginosa* – Biosurfactant – Rhamnolipid – Surface tension – Emulsification – Oil recovery

Lin Xu, Mingfang Luo, Chengying Jiang, Xuetuan Wei, Peng Kong, Xiangfeng Liang, Junmei Zhao, Liangrong Yang and Huizhou Liu. In Vitro Reduction of Hexavalent Chromium by Cytoplasmic Fractions of *Pannonibacter phragmitetus* LSSE-09 under Aerobic and Anaerobic Conditions. Applied Biochemistry and Biotechnology, Vol.166(4) (2012): 933-941

Hexavalent chromate reductase was characterized and was found to be localized in the cytoplasmic fraction of a chromium-resistant bacterium *Pannonibacter phragmitetus* LSSE-09. The Cr(VI) reductase activity of cell-free extract (S_{12}) was significantly improved by external electron donors, such as NADH, glucose, acetate, formate, citrate, pyruvate, and lactate. The reductase activity was optimal at pH 7.0 with NADH as the electron donor. The aerobic and anaerobic Cr(VI)-reduction enhanced by 0.1 mM NADH were respectively 3.5 and 3.4 times as high as that without adding NADH. The Cr(VI) reductase activity was inhibited by Mn^{2+}, Cd^{2+}, Fe^{3+}, and Hg^{2+}, whereas Cu^{2+} enhanced the chromate reductase activity by 29% aerobically and 33% anaerobically. The aerobic and anaerobic specific Michaelis–Menten constant K_m of S_{12} fraction was estimated to be 64.95 and 47.65 μmol L^{-1}, respectively. The soluble S_{150} fractions showed similar activity to S_{12} and could reduce 39.7% and 53.4% of Cr(VI) after 1 h of incubation aerobically and anaerobically while the periplasmic contents showed no obvious reduction activity, suggesting an effective enzymatic mechanism of Cr(VI) reduction in the cytoplasmic fractions of the bacterium. Results suggest that the enzymatic reduction of Cr(VI) could be useful for Cr(VI) detoxification in wastewater.

Keywords: *Pannonibacter phragmitetus* LSSE-09 – Cr(VI) reduction – Chromate reductase – Cytoplasmic fractions

Marina V. Donova and Olga V. Egorova. Microbial steroid transformations: current state and prospects. Applied Microbiology and Biotechnology, Vol. 94(6) (2012): 1423-1447

Studies of steroid modifications catalyzed by microbial whole cells represent a well-established research area in white biotechnology. Still, advances over the last decade in genetic and metabolic engineering, whole-cell biocatalysis in non-conventional media, and process monitoring raised research in this field to a new level. This review summarizes the data on microbial steroid conversion obtained since 2003. The key reactions of structural steroid functionalization by microorganisms are highlighted including sterol side-chain degradation, hydroxylation at various positions of the steroid core, and redox reactions. We also describe methods for enhancement of bioprocess productivity, selectivity of target reactions, and application of microbial transformations for production of valuable pharmaceutical ingredients and precursors. Challenges and prospects of whole-cell biocatalysis applications in steroid industry are discussed.

Keywords: Steroid – Microbial transformation – Bioconversion – Sterol – Side-chain degradation – Hydroxylation – Dehydrogenation – Sterol catabolism – Whole-cell biocatalysis

Shaohua Chen, Peng Geng, Ying Xiao and Meiying Hu. Bioremediation of β-cypermethrin and 3-phenoxybenzaldehyde contaminated soils using *Streptomyces aureus* HP-S-01. Applied Microbiology and Biotechnology, Vol. 94(2) (2012): 505-515

Using laboratory and field experiments, the ability of *Streptomyces aureus* HP-S-01 to eliminate β-cypermethrin (β-CP) and its metabolite 3-phenoxybenzaldehyde (3-PBA) in soils was investigated. In the laboratory, 80.5% and 73.1% of the initial dose of β-CP and 3-PBA (50 mg kg^{-1}) was removed in sterilized soils within 10 days, respectively, while in the same period, disappearance rate of β-CP and 3-PBA in non-sterilized soils was higher and reached 87.8% and 79.3%, respectively. Furthermore, the disappearance process followed the first-order kinetics and the half-life ($T_{1/2}$) for β-CP and 3-PBA reduced by 20.3–52.9 and 133.7–186.8 days, respectively, as compared to the controls. The addition of sucrose to the soils enhanced the ability of strain HP-S-01 to eliminate β-CP and 3-PBA. Similar results were observed in the field experiments. The introduced strain HP-S-01 quickly adapted to the environment and rapidly removed β-CP and 3-PBA without any lag phases in the field experiments. Compared with the controls, 47.9% and 67.0% of applied dose of β-CP and 3-PBA was removed from the soils without extra carbon sources and 52.5% and 73.3% of β-CP and 3-PBA was eliminated in soils supplemented with sucrose within 10 days, respectively. Analysis of β-CP degradation products in soil indicated that the tested strain transform β-CP to 3-PBA and α-hydroxy-3-phenoxy-benzeneacetonitrile. However, both intermediates were transient and they disappeared after 10 days. Therefore, the selected actinomyces strain HP-S-01 is suitable for the efficient and rapid bioremediation of β-CP contaminated soils.

Keywords: β-Cypermethrin – 3-Phenoxybenzaldehyde – Bioremediation – *Streptomyces aureus* HP-S-01 – Kinetics – Soil

Sachin P. Bachate, Rashmi M. Khapare and Kisan M. Kodam. Oxidation of arsenite by two β-proteobacteria isolated from soil. Applied Microbiology and Biotechnology, Vol. 93(5) (2012): 2135-2145

Two heterotrophic As(III)-oxidizing bacteria, SPB-24 and SPB-31 were isolated from garden soil. Based on 16S rRNA gene sequence analysis, strain SPB-24 was closely related to genus *Bordetella*, and strain SPB-31 was most closely related to genus *Achromobacter*. Both strains exhibited high As(III) (15 mM for SPB-24 and 40 mM for SPB-31) and As(V) (>300 mM for both strains) resistance. Both strains oxidized 5 mM As(III) in minimal medium with oxidation rate of 554 and 558 μM h^{-1} for SPB-24 and SPB-31, respectively. Washed cells of both strains oxidized As(III) over broad pH and temperature range with optimum pH 6 and temperature 42°C for both strains. The As(III) oxidation kinetic by washed cells showed K_m and V_{max} values of 41.7 μM and 1,166 μM h^{-1} for SPB-24, 52 μM and 1,186 μM h^{-1} for SPB-31. In the presence of minimal amount of carbon source, the strains showed high As(III) oxidation rate and high specific arsenite oxidase activity. The ability of strains to resist high concentration of arsenic and oxidize As(III) with highest rates reported so far makes them potential candidates for bioremediation of arsenic-contaminated environment.

Keywords: Arsenite oxidation – *Achromobacter* – *Bordetella*

Weiwei Zhang, Lingxin Chen and Dongyan Liu. Characterization of a marine-isolated mercury-resistant *Pseudomonas putida* strain SP1 and its potential application in marine mercury reduction. Applied Microbiology and Biotechnology, Vol. 93(3) (2012): 1305-1314

The *Pseudomonas putida* strain SP1 was isolated from marine environment and was found to be resistant to 280 μM $HgCl_2$. SP1 was also highly resistant to other metals, including $CdCl_2$, $CoCl_2$, $CrCl_3$, $CuCl_2$, $PbCl_2$, and $ZnSO_4$, and the antibiotics ampicillin (Ap), kanamycin (Kn), chloramphenicol (Cm), and tetracycline (Tc). *mer* operon, possessed by most mercury-resistant bacteria, and other diverse types of resistant determinants were all located on the bacterial chromosome. Cold vapor atomic absorption spectrometry and a volatilization test indicated that the isolated *P. putida* SP1 was able to volatilize almost 100% of the total mercury it was exposed to and could potentially be used for bioremediation in marine environments. The optimal pH for the growth of *P. putida* SP1 in the presence of $HgCl_2$ and the removal of $HgCl_2$ by *P. putida* SP1 was between 8.0 and 9.0, whereas the optimal pH for the expression of *merA*, the mercuric reductase enzyme in *mer* operon that reduces reactive Hg^{2+} to volatile and relatively inert monoatomic Hg^0 vapor, was around 5.0. LD_{50} of *P. putida* SP1 to flounder and turbot was 1.5×10^9 CFU. Biofilm developed by *P. putida* SP1 was 1- to 3-fold lower than biofilm developed by an aquatic pathogen *Pseudomonas fluorescens* TSS. The results of this study indicate that *P. putida* SP1 is a low virulence strain that can potentially be applied in the bioremediation of $HgCl_2$ contamination over a broad range of pH.

Keywords: *Pseudomonas putida* – Marine environment – *mer* operon – Bioremediation of $HgCl_2$ contamination

Carla M. Zammit, Stefanie Mangold, Venkateswara rao Jonna, Lesley A. Mutch, Helen R. Watling, Mark Dopson and Elizabeth L. J. Watkin. Bioleaching in brackish waters—effect of chloride ions on the acidophile population and proteomes of model species. Applied Microbiology and Biotechnology, Vol. 93(1) (2012): 319-329

High concentrations of chloride ions inhibit the growth of acidophilic microorganisms used in biomining, a problem particularly relevant to Western Australian and Chilean biomining operations. Despite this, little is known about the mechanisms acidophiles adopt in order to tolerate high chloride ion concentrations. This study aimed to investigate the impact of increasing concentrations of chloride ions on the population dynamics of a mixed culture during pyrite bioleaching and apply proteomics to elucidate how two species from this mixed culture alter their proteomes under chloride stress. A mixture consisting of well-known biomining microorganisms and an enrichment culture obtained from an acidic saline drain were tested for their ability to bioleach pyrite in the presence of 0, 3.5, 7, and 20 g□L^{-1} NaCl. Microorganisms from the enrichment culture were found to out-compete the known biomining microorganisms, independent of the chloride ion concentration. The proteomes of the Gram-positive acidophile *Acidimicrobium ferrooxidans* and the Gram-negative acidophile *Acidithiobacillus caldus* grown in the presence or absence of chloride ions were investigated. Analysis of differential expression showed that acidophilic microorganisms adopted several changes in their proteomes in the presence of chloride ions, suggesting the following strategies to combat the NaCl stress: adaptation of the cell membrane, the accumulation of amino acids possibly as a form of osmoprotectant, and the expression of a YceI family protein involved in acid and osmotic-related stress.

Keywords: Biomining – Chloride – Proteomics – Brackish – Membrane

N. Witters[a], R.O. Mendelsohn[b], S. Van Slycken[c], N. Weyens[a], E. Schreurs[a], E. Meers[c], F. Tack[c], R. Carleer[a], J. Vangronsveld[a]. ([a] Centre for Environmental Sciences (CMK), Hasselt University, Agoralaan, Building D, 3590 Diepenbeek, Belgium, [b] School of Forestry &

Environmental Studies (F&ES), Yale University, 195 Prospect Street, New Haven, CT 06511, USA, [c] Department of Applied Analytical and Physical Chemistry, Ghent University, Coupure 653, 9000 Ghent, Belgium). Phytoremediation, a sustainable remediation technology? Conclusions from a case study. I: Energy production and carbon dioxide abatement. Biomass and Bioenergy, Vol. 39(2012) :454–469

This study examines the renewable energy production of crops used for phytoremediation. Our analysis is based on a case study in the Campine region (Belgium and The Netherlands), where agricultural soils are diffusely contaminated with cadmium, lead, and zinc, with an enhanced risk for uptake of these metals in crops and leaching to the groundwater. However, the area has such a large extent (700 km^2) that conventional remediation is not applicable. Cultivation of crops for energy purposes on such land offers the opportunity to come up with an approach that efficiently uses contaminated agricultural land and that can be beneficial for both farmer and society. Performing a Life Cycle Analysis (LCA), we examined the energy and CO_2 abatement potential of willow (*Salix* spp.), silage maize (*Zea mays* L.), and rapeseed (*Brassica napus* L.) originating from contaminated land. Taking into account the marginal impact of the metals in the biomass on the energy conversion efficiency and on the potential use of the biomass and its rest products after conversion, digestion of silage maize with combustion of the contaminated digestate shows the best energetic and CO_2 abating perspectives. The replacement of cokes based electricity by willow is more efficient in CO_2 abatement than willow used in a Combined Heat and Power (CHP) unit, despite lower net energy production in the former option. Willow reaches the same energy production and same CO_2 abatement per hectare per year as silage maize when its biomass yield is respectively 13 and 8.7 Mg dm ha^{-1} y^{-1}.

Keywords: Contamination; Energy maize (Zea mays); Life cycle analysis (LCA); Metal; Rapeseed, (Brassica napus); Willow (Salix spp)

N. Witters[a], R. Mendelsohn[b], S. Van Passel[a], S. Van Slycken[c], N. Weyens[a], E. Schreurs[a], E. Meers[c], F. Tack[c], B. Vanheusden[a], J. Vangronsveld[a]. ([a] Centre for Environmental Sciences (CMK), Hasselt University, Agoralaan, Building D, 3590 Diepenbeek, Belgium, [b] School of Forestry & Environmental Studies (F&ES), Yale University, 195 Prospect Street, New Haven, CT 06511, USA, [c] Department of Applied Analytical and Physical Chemistry, Ghent University, Coupure 653, 9000 Ghent, Belgium). Phytoremediation, a sustainable remediation technology? II: Economic assessment of CO_2 abatement through the use of phytoremediation crops for renewable energy production. Biomass and Bioenergy, Vol. 39(2012): 470–477

Phytoremediation could be a sustainable remediation alternative for conventional remediation technologies. However, its implementation on a commercial scale remains disappointing. To emphasize its sustainability, this paper examines whether and how the potential economic benefit of CO_2 abatement for different crops used for phytoremediation or sustainable land management purposes could promote phytotechnologies. Our analysis is based on a case study in the Campine region, where agricultural soils are contaminated with mainly cadmium. We use Life Cycle Analysis to show for the most relevant crops (willow (*Salix* spp), energy maize (*Zea mays*), and rapeseed (*Brassica napus*)), that phytoremediation, used for renewable energy production, could abate CO_2. Converting this in economic numbers through the Marginal Abatement Cost of CO_2 (€ 20 ton^{-1}) we can integrate this in the economic analysis to compare

phytoremediation crops among each other, and phytoremediation with conventional technologies. The external benefit of CO_2 abatement when using phytoremediation crops for land management ranges between € 55 and € 501 per hectare. The purpose of these calculations is not to calculate a subsidy for phytoremediation. There is no reason why one would prefer phytoremediation crops for renewable energy production over "normal" biomass. Moreover, subsidies for renewable energy already exist. Therefore, we should not integrate these numbers in the economic analysis again. However, these numbers could contribute to making explicit the competitive advantage of phytoremediation compared to conventional remediation technologies, but also add to a more sustainably funded decision on which crop should be grown on contaminated land.

Keywords: Marginal abatement cost (MAC) CO2; Metal contamination; Subsidy; Energy crops; Adapted gross income (AGI); Phytoremediation

Daniela M. A. Leles, Diego A. Lemos, Ubirajara C. Filho, Lucienne L. Romanielo, Miriam M. de Resende and Vicelma L. Cardoso. Evaluation of the bioremoval of Cr(VI) and TOC in biofilters under continuous operation using response surface methodology. Biodegradation, Vol. 23(3) (2012): 441-454

In the present study, the bioremoval of Cr(VI) and the removal of total organic carbon (TOC) were achieved with a system composed by an anaerobic filter and a submerged biofilter with intermittent aeration using a mixed culture of microorganisms originating from contaminated sludge. In the aforementioned biofilters, the concentrations of chromium, carbon, and nitrogen were optimized according to response surface methodology. The initial concentration of Cr(VI) was 137.35 mg l^{-1}, and a bioremoval of 85.23% was attained. The optimal conditions for the removal of TOC were 4 to 8 g l^{-1} of sodium acetate, >0.8 g l^{-1} of ammonium chloride and 60 to 100 mg l^{-1} of Cr(VI). The results revealed that ammonium chloride had the strongest effect on the TOC removal, and 120 mg l^{-1} of Cr(VI) could be removed after 156 h of operation. Moreover, 100% of the Cr(VI) and the total chromium content of the aerobic reactor output were removed, and TOC removals of 80 and 87% were attained after operating the anaerobic and aerobic reactors for 130 and 142 h, respectively. The concentrations of cells in both reactors remained nearly constant over time. The residence time distribution was obtained to evaluate the flow through the bioreactors.

Keywords: Cr(VI) bioremoval – Biofilters – Anaerobic and aerobic processes – Kinetic study – Hydrodynamic study

Pensri Plangklang, Alissara Reungsang and Wisarut Suphannafai. Bioremediation of carbofuran contaminated soil under saturated condition: soil column study. Biodegradation, Vol. 23(3) (2012): 473-485

Disturbed soil columns, 5.8 cm in diameter and 25 cm in length, were used as a basic model to simulate the movement of carbofuran in rice field soil under saturated conditions. Bioaugmentation using a specific carbofuran degrader, *Burkholderia* sp. PCL3, in free and immobilized cell forms and biostimulation using rice straw as organic amendment were applied with the aim of enhancing the degradation of carbofuran in soil and to prevent the movement of carbofuran along with the flow through. In the abiotic control and the treatment with only indigenous microorganisms, the mass recovery percentage of carbofuran in the effluent was 52.1 and 22.5%, respectively. The application of bioaugmentation or biostimulation significantly

enhanced carbofuran degradation in soil and reduced the movement of carbofuran as indicated by a low mass recovery percentage of carbofuran in the effluent of 14.6–15.5%. A low efficiency of carbofuran removal was obtained from the soil column with bioaugmentation together with biostimulation treatments in which the mass recovery percentage of carbofuran in the effluent was in the range of 22.1–22.6%. Sorption of carbofuran to soil, rice straw and corncob, formation of carbofuran metabolite and colony forming unit (CFU) and pH variation with the time were also investigated during column operation.

Keywords: Soil column – Bioremediation – Carbofuran – Saturated zone

O. P. Abioye, P. Agamuthu and A. R. Abdul Aziz. Phytotreatment of soil contaminated with used lubricating oil using *Hibiscus cannabinus*. Biodegradation, Vol. 23(2) (2012): 277-286

Soil contamination by hydrocarbons, especially by used lubricating oil, is a growing problem in developing countries, which poses a serious threat to the environment. Phytoremediation of these contaminated soils offers environmental friendly and a cost effective method for their remediation. *Hibiscus cannabinus* was studied for the remediation of soil contaminated with 2.5 and 1% used lubricating oil and treated with organic wastes [banana skin (BS), brewery spent grain (BSG) and spent mushroom compost (SMC)] for a period of 90 days under natural conditions. Loss of 86.4 and 91.8% used lubricating oil was recorded in soil contaminated with 2.5 and 1% oil and treated with organic wastes respectively at the end of 90 days. However, 52.5 and 58.9% oil loss was recorded in unamended soil contaminated with 2.5 and 1% oil, respectively. The plant did not accumulate hydrocarbon from the soil but shows appreciable accumulation of Fe and Zn in the root and stem of *H. cannabinus* at the end of the experiment. The first order kinetic rate of uptake of Fe and Zn in *H. cannabinus* was higher in organic wastes amendment treatments compared to the unamended treatments, which are extremely low. The results of this study suggest that *H. cannabinus* has a high potential for remediation of hydrocarbon and heavy metal contaminated soil.

Keywords: *Hibiscus cannabinus* – Used lubricating oil – Organic wastes – Hydrocarbons – Bioaccumulation

Arvind Sinha and Sunil Kumar Khare. Mercury bioremediation by mercury accumulating *Enterobacter* sp. cells and its alginate immobilized application. Biodegradation, Vol. 23(1) (2012): 25-34

The effective microbial remediation of the mercury necessitates the mercury to be trapped within the cells without being recycled back to the environment. The study describes a mercury bioaccumulating strain of *Enterobacter* sp., which remediated mercury from the medium simultaneous to its growth. The transmission electron micrographs and electron dispersive X-ray analysis revealed the accumulation of remediated mercury as nano-size particles in the cytoplasm as well as on the cell wall. The *Enterobacter* sp. in the present work was able to accumulate mercury, without being engineered in its native form. The possibility of recovering the accumulated mercury from the cells is also indicated. The applicability of the alginate immobilized cells in removing mercury from synthetic and complex industrial effluent in a batch mode was amply demonstrated. The initial load of 7.3 mg l^{-1} mercury in the industrial effluent

was completely removed in 72 h. The cells immobilized in calcium alginate were similarly effective in the complete removal of 5 mg l^{-1} $HgCl_2$ of mercury from the synthetic effluent in less than 72 h. The immobilized cells could be reused for multiple cycles.

Keywords: *Enterobacter* sp. – Mercury bioremediation – Mercury bioaccumulation – Immobilized cell

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Cadmium is the most common toxic metal threatening safe rice supply. Rice straw has the potential to remove Cd from large-scale effluent contaminated by heavy metals since it exhibited a short biosorption equilibrium time of 5 min, high biosorption capacity (13.9 mg g^{-1}) and high removal efficiency at a pH range of 2.0–6.0. The main Cd biosorption mechanism was Cd^{2+} ion exchange with K^+, Na^+, Mg^{2+} and Ca^{2+}, together with chelation with functional groups such as C═C, C—O, O—H and carboxylic acids. When 0.5% (w/v) rice straw was exposed to 50 mg mL^{-1} $CdSO_4$ solution with shaking at 150 r min^{-1} for 3 h, about 80% of the aquatic Cd was absorbed and the Cd content in rice straw reached 8–10 mg g^{-1}, suggesting that the metal-enriched rice straw could become high quality bio-ore by virtue of the industrial mining grade of its metal content and easy metal recovery.

Keywords: Rice straw; Cadmium biosorption; Pseudo-second order kinetic model; Isothermal model; Biosorption mechanism

Mahwish Asgher. Biosorption of Reactive Dyes: A Review. Water, Air, & Soil Pollution, Vol. 223(5) (2012) : 2417-2435

Development of treatment technologies to alleviate water pollution has been a challenging and demanding task for researchers. Furthermore, synthetic dyes fabricated of complex aromatic structures turned out to be a great hazard as they impart color to water reservoirs making them abhorrent for human use. Reactive dyes being water soluble prove difficult to be eliminated by conventional treatment technologies. In recent times, biosorption has gained prominence as a finishing technology to remove pollutants being cost-effective and environment friendly. This paper describes the hazards posed by dyeing effluents, exclusively reactive dyes, on the environment and use of various biosorbents to remove reactive dyes from aqueous solution under optimum physicochemical parameters. Enhancement of biosorption capacity by chemical treatment and immobilization; equilibrium, kinetic and thermodynamic modeling of biosorption process; characterization by FTIR and SEM and regeneration of biosorbents is also plainly and comprehensively discussed.

Keywords: Biosorption – Reactive dyes – Chemical modification – Immobilization: equilibrium, kinetic and thermodynamic modeling – Regeneration

Xi Liu, Hongyi Ao, Xiong Xiong, Jinguang Xiao and Jiantong Liu. Arsenic Removal from Water by Iron-Modified Bamboo Charcoal. Water, Air, & Soil Pollution, Vol.223(3) (2012): 1033-1044

The effectiveness of a novel and low-cost adsorbent, iron-modified bamboo charcoal (BC-Fe), for arsenic removal from aqueous systems was evaluated in this study. The BC-Fe was synthesized by loading iron onto bamboo charcoal via soaking in a ferric salt solution. The BC-Fe possessed a porous structure with a surface area of 277.895 m^2/g. The adsorption characteristics of arsenic onto BC-Fe were further investigated at various pHs, contact times, arsenic concentrations, and adsorbent doses in batch tests. The corresponding optimum equilibrium pH ranges for As(III) and As(V) removal were 4–5 and 3–4, respectively. The equilibrium times for As(III) and As(V) adsorption were 30 and 35.5 h, respectively. The arsenic removal was strongly dependent on the initial adsorbate concentration and adsorbent dosage. The maximum arsenic removal capacities of BC-Fe under the experimental conditions were 7.237 and 19.771 mg/g for As(III) and As(V), respectively. The pseudo-second-order kinetic model and Freundlich isotherm explained the kinetic and equilibrium of both the As(III) and As(V) adsorbent processes, respectively. Based on these results, the BC-Fe developed in this study is a promising material for the treatment of arsenic-contaminated water.

Keywords: Adsorption – Arsenate – Arsenite – Bamboo charcoal – Freundlich isotherm

P. Timothy Tate, Won Sik Shin, John H. Pardue and W. Andrew Jackson. Bioremediation of an Experimental Oil Spill in a Coastal Louisiana Salt Marsh. Water, Air, & Soil Pollution, Vol. 223(3) (2012) : 1115-1123

The massive oil release from the Deep Water Horizon disaster has reemphasized the need to remediate oil impacted marshes. Due to the physically fragile nature of salt water marshes, bioremediation is often proposed as an appropriate technology and nutrient amendment is often proposed as a means of accelerating biodegradation of crude oil. However, no information is currently available concerning the efficacy of in situ nutrient amendments in Gulf Coast salt marshes. An experimental crude oil spill (142 l over 100 m^2) was conducted to evaluate the efficacy of nitrogen amendment to stimulate bioremediation in a *Spartina alterniflora* dominated Louisiana salt marsh. A randomized complete block design with replication (n=10) was utilized to test the hypothesis that additions of fast-release ammonium nitrate (60 g N/m^2) and slow-release urea (30 g N/m^2) fertilizers could enhance biodegradation of selected crude oil components in the marsh. Crude oil degradation was monitored by analyzing sediment samples for branched and unbranched alkanes over the 180-day study period. The compound/hopane ratio was used to correct for nonbiological losses. No consistent statistically significant effect of fertilizer addition on degradation rates was observed, despite success in increasing the porewater ammonium and NaCl-extractable ammonium over the time frame of the trial. Intrinsic pseudo-first order degradation rates of alkanes in all plots were substantial (0.003–0.008 day^{-1}). Existing, background levels of N did not appear to limit biodegradation rates in *Spartina*-dominated salt marshes. These results suggest that nutrient amendments will not be successful in stimulation biodegradation of crude oil in these systems.

Keywords: Biodegradation – Oil Spill – Salt marsh

Radha Rani, Priyanka Padole, Asha Juwarkar and Tapan Chakrabarti. Phytotransformation of Phorate by *Brassica juncea* (Indian Mustard). Water, Air, & Soil Pollution, Vol. 223(3) (2012) : 1383-1392

Over 5 days, *Brassica juncea* removed 54% of the highly toxic insecticide phorate from the medium with the formation of phorate sulfoxide in small quantity. The loss of phorate from the medium followed first-order kinetics. The half-life of phorate disappearance from water decreased by ~4.5-fold in the presence of *B. juncea*. Mild phorate phytotoxicity was evident from the elevated activities of the antioxidative enzymes like glutathione-disulfide reductase, glutathione S-transferase, superoxide dismutase, and catalase in the plants. Nevertheless, the ubiquitous antioxidative peroxidase was not significantly increased, nor the total glutathione content, due to phorate exposure. Phosphotriester bond hydrolysis and glutathione S-transferase-mediated conjugation seemed to be the key reactions for phorate metabolism by *B. juncea*. From the limited information available, for the first time, a tentative mapping of phytotransformation pathways was performed.

Keywords: Antioxidative response – *Brassica juncea* – Enzymes – Glutathione conjugation – Hydrolysis – Phytotransformation

Sachitra Kumar Ratha, Radha Prasanna, Vishal Gupta, Dolly Wattal Dhar and Anil Kumar Saxena. Bioprospecting and indexing the microalgal diversity of different ecological habitats of India. World Journal of Microbiology and Biotechnology, Vol. 28(4) (2012): 1657-1667

Our study reports the collection, biodiversity analyses, isolation and identification of microalgae from different habitats of India. Cyanophyceae and Chlorophyceae were the most dominant algal groups recorded, with the highest number being recorded for non-heterocystous cyanobacteria (48), followed by 44 unicellular forms. Sagar Island, Sunderbans recorded the greatest number of algae, and unicellular/colonial green algae were present in all the samples. Shannon's Diversity Index was highest in Koikhali, Sunderbans, followed by Rushikulya River, Odisha. Selective enrichment, purification through serial dilution followed by plating and regular observations led to the isolation of sixteen strains. Identification was done by using microscopic observations, supported with standard monographs and classified as belonging to seven genera (*Chlorella*, *Chlorococcum*, *Kirchneria*, *Scenedesmus*, *Chlamydomonas*, *Tetracystis* and *Ulothrix*). 18S rDNA sequencing was undertaken for four strains. The set of sixteen strains were screened under standard cultural conditions for their growth kinetics and *Chlorella sorokiniana* MIC-G5, followed by *Chlorella* sp. MIC-G4 exhibited the highest growth rates. The strain *Chlorococcum* sp. MIC-G2 recorded highest chlorophyll, while MIC-G3 ranked highest for carbohydrates. The study aided in identifying the dominant microalgae in the diverse habitats and characterizing their growth rate and carbohydrate content, providing a valuable germplasm for further utilization in agriculture and industry.

Keywords: Biodiversity – Chlorophyll – *Chlorella* – Diversity – Microalgae

Akbar Esmaeili and Mona Kalantari. Bioremoval of an azo textile dye, Reactive Red 198, by *Aspergillus flavus*. World Journal of Microbiology and Biotechnology, Vol. 28(3) (2012): 1125-1131

The objective of this paper was to study the potential for bioremoval of a textile dye, Reactive Red 198 (RR198), by a fungus isolated from soil collected from an effluent disposal area near a textile company. The fungus was identified as *Aspergillus flavus*, and its use as a low-cost live-cell biomass for the biodegradation of RR198 from contaminated water was investigated using batch studies. The effects of time, dye concentration, and pH as variable factors were examined in the process. Results showed that bioremoval of RR198 by *A. flavus* increased to over 84.96% with increasing time until equilibrium was reached after a period of 24 h. A low pH was the most effective, as were lower levels of dye concentration. The decolorization was determined by the decrease in the absorption maximums of this dye by UV–visible spectroscopy. *A. flavus* was shown to be an efficient fungus for removal of RR198 from wastewater.

Keywords: Color effluent – Bioremoval – Reactive Red 198 – *Aspergillus flavus*

Vanessa S. Cerqueira, Emanuel B. Hollenbach, Franciele Maboni, Flávio A. O. Camargo, Maria do Carmo R. Peralba and Fátima M. Bento. Bioprospection and selection of bacteria isolated from environments contaminated with petrochemical residues for application in bioremediation. World Journal of Microbiology and Biotechnology, Vol. 28(3) (2012):1203-1222

The use of microorganisms with hydrocarbon degrading capability and biosurfactant producers have emerged as an alternative for sustainable treatment of environmental passives. In this study 45 bacteria were isolated from samples contaminated with petrochemical residues, from which 21 were obtained from *Landfarming* soil contaminated with oily sludge, 11 were obtained from petrochemical industry effluents and 13 were originated directly from oily sludge. The metabolization capability of different carbon sources, growth capacity and tolerance, biosurfactant production and enzymes detection were determined. A preliminary selection carried out through the analysis of capability for degrading hydrocarbons showed that 22% of the isolates were able to degrade all carbon sources employed. On the other hand, in 36% of the isolates, the degradation of the oily sludge started within 18–48 h. Those isolates were considered as the most efficient ones. Twenty isolates, identified based on partial sequencing of the 16S rRNA gene, were pre-selected. These isolates showed ability for growing in a medium containing 1% of oily sludge as the sole carbon source, tolerance in a medium containing up to 30% of oily sludge, ability for biosurfactant production, and expression of enzymes involved in degradation of aliphatic and aromatic compounds. Five bacteria, identified as *Stenotrophomonas acidaminiphila* BB5, *Bacillus megaterium* BB6, *Bacillus cibi*, *Pseudomonas aeruginosa*, and *Bacillus cereus* BS20 were shown to be promising for use as inoculum in bioremediation processes (bioaugmentation) of areas contaminated with petrochemical residues since they can use oily sludge as the sole carbon source and produce biosurfactants.

Keywords: Bacteria – Biodegradation – Biosurfactant – Oily sludge – Hydrocarbon

Ling-yun Ji, Wei-wei Zhang, Dong Yu, Yan-ru Cao and Heng Xu. Effect of heavy metal-solubilizing microorganisms on zinc and cadmium extractions from heavy metal contaminated soil with *Tricholoma lobynsis*. World Journal of Microbiology and Biotechnology, Vol. 28(1) (2012): 293-301

The macrofungus, *Tricholoma lobynsis*, was chosen to remedy Zn–Cd–Pb contaminated soil. To enhance its metal-extracting efficiency, two heavy metal resistant microbes M6 and K1 were applied owing to their excellent abilities to solubilize heavy metal salts. The two isolated microbial strains could also produce indole acetic acid (IAA), siderophore and solubilize inorganic phosphate, but neither of them showed 1-aminocyclopropane-1-carboxylate deaminase activity. The strains M6 and K1 were identified as *Serratia marcescens* and *Rhodotorula mucilaginosa* based on 16S rDNA and ITS sequence analysis respectively. Pot experiment showed that spraying to *T. lobynsis*-inoculated soil with M6 and K1 respectively could increase total Cd accumulations of this mushroom by 216 and 61%, and Zn by 153 and 49% compared to the uninoculated control. Pb accumulation however, was too low (<1 mg kg^{-1}) to be determined. The results illustrated that special microbes and macrofungi can work together to remedy polluted soil as plant and plant growth promoting microbes do, probably because of excellent metal-accumulating abilities of macrofungi and IAA-siderophore production, phosphate solubilization abilities of the assisted-microbes. This kind of macrofungi-microbe interaction can be developed into a novel bioremediation strategy.

Keywords: Heavy metals – *Tricholoma lobynsis* – Bioaugmentation – Plant growth promoting microbes

Christine Lors[a, b, c], Denis Damidot[a, b], Jean-François Ponge[d], Frédéric Périé[e]. ([a] Université Lille Nord de France, 1 bis rue Georges Lefèvre, 59044 Lille Cedex, France, [b] EM Douai, LGCgE-MPE-GCE, 941 rue Charles-Bourseul, 59500 Douai, France, [c] National Research Center on Polluted Sites and Soils, 930 Boulevard Lahure, BP 537, 59505 Douai Cedex, France, [d] Muséum National d'Histoire Naturelle, CNRS UMR 7179, 4 avenue du Petit-Château, 91800 Brunoy, France, [e] TOTAL, Pôle R&D Mont Lacq, B.P. 47, 64170 Lacq, France). Comparison of a bioremediation process of PAHs in a PAH-contaminated soil at field and laboratory scales. Environmental Pollution, Vol.165(2012) : 11–17

A laboratory experiment was carried on the same initial soil and at the same time than a windrow treatment in order to compare results at field and laboratory scales for a soil mainly contaminated with PAHs. After 6 months, laboratory experiments gave similar but less scattered results than those obtained in the field indicating that the field biotreatment was well optimised. The total amount of PAHs degraded after 6 months was ca. 90% and degradation rates followed a negative exponential trend. Relative degradation rates of 3- and 4-ring PAHs were about 32 and 7.2 times greater than those of 5- and 6-ring PAHs, respectively. With respect to the bacterial community, bacteria belonging to *Gamma-proteobacteria* persisted whereas *Beta-proteobacteria* appeared after three months of biotreatment when PAH concentration was low enough to render the soil non-ecotoxic.

Keywords: Bioremediation; Contaminated soils; Polycyclic aromatic hydrocarbons (PAHs); Laboratory and field experiments; Bacterial diversity

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2JQ, United Kingdom, [e] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China). Phytochelatins play a key role in arsenic accumulation and tolerance in the aquatic macrophyte *Wolffia globosa*. Environmental Pollution, Vol. 165(2012) :18–24

The rootless duckweed *Wolffia globosa* can accumulate and tolerate relatively large amounts of arsenic (As); however, the underlying mechanisms were unknown. *W. globosa* was exposed to different concentrations of arsenate with or without l-buthionine sulphoximine (BSO), a specific inhibitor of γ-glutamylcysteine synthetase. Free thiol compounds and As(III)–thiol complexes were identified and quantified using HPLC – high resolution ICP-MS – accurate mass ESI-MS. Without BSO, 74% of the As accumulated in the duckweed was complexed with phytochelatins (PCs), with As(III)–PC_4 and As(III)–PC_3 being the main species. BSO was taken up by the duckweed and partly deaminated. The BSO treatment completely suppressed the synthesis of PCs and the formation of As(III)–PC complexes, and also inhibited the reduction of arsenate to arsenite. BSO markedly decreased both As accumulation and As tolerance in *W. globosa*. The results demonstrate an important role of PCs in detoxifying As and enabling As accumulation in *W. globosa*.

Keywords: Arsenic; Arsenic tolerance; Arsenic speciation; Phytochelatins; Wolffia globosa

Biotransformation

Lijuan Yu, Fang Gao, Liping Yang, Lei Xu and Zhaohui Wang, et al. Journal of Industrial Microbiology & Biotechnology, Vol.39(2) (2012): 299-305

The biotransformation of puerarin catalyzed by *Bacillus cereus* NT02 was studied. A primary screening was carried out using 307 strains of bacteria isolated from soil which were able to grow in the presence of puerarin. Strain NT02, identified as *B. cereus*, was able to convert puerarin into puerarin-6″-*O*-phosphate. Under the optimum conditions, resting cells of *B. cereus* NT02 converted 27% of added 0.4 g/l puerarin into puerarin-6″-*O*-phosphate that was characterized by MS, ^{13}C NMR, ^{31}P NMR. The activity of puerarin-6″-*O*-phosphate was 25 times higher than that of puerarin in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging system. The water solubility of puerarin-6″-*O*-phosphate was 85.4 times higher than that of puerarin.

Keywords : *Bacillus cereus* – Biotransformation – Puerarin – Puerarin-6″-*O*-phosphate – Antioxidant

Suchitra Banerjee, Sailendra Singh, Laiq Ur Rahman. (Plant Biotechnology Division, Central Institute of Medicinal and Aromatic Plants, (CIMAP-CSIR), Kukrail Picnic Spot Road, P.O. CIMAP, Lucknow 226015, India). Biotransformation studies using hairy root cultures — A review. Biotechnology Advances, Vol. 30(3) (2012): 461–468

Agrobacterium rhizogenes induced hairy root cultures are entering into a new juncture of functional research in generating pharmaceutical lead compounds by bringing about chemical

transformations aided through its inherent enzyme resources. Rational utilization of hairy root cultures as highly effective biotransformation systems has come into existence in the last twenty years involving a wide range of plant systems as well as exogenous substrates and diverse chemical reactions. To date, hairy root cultures are preferred over plant cell/callus and suspension cultures as biocatalyst due to their genetic/biochemical stability, hormone-autotrophy, multi-enzyme biosynthetic potential mimicking that of the parent plants and relatively low-cost cultural requirements. The resultant biotransformed molecules, that are difficult to make by synthetic organic chemistry, can unearth notable practical efficacies by acquiring improved physico-chemical properties, bioavailability, lower toxicity and broader therapeutic properties. The present review summarizes the overall reported advances made in the area of hairy root mediated biotransformation of exogenous substrates with regard to their reaction types, plant systems associated, bacterial strains/molecules involved and final product recovery.

Keywords: Biotransformation; Exogenous substrate; Hairy root cultures; Reaction types; Product recovery; Plant systems

Lin-Hu Quan, Jin-Woo Min, Dong-Uk Yang, Yeon-Ju Kim and Deok-Chun Yang. Enzymatic biotransformation of ginsenoside Rb1 to 20(*S*)-Rg3 by recombinant β-glucosidase from *Microbacterium esteraromaticum*. Applied Microbiology and Biotechnology, Vol. 94(2) (2012):377-384

Microbacterium esteraromaticum was isolated from ginseng field. The β-glucosidase gene (*bgp1*) from *M. esteraromaticum* was cloned and expressed in *Escherichia coli* BL21 (DE3). The *bgp1* gene consists of 2,496 bp encoding 831 amino acids which have homology to the glycosyl hydrolase family 3 protein domain. The recombinant β-glucosidase enzyme (Bgp1) was purified and characterized. The molecular mass of purified Bgp1 was 87.5 kDa, as determined by SDS-PAGE. Using 0.1 mg ml^{-1} enzyme in 20 mM sodium phosphate buffer at 37°C and pH 7.0, 1.0 mg ml^{-1} ginsenoside Rb1 was transformed into 0.444 mg ml^{-1} ginsenoside Rg3 within 6 h. The Bgp1 sequentially hydrolyzed the outer and inner glucose attached to the C-20 position of ginsenosides Rb1. Bgp1 hydrolyzed the ginsenoside Rb1 along the following pathway: Rb1→Rd→20(*S*)-Rg3. This is the first report of the biotransformation of ginsenoside Rb1 to ginsenoside 20(*S*)-Rg3 using the recombinant β-glucosidase.

Keywords: Biotransformation – β-glucosidase – Ginsenoside Rb1 – Ginsenoside 20(*S*)-Rg3

Jianqiao Wang, Hirofumi Hirai and Hirokazu Kawagishi. Biotransformation of acetamiprid by the white-rot fungus *Phanerochaete sordida* YK-624. Applied Microbiology and Biotechnology, Vol. 93(2) (2012): 831-835

Acetamiprid (ACE) belongs to the neonicotinoid class of systemic broad-spectrum insecticides, which are the most highly effective and largest-selling insecticides worldwide for crop protection. As neonicotinoid insecticides persist in crops, biotransformation of these insecticides represents a promising approach for improving the safety of foods. Here, the elimination of ACE from a liquid medium by the white-rot fungus *Phanerochaete sordida* YK-624 was examined. Under ligninolytic and non-ligninolytic conditions, 45% and 30% of ACE were eliminated, respectively, after 15 days of incubation. High-resolution electrospray ionization mass spectra and nuclear magnetic resonance analyses of a metabolite identified in the culture supernatant suggested that ACE was *N*-demethylated to (*E*)-N^{1}-[(6-chloro-3-pyridyl)-methyl]-N^{2}-cyano-

acetamidine, which has a much lower toxicity than ACE. In addition, we investigated the effect of the cytochrome P450 inhibitor piperonyl butoxide (PB) on the elimination of ACE. The elimination rate of ACE by *P. sordida* YK-624 was markedly reduced by the addition of either 0.01 or 0.1 mM PB to the culture medium. These results suggest that cytochrome P450 plays an important role in the *N*-demethylation of ACE by *P. sordida* YK-624.

Keywords: Acetamiprid – Detoxification – *Phanerochaete sordida* YK-624 – *N*-demethylation – White-rot fungi

Nancy N. Perreault, Dominic Manno, Annamaria Halasz, Sonia Thiboutot, Guy Ampleman and Jalal Hawari. Aerobic biotransformation of 2,4-dinitroanisole in soil and soil *Bacillus* sp. Biodegradation, Vol. 23(2) (2012): 287-295

2,4-Dinitroanisole (DNAN) is a low sensitive melt-cast chemical being tested by the Military Industry as a replacement for 2,4,6-trinitrotoluene (TNT) in explosive formulations. Little is known about the fate of DNAN and its transformation products in the natural environment. Here we report aerobic biotransformation of DNAN in artificially contaminated soil microcosms. DNAN was completely transformed in 8 days in soil slurries supplemented with carbon and nitrogen sources. DNAN was completely transformed in 34 days in slurries supplemented with carbons alone and persisted in unamended microcosms. A strain of *Bacillus* (named 13G) that transformed DNAN by co-metabolism was isolated from the soil. HPLC and LC–MS analyses of cell-free and resting cell assays of *Bacillus* 13G with DNAN showed the formation of 2-amino-4-nitroanisole as the major end-product via the intermediary formation of the arylnitroso (ArNO) and arylhydroxylamino (ArNHOH) derivatives, indicating regioselective reduction of the *ortho*-nitro group. A series of secondary reactions involving ArNO and ArNHOH gave the corresponding azoxy- and azo-dimers. Acetylated and demethylated products were identified. Overall, this paper provides the evidence of fast DNAN transformation by the indigenous microbial populations of an amended soil with no history of contamination with explosives and a first insight into the aerobic metabolism of DNAN by the soil isolate *Bacillus* 13G.

Keywords: DNAN – Nitroaromatic – Explosive – Biodegradation – Nitroreduction

Mojtaba Shamsipur, Ali Akbar Miran Beigi, Mohammad Teymouri, Tahereh Poursaberi, S. Mojtaba Mostafavi, Parviz Soleimani, Fereshteh Chitsazian and Shahram Abolhassan Tash. Biotransformation of methyl *tert*-butyl ether by human cytochrome P450 2A6. Biodegradation, Vol. 23(2) (2012): 311-318

Methyl tert-butyl ether (MTBE) is widely used as gasoline oxygenate and octane number enhancer for more complete combustion in order to reduce the air pollution caused by motor vehicle exhaust. The possible adverse effects of MTBE on human health are of major public concern. However, information on the metabolism of MTBE in human tissues is scarce. The present study demonstrates that human cytochrome P450 2A6 is able to metabolize MTBE to tert-butyl alcohol (TBA), a major circulating metabolite and marker for exposure to MTBE. As CYP2A6 is known to be constitutively expressed in human livers, we infer that it may play a significant role in metabolism of gasoline ethers in liver tissue.

Keywords: Biotransformation – MTBE – Cytochrome P-450 – Metabolism – HS-GC/MS – Formaldehyde – TBA

Pankaj Kumar Arora and Rakesh Kumar Jain. Biotransformation of 4-chloro-2-nitrophenol into 5-chloro-2-methylbenzoxazole by a marine *Bacillus* sp. strain MW-1. Biodegradation, Vol. 23(2) (2012): 325-331

Decolourization, detoxification and biotransformation of 4-chloro-2-nitrophenol (4C2NP) by *Bacillus* sp. strain MW-1 were studied. This strain decolorized 4C2NP only in the presence of an additional carbon source. On the basis of thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), 4-chloro-2-aminophenol, 4-chloro-2-acetaminophenol and 5-chloro-2-methylbenzoxazole were identified as metabolites. Resting cells depleted 4C2NP with stoichiometric formation of 5-chloro-2-methyl benzoxazole. This is the first report of the formation of 5-chloro-2-methylbenzoxazole from 4C2NP by any bacterial strain.

Keywords: 4-chloro-2-nitrophenol – 4-chloro-2-acetaminophenol – 5-chloro-2-methylbenzoxazole – Biotransformation – Detoxification

A. I. Rodarte-Morales, G. Feijoo, M. T. Moreira and J. M. Lema. Biotransformation of three pharmaceutical active compounds by the fungus *Phanerochaete chrysosporium* in a fed batch stirred reactor under air and oxygen supply. Biodegradation, Vol. 23(1) (2012): 145-156

White-rot fungi are a group of microorganisms capable of degrading xenobiotic compounds, such as polycyclic aromatic hydrocarbons or synthetic dyes, by means of the action of extracellular oxidative enzymes secreted during secondary metabolism. In this study, the transformation of three anti-inflammatory drugs: diclofenac, ibuprofen and naproxen were carried out by pellets of *Phanerochaete chrysosporium* in fed-batch bioreactors operating under continuous air supply or periodic pulsation of oxygen. The performance of the fungal reactors was steady over a 30-day treatment and the effect of oxygen pulses on the pellet morphology was evidenced. Complete elimination of diclofenac was achieved in the aerated and the oxygenated reactors, even with a fast oxidation rate in the presence of oxygen (77% after 2 h), reaching a total removal after 23 h. In the case of ibuprofen, this compound was completely oxidized under air and oxygen supply. Finally, naproxen was oxidized in the range of 77 up to 99% under both aeration conditions. These findings demonstrate that the oxidative capability of this microorganism for the anti-inflammatory drugs is not restricted to an oxygen environment, as generally accepted, since the fungal reactor was able to remove these compounds under aerated and oxygenated conditions. This result is very interesting in terms of developing viable reactors for the oxidation of target compounds as the cost of aeration can be significantly reduced.

Keywords: Pharmaceutical – White-rot fungi (WRF) – Degradation – Diclofenac – Ibuprofen – Naproxen

Ming-Jia Yang, Xiang-Jing Wang, Zhong-Yi Yang, Jing An, Wen-Sheng Xiang and Ji Zhang. Bioconversion of ethyl (*R*)-4-cyano-3-hydroxybutyate into (*R*)-ethyl-3-hydroxyglutarate via an indirect pathway by *Rhodococcus boritolerans*. Biotechnology Letters, Vol. 34(5) (2012): 901-905

(*R*)-Ethyl-3-hydroxyglutarate, (*R*)-3, is an intermediate in the synthesis of the statin side chain. Here, a new two-step, indirect biotransformation pathway involving the formation of ethyl (*R*)-4-carbamoyl-3-hydroxybutanoate, (*R*)-2, as an intermediate for (*R*)-3 production was developed using *Rhodococcus boritolerans* with ethyl (*R*)-4-cyano-3-hydroxybutyate, (*R*)-1, as substrate. Maximum conversion was with 10 g (*R*)-1/l, 7 g cells/l (dry wt), pH 7.5 and 25°C. A yield of 98 ± 0.5% (w/w) was attained within 8 h.

Keywords: Ethyl (*R*)-4-carbamoyl-3-hydroxybutanoate – Ethyl (*R*)-4-cyano-3-hydroxybutyate – (*R*)-Ethyl-3-hydroxyglutarate – *Rhodococcus boritolerans*

Lin-Hu Quan, Jin-Woo Min, Subramaniyam Sathiyamoorthy, Dong-Uk Yang, Yeon-Ju Kim and Deok-Chun Yang. Biotransformation of ginsenosides Re and Rg1 into ginsenosides Rg2 and Rh1 by recombinant β-glucosidase. Biotechnology Letters. Vol. 34(5) (2012): 913-917

Ginsenosides Re and Rg1 were transformed by recombinant β-glucosidase (Bgp1) to ginsenosides Rg2 and Rh1, respectively. The *bgp1* gene consists of 2,496 bp encoding 831 amino acids which have homology to the glycosyl hydrolase families 3 protein domain. Using 0.1 mg enzyme ml^{-1} in 20 mM sodium phosphate buffer at 37°C and pH 7.0, the glucose moiety attached to the C-20 position of ginsenosides Re and Rg1, was removed: 1 mg ginsenoside Re ml^{-1} was transformed into 0.83 mg Rg2 ml^{-1} (100% molar conversion) after 2.5 h and 1 mg ginsenoside Rg1 ml^{-1} was transformed into 0.6 mg ginsenoside Rh1 ml^{-1} (78% molar conversion) in 15 min. Using Bgp1 enzyme, almost all initial ginsenosides Re and Rg1 were converted completely to ginsenosides Rg2 and Rh1. This is the first report of the conversion of ginsenoside Re to ginsenoside Rg2 and ginsenoside Rg1 to ginsenoside Rh1 using the recombinant β-glucosidase.

Keywords: Biotransformation – β-Glucosidase – Ginsenoside Rg2 – Ginsenoside Rh1

Zhiqiang Zheng, Huazhong Li, Lun Li and Weilan Shao. Biobleaching of wheat straw pulp with recombinant laccase from the hyperthermophilic *Thermus thermophilus*. Biotechnology Letters, Vol. 34(3) (2012): 541-547

The recombinant laccase from *Thermus thermophilus* was applied to the biobleaching of wheat straw pulp. The best bleaching effect was when the pulp was treated with 3 U laccase g^{-1} dry pulp at 90°C, pH 4.5, 8% consistency for 1.5 h. Under these conditions, the pulp brightness was increased by 3.3% ISO, and the pulp kappa number was decreased by 5.6 U. Enzymatic treatment improved the bleachability of wheat straw pulp but caused no damage to the pulp fibers. The use of enzyme-treated pulp saved 25% H_2O_2 consumption in subsequent peroxide bleaching without decreasing the final brightness. Pulp biobleaching in the presence of 5 mM ABTS further increased the pulp brightness by 1.5% ISO. This is the first report on the application of laccase from *T. thermophilus* in the pulp and paper sector.

Keywords: Biobleaching – Recombinant laccase – *Thermus thermophilus* – Wheat straw pulp

Cristina Chuck-Hernandez, Mayeli Peralta-Contreras, Gabriela Bando-Carranza, Mariano Vera-Garcia, Nallely Gaxiola-Cuevas, Ranses Tamayo-Limon, Feliznando

Cardenas-Torres, Esther Perez-Carrillo and Sergio O. Serna-Saldivar. Bioconversion into ethanol of decorticated red sorghum (*Sorghum bicolor* L. Moench) supplemented with its phenolic extract or spent bran. Biotechnology Letters, Vol. 34(1) (2012): 97-102

The effect of extracted phenolics or spent bran added to decorticated red sorghum kernels during fuel ethanol production was studied and compared to maize and whole red and white sorghums. After liquefaction, free amino nitrogen ranged from 65 to 101 mg/l and at the end of saccharification all mashes had approx. 80 g glucose and 2–5 g maltose/100 g meal (dry basis). Saccharified worts were fermented giving 50–90 ml ethanol/l. The lowest fermentation efficiency (76%) was obtained in the white sorghum. Ethanol yields indicate that sorghum bran or its associated phenolics did not significantly affect the efficiency of the sequential steps involved in ethanol production. Red sorghum is a good alternative to maize to produce ethanol and the difference regarding white sorghum and maize was mainly due to endosperm protein structure and composition.

Keywords: Bioethanol – Phenolic extract – Sorghum – Sorghum bran – Sorghum decortication

Su Lin Lim[a], Ta Yeong Wu[a], Edwin Yih Shyang Sim[a], Pei Nie Lim[a], Charles Clarke[b]. ([a]Chemical Engineering Discipline, School of Engineering, Monash University, Jalan Lagoon Selatan, Bandar Sunway, 46150 Selangor Darul Ehsan, Malaysia, [b] School of Science, Monash University, Jalan Lagoon Selatan, Bandar Sunway, 46150 Selangor Darul Ehsan, Malaysia). Biotransformation of rice husk into organic fertilizer through vermicomposting. Ecological Engineering, Vol. 41(2012): 60–64

Rice husk (RH) is an abundant agricultural solid waste as a result of rice-milling process. The present study investigated the potential of converting RH amended with market refused fruit (market refused banana (B), honeydew (H) or papaya (P)) into vermicompost using *Eudrilus eugeniae*. RH was mixed with market refused fruit in an equal ratio to produce three different treatments (1B:1RH, 1H:1RH and 1P:1RH) for laboratory screening of solid wastes. Generally, the application of *E. eugeniae* permitted an increase in calcium (6.9–99.0%), potassium (15.0–121.4%), phosphorus (2.4–49.5%) and carbon (6.5–69.0%) in final vermicompost after 9 weeks of vermicomposting. However, decreases in magnesium (3.7–45.7%) and nitrogen (6.9–23.7%) were also observed in final vermicomposts. Among all the RH treatments, RH which was mixed with market refused papaya (1P:1RH) showed better quality vermicompost with higher nutritional status. It was also found that RH which was amended by market refused fruit (1B:1RH, 1H:1RH or 1P:1RH), especially market refused papaya, encouraged the growth of earthworm as compared to the treatment with RH alone. The present data reveal that vermicomposting is a feasible technology for bio-transforming RH into value-added material, namely vermicompost.

Keywords: Eudrilus eugeniae; Market refused fruit; Rice husk; Vermicompost; Solid waste management

Biomarker

Rim Ladhar-Chaabouni, Monia Machreki-Ajmi and Amel Hamza-Chaffai. Use of metallothioneins as biomarkers for environmental quality assessment in the Gulf of Gabès (Tunisia) Environmental Monitoring and Assessment, Vol.184(4) (2012): 2177-2192

Detection and assessment of the impact of pollution on biological resources imply increasing research on early-warning markers such as metallothioneins (MTs) in metal exposure. In this paper, we have collated published information on the use of metallothioneins and metallothionein-like proteins (MTLPs) as biomarkers for environmental quality assessment in the Gulf of Gabès. In this area, some species of fish and bivalve were used as bioindicators of pollution. In these species, an induction of MTs/MTLPs by the essential metals such as Cu and Zn and the non-essential metals such as Cd was observed by different authors who suggest the potential use of these proteins as biomarkers. However, MT concentrations can be influenced by many biotic (sex, maturity stages, and tissues) and abiotic factors (temperature, salinity, and pH). This is essentially the case in field studies where many parameters can randomly affect MT levels, so the endogeneous regulation of MTs must be considered before using MTs as an indicator of heavy metal exposure. Moreover, the use of biomarker cannot be examined independently of the evaluation of techniques that enable its quantification. Therefore, the approach to the use of MTs/MTLP as biomarkers of exposure for an assessment of the physiological status of aquatic organisms is discussed in this paper.

Keywords: Biomarker – Biomonitoring – Gulf of Gabès – Metallothionein – Review

C. D. S. Pereira[1,2,3,*], M. L. Martín-Díaz[3], M. G. M. Catharino[4], A. Cesar[1], R. B. Choueri[1,3], S. Taniguchi[2], D. M. S. Abessa[5], M. C. Bícego[2], M. B. A. Vasconcellos[4], A. C. D. Bainy[6], E. C. P. M. Sousa[2], T. A. DelValls[3]. Chronic contamination assessment integrating biomarkers' responses in transplanted mussels—A seasonal monitoring[†]. Environmental Toxicology, Vol. 27(5) (2012): 257–267

This study aimed to provide the first biomonitoring integrating biomarkers and bioaccumulation data in São Paulo coast, Brazil and, for this purpose, a battery of biomarkers of defense mechanisms was analyzed and linked to contaminants' body burden in a weigh-of-evidence approach. The brown mussel *Perna perna* was selected to be transplanted from a farming area (Caraguatatuba) to four possibly polluted sites: Engenho D'Água, DTCS (Dutos e Terminais do Centro-Oeste de São Paulo) oil terminal (Sao Sebastiao zone), Palmas Island, and Itaipu (It; Santos Bay zone). After 3 months of exposure in each season, mussels were recollected and the cytochrome P4501A (CYP1A)- and CYP3A-like activities, glutathione-S-transferase and antioxidants enzymes (catalase, glutathione peroxidase, and glutathione reductase) were analyzed in gills. The concentrations of polycyclic aromatic hydrocarbons, linear alkylbenzenes, and nonessential metals (Cr, Cd, Pb, and Hg) in whole tissue were also analyzed and data were linked to biomarkers' responses by multivariate analysis (principal component analysis—factor analysis). A representation of estimated factor scores was performed to confirm the factor descriptions and to characterize the studied stations. Biomarkers exhibited most significant

alterations all year long in mussels transplanted to It, located at Santos Bay zone, where bioaccumulation of organic and inorganic compounds was detected. This integrated approach using transplanted mussels showed satisfactory results, pointing out differences between sites, seasons, and critical areas, which could be related to land-based contaminants' sources. The influence of natural factors and other contaminants (e.g., pharmaceuticals) on biomarkers' responses are also discussed.

Keywords: biomarkers; bioaccumulation; *Perna perna*; multivariate analysis; biomonitoring

Bruno do Amaral Crispim, Jussara Oliveira Vaini, Alexeia Barufatti Grisolia, Tatiane Zaratini Teixeira and Rosilda Mara Mussury, et al. Biomonitoring the genotoxic effects of pollutants on *Tradescantia pallida* (Rose) D.R. Hunt in Dourados, Brazil. Environmental Science and Pollution Research, Vol. 19(3) (2012): 718-723

This study aimed to associate the intensity of vehicular traffic in the city of Dourados (Mato Grosso do Sul State, Brazil) with mutagenic effects and alterations in leaf physiology as measured by the quantity of micronuclei and the leaf surface parameters of *Tradescantia pallida*.

Five collections of inflorescences were undertaken for 24 weeks to determine the quantities of micronuclei using the *Tradescantia* Micronuclei (Trad-MCN) bioassay. Leaf surface parameters, including stomatal index (SI), stomatal density, and the size of the stomatal ostiole opening size (SO), were evaluated in addition to Trad-MCN. Collections were made at four sampling points with different vehicular traffic intensities. Statistical analyses were performed with SAS software using the Tukey's and Kruskal–Wallis test. Additionally, associations of the characteristics were verified using Pearson's simple correlation analysis.

Significant effects were observed with the Trad-MCN bioassay (p<0.01) that were related to the collection period and location, as well as significant differences (p <0.05) for the effects of the collection points using the Kruskal–Wallis test. In general, the locations with greatest vehicular traffic had plants with the greatest stomatal density values. The characteristics SI and SO did not demonstrate significant differences (p>0.05) in relation to the collection sites. The simple correlation analysis demonstrated a negative association (−0.65) between SI and Trad-MCN (p<0.05).

Plants growing in localities with more intense vehicular traffic had greater quantities of micronuclei as well as higher frequencies and average numbers of stomata than localities with less traffic, indicating the presence of atmospheric contaminants that damaged their DNA.

Keywords: Pollution – Stomatal density – Leaf anatomy foliar – Micronuclei – Mutagenesis – Stomatal index – Vehicular flux

Raimunda Nonata Fortes Carvalho-Neta, Audalio Rebelo Torres and Ana Lúcia Abreu-Silva. Biomarkers in Catfish *Sciades herzbergii* (Teleostei: Ariidae) from Polluted and Non-polluted Areas (São Marcos' Bay, Northeastern Brazil). Applied Biochemistry and Biotechnology, Vol.166(5) (2012): 1314-1327

Biomarkers based on specific enzyme activities and histological alterations are useful tools for evaluating toxicological effects of xenobiotics in wild fish. In this work, an experimental system of biomarkers with enzyme glutathione *S*-transferase (GST) and branchial lesions in catfish

(*Sciades herzbergii*) was mathematically modeled. The fish were collected along known pollution gradients (S1) and from areas regarded relatively free of anthropogenic input (S2) in São Marcos' Bay, Brazil. GST was measured spectrophotometrically, and branchial lesions were examined by light microscope. The databases from this analysis were compiled, and non-linear models were used to analyze the dependence of the enzyme activity on the areas of sampling and on selected biological parameters of the fish. Fish weight, length, and somatic indices (gonadosomatic index) were significant in the model of GST activity only in A2. Brachial lesions were significant in the model of GST activity only in A1. The obtained model indicates that when the GST ceases to act, serious branchial lesions are observed in the fish of the contaminated regions.

Keywords: Glutathione *S*-transferase – Branchial lesion – Gonadosomatic index – Biometry – Catfish – *Sciades herzbergii*

Márcia Isabel Käffer[a, b], Andréa T. Lemos[a, c], Miriam Anders Apel[d], Jocelita Vaz Rocha[c], Suzana Maria de Azevedo Martins[b], Vera Maria Ferrão Vargas[a, c]. ([a] Programa de Pós-Graduação em Ecologia, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil, [b] Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul, C.P. 1188, CEP 90690-000 Porto Alegre, RS, Brazil, [c] Programa de Pesquisas Ambientais, Fundação Estadual de Proteção Ambiental Henrique L. Roessler (FEPAM), Avenida Salvador França, 1707, CEP 90690-000 Porto Alegre, RS, Brazil, [d] Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil). Use of bioindicators to evaluate air quality and genotoxic compounds in an urban environment in Southern Brazil. Environmental Pollution, Vol. 163(2012) : 24–31

Biological indicators are widely used to monitor genotic compounds and air quality in urban environments. *Parmotrema tinctorum* and *Teloschistes exilis* have been used to verify the presence of pollutants and analyze morphophysiological alterations in the thallus of species caused by their action. Species were exposed for seven months, in an urban area, in southern Brazil. Mutagenicity and cytotoxicity of PM10 organic extracts were assessed in the *Salmonella*/microsome assay at two stations. High concentrations of S, Pb, Cr, Zn and Hg were registered in the last period of exposure and more significant morphophysiological damages were verified in the lichens. Generally a higher mutagenic activity is observed in organic extracts of airborne particulate matter during the first months and in the third period of exposure of lichens. In addition, nitro compounds was detected through nitro-sensitive strains. Lichens and mutagenic biomarkers enabled the evaluation of air quality and the presence of environmentally-aggressive compounds.

Keywords: Airborne particulate matter; Biomonitoring; Chlorophyll; Mutagenicity

Biofertilizer

Deepika Singh, Surindra Suthar. (School of Environment & Natural Resources, Doon University, Dehradun 248001, India). Vermicomposting of herbal pharmaceutical industry solid wastes. Ecological Engineering, Vol. 39(2012) : 1–6

The efforts were made in this study to decompose the herbal pharmaceutical industrial waste (HPIW) using earthworm *Eisenia fetida* (Savigny) under laboratory conditions. To achieve the objectives HPIW was mixed with cow dung (CD) in different ratios to produce five different waste mixtures for vermicomposting: T_1 – CD (100%), T_2 – HPIW (25%) + CD (75%), T_3 – HPIW (50%) + CD (50%), T_4 – HPIW (75%) + CD (25%) and T_5 – HPIW (100%). Vermicomposting caused significant changes in vermibed characteristics. In all waste mixtures, a decrease in pH, organic C, C:P ratio and C:N ratio, but increase total N, available P and exchangeable K was recorded. C:N ratio of end material (vermicompost) was within the agronomic preferable limit (>15). T_3 and T_4 vermibeds showed better mineralization and waste decomposition rate during vermicomposting. *E. fetida* produced cocoons in the ranges of 81.0 ± 9.54 (T_1)–306.33 ± 14.31 (T_4) in all vermibeds. Results clearly suggested that vermicomposting could be an efficient technology to convert HPIW into some value-added products for ecological restoration practices.

Keywords: *Eisenia fetida*; Industrial waste; Vermicompost; Composting; Waste recycling

Biocomposting

Anbuselvi, S., Rebecca, J. (Bharath University, Chennai 73, India). A comparative study on the biodegradation of coir waste by three different species of Marine cyanobacteria. Journal of Applied Sciences Research, Vol. 5(12) (2009): 2369-2374

Now a days imbalanced use of chemical fertilizers and pesticides on soil and in plant are not only harmful to soil micro flora and fauna but also reduce the progressive productivity potential of the land. Coir waste contain high amount of cellulose and lignin. So it cannot be easily degraded by soil and cause environmental pollution. In order to overcome this problem and increase the nitrogen content of the soil, biocomposting process is a viable means of converting various organic wastes generated from the industry and the agriculture sectors into beneficial products such as biofertilizer and as a soil conditioner. This is an appropriate method was employed to utilize the coir waste in a profitable manner to reduce the environmental hazards. This study was conducted to identify the efficiency of the degradation work among the Phormidium sp, Oscillatoria sp and Anabaena azollae sps. The field trial was also carried out by using the above selected organisms with coir waste to make value added product such as manure or Biofertilizer

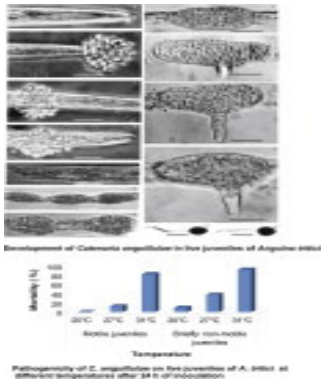
Keywords: Biocomposting process; Biodegradation; Cyano bacteria species; Physical and chemical parameters; Thin layer chromatography

Biopesticides

K.P. Singh, S.S. Vaish, Niranjan Kumar, K.D. Singh, Minakshi Kumari. (Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221 005, India). *Catenaria anguillulae* as an efficient biological control agent of *Anguina tritici in vitro.* Biological Control, Vol.61(3) (2012): 185–193

During the course of our investigation on the selective isolation and pathogenicity tests of *Catenaria anguillulae* against plant parasitic nematodes, actively motile second stage juveniles (J_2s) of *Anguina tritici* obtained from wheat galls collected from Leh, Kashmir, India were found to be severely infected by zoospores of this fungus. The motile J_2s of *A. tritici* suffered nearly 82% mortality at 31 ± 1 °C after 24 h of exposure while the mortality decreased to 77% at 20 ± 1 °C on the 6th day. Pathogenicity trial of 13 isolates of *C. anguillulae* against the motile J_2s revealed that this nematode is highly susceptible to infection. Comparative susceptibility of the plant parasitic nematodes to *C. anguillulae* further revealed that the fungus is more virulent to *A. tritici* than the other nematodes. These findings firmly established that the *C. anguillulae* is a highly virulent biological control agent of *A. tritici* which resolved the controversial issue about its virulence against a nematode belonging to order Tylenchida. Motile and non-motile J_2s of *A. tritici* are equally good for the selective isolation of *C. anguillulae*. However, larger sporangia are produced in the motile J_2s, hence, motile J_2s should be preferred over the non-motile ones. Observations of the developmental stages of *C. anguillulae* in living J_2s of *A. tritici* revealed that the development from zoospore colonization to release of zoospores from mature sporangia was completed in 22–24 h at 31 ± 1 °C, whereas, at 20 ± 1 °C the developmental stages were greatly delayed requiring 72–75 h for zoospore liberation. Pathogenicity and development of *C. anguillulae* in living J_2s of *A. tritici* also proved that the fungus is a wonderful experimental tool which can be used as an example of excellent biological control in the class room.

Graphical abstract

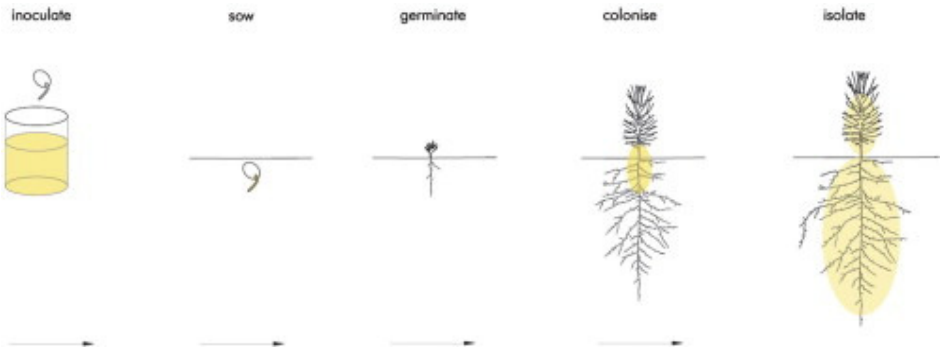


Keywords: *Anguina tritici*; Biological control agent; *Catenaria anguillulae*; Experimental tool; Facultative endoparasite of nematodes; Nematophagous fungi; Pathogenicity; Selective isolation; Sporangia; Sporogenesis; Zoospore; Wheat seed gall/ear cockle nematode

Michael Brownbridge[a, 1], Stephen D. Reay[b], Tracey L. Nelson[a], Travis R. Glare[c], ([a]AgResearch Ltd., Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand, [b] Silver Bullet Forest Research, Auckland, New Zealand, [c] Bio-protection Research Centre, P.O. Box 84, Lincoln University, Lincoln 7647, New Zealand). **Persistence of *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte following inoculation of radiata pine seed and seedlings. Biological Control, Vol.61(3) (2012): 194–200**

The entomopathogenic fungus *Beauveria bassiana* commonly causes disease on a range of insects, including bark beetle pests of plantation forest trees. However, using broadcast application of the fungus to control pest beetles in large scale plantation forests could be difficult to achieve economically. *B. bassiana* has also been found as an endophyte in plants, including the main commercially planted tree in New Zealand, *Pinus radiata*. In this study we investigated two methods to establish *B. bassiana* as endophytes of pine seedlings, seed coating and root dip. Two isolates previously isolated from within mature pines were used and the seedlings monitored for 9 months. Samples of unwashed, washed and surface sterilised roots, surface sterilised needles and soil were plated on semi-selective agar at 2, 4 and 9 months after inoculation. *B. bassiana* was successfully established in pine seedlings using both root dip and seed coating. The fungus was found in soil, non-sterile and sterilised samples at 2 and 4 months, but only one seedling of 30 was positive for fungus in surface sterilised samples after 9 months.

Graphical abstract

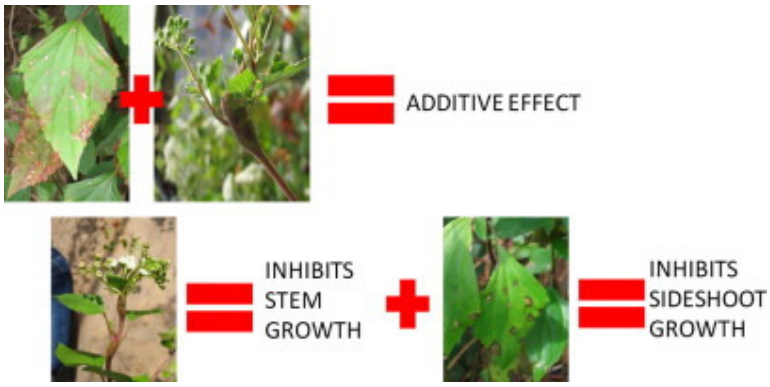


Keywords: Entomopathogens; *Beauveria* spp.; *Pinus radiata*; Endophytes

L. Buccellato, M.J. Byrne, E.T.F. Witkowski. (School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, Johannesburg, South Africa). Interactions between a stem gall fly and a leaf-spot pathogen in the biological control of *Ageratina adenophora*. Biological Control, Vol.61(3) (2012): 222–229

Many biological control projects involve the release of multiple agents. *Ageratina adenophora* (crofton weed) has two agents, *Procecidochares utilis*, a stem gall fly, and *Passalora ageratinae*, a leaf-spot fungal pathogen, released against it in South Africa. This study investigated whether both agents, individually or jointly, increased or decreased the impact on crofton weed under greenhouse conditions. Six-month-old plants were exposed to one of six treatments ($n = 15$ plants/treatment): control (no agents), pathogen-only, single-galled only, double-galled only, pathogen-single-galled, and pathogen-double-galled, all for a period of 6 months. Individually, both of the agents reduced stem height and percentage of live leaves, but there was no synergistic effect of the two agents together. Pathogen-double-galled stems had significantly fewer pathogen-infected leaves relative to the other pathogen-infected treatment stems, suggesting a negative interaction between the two agents on pathogen establishment. Pathogen infection did not affect the size of the galls. Double galling by the fly inhibited stem growth above the gall position on the stem. Crofton weed compensated for galling by increasing the number of sideshoots. The pathogen inhibited sideshoot growth, thereby curbing the plant's ability to offset galling. Overall, there was an additive interaction between the two agents which enhances their usefulness as biocontrol agents of crofton weed.

Graphical abstract

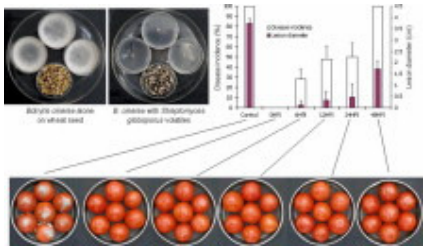


Keywords : Crofton weed; Insect–plant-pathogen interaction; Multiple agents; *Procecidochares utilis*; *Passalora ageratinae*

Qili Li[a, b], Ping Ning[c], Lu Zheng[a], Junbin Huang[a], Guoqing Li[a], Tom Hsiang[d]. ([a] The Key Lab of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan, Hubei 430070, China, [b] Institute of Plant Protection, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi 530007, China, [c] Department of Biotechnology, Guangxi Agricultural Vocation-Technical College, Nanning, Guangxi 530007, China, [d] School of Environmental Sciences, University of Guelph, Guelph, ON, Canada N1G 2W1). Effects of volatile substances of *Streptomyces globisporus* JK-1 on control of *Botrytis cinerea* on tomato fruit. Biological Control, Vol. 61(2) (2012): 113–120

Volatile substances produced by *Streptomyces globisporus* JK-1 grown on autoclaved wheat seed inhibited *Botrytis cinerea* growth and development both on media and in inoculated tomato fruit. The volatiles suppressed mycelial growth of various plant pathogens *in vitro*, especially that of *B. cinerea* and *Sclerotinia sclerotiorum*. Conidial germination and sporulation of *B. cinerea* were also suppressed. Disease incidence and severity on wound-inoculated tomato fruit (*Lycopersicon esculentum*) were inhibited when fumigated with 120 g wheat seed culture of *S. globisporus* JK-1 per liter of airspace in treatment containers. Suppression of the infection process of *B. cinerea* on tomato fruit was observed via scanning microscopy, showing inhibition of conidial germination and of appressorial formation on tomato fruit, as well as abnormal morphology of appressoria and conidia. The viability of the conidia obtained from the volatile-treated and non-treated disease lesions was tested with the vital stains fluorescein diacetate (FDA) and propidium iodide (PI). Conidia fumigated with 30, 60 or 120 g/L wheat seed culture of *S. globisporus* JK-1 at 20 °C for 6 days showed 46.0%, 69.8%, or 80.9% reduction in viability, respectively. Transmission electron microscopy of fumigated and untreated *B. cinerea* showed excessive vesiculation or thickened cell walls in exposed conidia and increased vesiculation or strong retraction of plasma membrane in exposed hyphae. These results provide a better understanding of the mode of action of volatiles from JK-1 on *B. cinerea*. The inhibition growth of *B. cinerea* both *in vitro* and *in vivo* showed that volatiles from *S. globisporus* JK-1 have the potential for control of postharvest grey mold of tomato fruits through fumigant action.

Graphical abstract

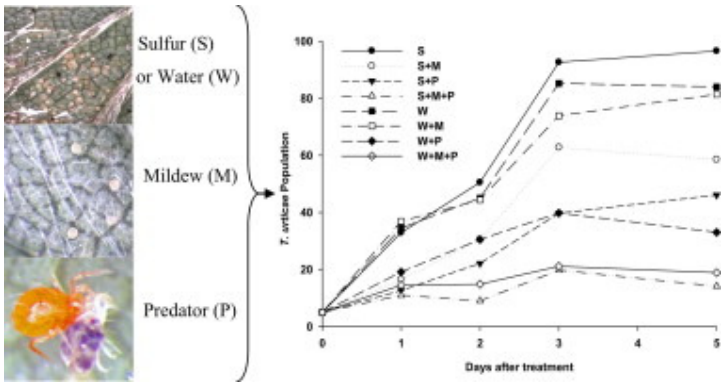


Keywords: *Streptomyces globisporus* JK-1; Volatiles; *Botrytis cinerea*; Infection process; Ultrastructure

Belachew Asalf[a, b], Nina Trandem[a], Arne Stensvand[a], Vitalis W. Wekesa[a, d], Gilberto J. de Moraes[c], Ingeborg Klingen[a]. ([a] Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, N-1432 Ås, Norway, [b] Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, N-1432 Ås, Norway, [c] ESALQ-Universidade de São Paulo, 13418-900 Piracicaba, SP, Brazil, [d] Kenya Polytechnic University College, P.O. Box 52428-00200, Nairobi, Kenya). **Influence of sulfur, powdery mildew, and the predatory mite *Phytoseiulus persimilis* on two-spotted spider mite in strawberry. Biological Control, Vol.61(2) (2012): 121–127**

Strawberry plants frequently suffer from simultaneous or sequential attacks of powdery mildew (*Podosphaera aphanis*) and the two-spotted spider mite (*Tetranychus urticae*), and for many years control of these two plant-damaging organisms have been achieved by use of sulfur and the predatory mite *Phytoseiulus persimilis*, respectively. Sulfur, predatory mites, and powdery mildew have long been studied separately regarding their impacts on spider mites, but knowledge of their combined influence in that context is also needed to aid integrated pest and disease management. Therefore, we conducted controlled laboratory experiments to study the main and the interaction effects of sulfur, *P. persimilis*, *P. aphanis*, and *T. urticae* in strawberry leaf disc bioassays. The results showed that the predatory mite, powdery mildew, and sulfur had additive effects on reducing egg number and population growth of *T. urticae*. Compared to the control, populations of *T. urticae* on the leaf discs were decreased by 83%, 76%, and 61% after 5 days of treatment with sulfur + powdery mildew + predatory mites, mildew + predatory mites, and predatory mites alone, respectively. The survival and egg production of *T. urticae* females was affected to the greatest extent by *P. persimilis*, followed by powdery mildew. Residual sulfur had a short-term suppressive effect on *T. urticae* egg production but did not influence survival and reproduction of *P. persimilis*. If these findings also apply under field conditions, controlling powdery mildew will not reduce the need for spider mite control in strawberry, as has been suggested for other fruit crops.

Graphical abstract

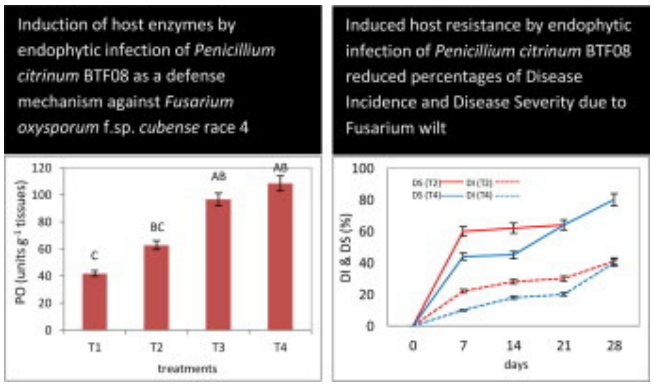


Keywords: Biological control; *Fragaria* × *ananassa*; Integrated pest management; *Phytoseiulus persimilis*; *Podosphaera aphanis*; Sulfur; *Tetranychus urticae*

A.S.Y. Ting[a, b], S.W. Mah[a], C.S. Tee[c]. ([a] Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Jalan Genting Kelang, Setapak, 53300 Kuala Lumpur, Malaysia, [b] School of Science, Monash University Sunway Campus, Jalan Lagoon Selatan, 46150 Bandar Sunway, Selangor Darul Ehsan, Malaysia, [c] Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar Perak, Malaysia). **Evaluating the feasibility of induced host resistance by endophytic isolate *Penicillium citrinum* BTF08 as a control mechanism for Fusarium wilt in banana plantlets. Biological Control, Volume 61(2) (2012): 155–159**

Penicillium citrinum BTF08 was evaluated for its biocontrol potential against the pathogenic *Fusarium oxysporum* f. sp. *cubense* race 4 (FocR4) in banana plantlets via induction of host resistance. Disease assessments were recorded weekly and their biochemical markers (peroxidase, polyphenoloxidase, phenylalanine ammonia lyase) assayed. Results showed colonization of *P. citrinum* BTF08 resulted in significant levels of PO and PPO that were detected in plantlets pre-treated with *P. citrinum* BTF08. These levels of 98.35 units g^{-1} tissues and 57.25 units g^{-1} tissues were higher compared to levels assayed from plantlets infected with only pathogenic FocR4 with 63.7 units g^{-1} tissues and 45.80 units g^{-1} tissues, for PO and PPO, respectively. The elevated levels of PO and PPO suggest the occurrence of induced host resistance in *P. citrinum* BTF08-treated plantlets, and this benefited the plantlets, as they recorded lower percentages of disease incidence and severity, and delayed symptom progression. However, at the end of 28 days, all plantlets succumbed to Fusarium wilt with 80% disease incidence and 42% disease severity. Our study is the first to document the induction of host resistance by *P. citrinum* BTF08 and their efficacy to suppress wilt incidence.

Graphical abstract



Keywords: Biocontrol; Disease incidence; Disease severity; *Fusarium oxysporum* f. sp. *cubense* race 4; Host resistance; *Penicillium citrinum*

Francis W.M.R. Schwarze[a], Frederick Jauss[a], Chris Spencer[b], Craig Hallam[b], Mark Schubert[a]. ([a] EMPA, Swiss Federal Laboratories for Materials Science and Technology, Wood Laboratory, Section Wood Protection and Biotechnology, Lerchenfeldstrasse. 5, CH-9014 St. Gallen, Switzerland, [b] ENSPEC, Unit 2/13 Viewtech Place, Rowville, Victoria 3178, Australia). Evaluation of an antagonistic *Trichoderma* strain for reducing the rate of wood decomposition by the white rot fungus *Phellinus noxius*. Biological Control, Vol.61(2) (2012): 160–168

The objective of these *in vitro* studies was to identify a *Trichoderma* strain that reduces the rate of wood decomposition by the white rot fungus *Phellinus noxius* and *Ganoderma australe*. For this purpose, dual culture and interaction tests in wood blocks of three hardwoods, *Delonix regia*, *Ficus benjamina*, *Jacaranda mimosifolia*, and one softwood, *Araucaria bidwillii*, as well as investigations of fungal growth under different environmental conditions, were performed. The effect of *Trichoderma ghanense*, *two strains of T. harzianum* and *T. reesei* on wood colonization and decomposition by four *P. noxius* strains and *G. australe* were quantitatively analyzed by measuring the dry weight loss of wood. All *Trichoderma* species and wood-decay fungi showed optimum growth at a mean temperature of 25–35 °C and a high water activity (a_w) of 0.998. At 35 °C and a_w 0.928, no growth was recorded for any of the wood-decay fungi after 1 week, whereas most *Trichoderma* species were still actively growing. The different *Trichoderma* species all showed an antagonistic potential against *P. noxius* in the *in vitro* studies. The species of wood-decay fungi showed significant differences in their sensitivity when challenged by the volatile organic compounds (VOCs) of *Trichoderma* species. Reduction in the rate of wood decomposition by different *Trichoderma* species against all wood-decay fungi varied strongly according to the specific plant host. *T. harzianum* 121009 and *T. atroviride* 15603.1 showed the highest reduction in weight losses. *P. noxius* 169 strongly decomposed untreated and pretreated wood of *D. regia*, whereas weight losses of *F. benjamina* and *J. mimosifolia* pretreated with *Trichoderma* strains were significantly lower. Weight losses by *G. australe* were significantly reduced for *A. bidwillii*, *D. regia* and *F. benjamina* by all *Trichoderma* species, but no affect was recorded for *J. mimosifolia*. The *in vitro* studies show that only after careful monitoring (i.e. selecting the appropriate strain for the target pathogen and its niche (wood species) can *Trichoderma* species be used to significantly reduce the growth and rate of wood decomposition by different *P. noxius* strains.

Graphical abstract

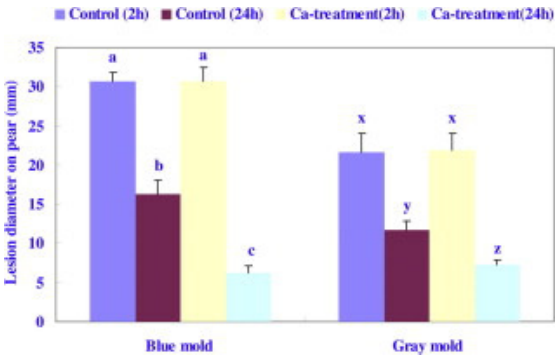
Ficus bejamina infected with *Phellinus noxius*. Dual culture test.

Keywords: Biological control; White rot; *Ganoderma australe*; Dry weight loss; Interaction tests in wood blocks

Ting Yu, Chen Yu, Huangping Lu, Mahbuba Zunun, Fangxia Chen, Tao Zhou, Kuang Sheng, Xiaodong Zheng. (Department of Food Science and Nutrition, Zhejiang University, Hangzhou 310058, People's Republic of China). Effect of *Cryptococcus laurentii* and calcium chloride on control of *Penicillium expansum* and *Botrytis cinerea* infections in pear fruit. Biological Control, Vol.61(2) (2012): 169–175

This study was conducted to evaluate the efficacy of the biocontrol yeast *Cryptococcus laurentii* and calcium chloride ($CaCl_2$) in suppressing the blue and gray mold rots in pear fruit wounds and the possible mechanisms involved. The results from the presented investigation showed that combined treatment of pear fruit wounds with *C. laurentii* and $CaCl_2$ was a much better approach for inhibition of *Penicillium expansum* and *Botrytis cinerea* infections than *C. laurentii* or $CaCl_2$ alone. $CaCl_2$ neither affected the growth of *C. laurentiiin vitro* or *in vivo*, nor directly inhibited the mold rots in pear fruit. However, $CaCl_2$ was shown to elicit the fruit resistance to mold rots when the time interval between $CaCl_2$-treatment and pathogen-inoculation was increased up to 24 h, being associated with an activation of the peroxidase activity of pear fruit. Therefore, it could be proposed that the mechanism by which $CaCl_2$ reinforced the biocontrol efficacy of *C. laurentii* was mainly due to its ability to induce the fruit natural resistance.

Graphical abstract

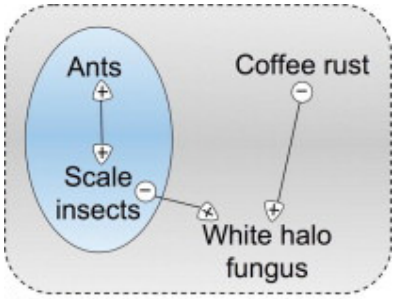


Keywords: *Cryptococcus laurentii*; Calcium chloride ($CaCl_2$); Pear; *Penicillium expansum*; *Botrytis cinerea*; Biocontrol; Postharvest

Doug Jackson[a], Jane Skillman[b], John Vandermeer[a, b]. ([a] Department of Ecology and Evolutionary Biology, 830 N. University, University of Michigan, Ann Arbor, MI 48109, United States, [b] School of Natural Resources and Environment, 440 Church St., University of Michigan, Ann Arbor, MI 48109, United States). Indirect biological control of the coffee leaf rust, *Hemileia vastatrix*, by the entomogenous fungus *Lecanicillium lecanii* in a complex coffee agroecosystem. Biological Control, Vol. 61(1) (2012): 89–97

The entomopathogenic and mycoparasitic fungus *Lecanicillium lecanii* is known to attack both the green coffee scale, *Coccus viridis*, and coffee leaf rust, *Hemileia vastatrix*. Using multi-year surveys of *L. lecanii* and *H. vastatrix* prevalence, we demonstrate a previously unreported, one-year time lag between local epizootics (outbreaks) of *L. lecanii* and significant suppression of *H. vastatrix*. Epizootics of *L. lecanii* are associated with large populations of *C. viridis*, which are in turn associated with colonies of their mutualistic partner, the arboreal-nesting ant *Azteca instabilis*. Therefore, these results suggest that effective conservation biological control of *H. vastatrix* using *L. lecanii* will be enhanced by an understanding of the self-organization process that gives rise to the emergent spatial distribution of the *A. instabilis–C. viridis* mutualism in this complex coffee agroecosystem.

Graphical abstract



Facilitation by a keystone mutualism of coffee rust biocontrol throughout a coffee agroecosystem

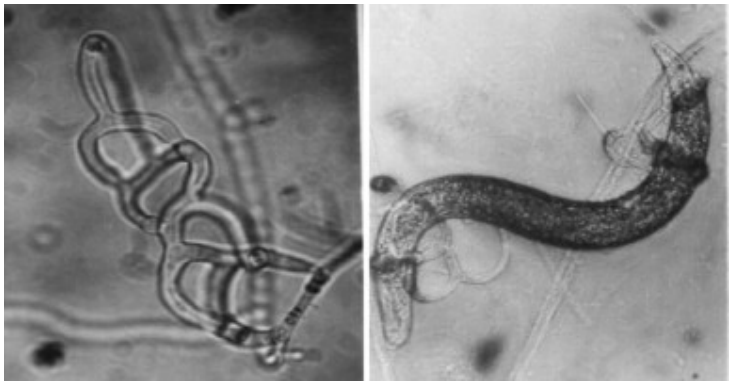
Keywords: Conservation biological control; Entomopathogen; Mycoparasite; *Lecanicillium lecanii*; *Hemileia vastatrix*

Udai B. Singh[a], Asha Sahu[b], R.K. Singh[a], Dhananjaya P. Singh[c], Kamlesh K. Meena[c], J.S. Srivastava[a], Renu[c], M.C. Manna[b]. ([a] Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221 005, India, [b] Division of Soil Biology, Indian Institute of Soil Science, Nabibagh, Berasia Road, Bhopal 462 038, India, [c] National Bureau of Agriculturally Important Microorganisms, Kushmaur, Maunath Bhanjan 275 101, India). Evaluation of biocontrol potential of *Arthrobotrys oligospora* against *Meloidogyne graminicola* and *Rhizoctonia solani* in Rice (*Oryza sativa* L.). Biological Control, Vol. 60(3) (2012): 262–270

The nematode trapping and mycoparasitic potential of *Arthrobotrys oligospora* was tested *in vitro* against *Meloidogyne graminicola* and *Rhizoctonia solani*, respectively. Five isolates of *A.*

oligospora were isolated from different locations of India. Diversity of the trapping structures is large and highly dependent on the environmental condition and nature of the fungus. In *A. oligospora*, a three-dimensional adhesive net (in response to nematode) and hyphal coils developed around the hyphae of *R. solani*. *In vitro* trap formation and predacity were tested against second-stage juveniles of *M. graminicola* (J_2) and the interactions between *A. oligospora* and *R. solani* were recorded. Under field conditions, we demonstrated the biocontrol potential of *A. oligospora* against *R. solani* causing sheath blight of rice (*Oryza sativa*) for the first time. All the isolates of *A. oligospora* parasitized and killed *M. graminicola* and *R. solani.* Application of *A. oligospora*, isolate VNS-1, in soil infested with *M. graminicola* and *R. solani* reduced the number of root knot by 57.58–62.02%, sheath blight incidence by 55.68–59.32% and lesion length by 54.91–66.66% under green house and miniplot (field) conditions. Applications of *A. oligospora* to the soil increased plant growth: shoot length by 56.4–68.8%, root length by 44.0–54.55%, fresh weight of shoot and root by 62.91–65.4% and 38.9–44.19%, respectively, as compared to the plants grown in nematode infested soil.

Graphical abstract



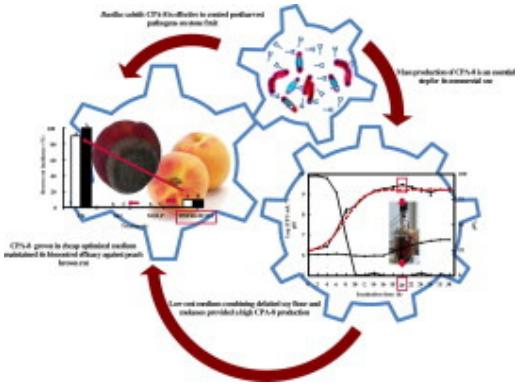
Keywords: Nematode trapping fungi; Mycoparasitism; *Arthrobotrys oligospora*; *Meloidogyne graminicola*; *Rhizoctonia solani*; Root knot

V. Yánez-Mendizábal[a], I. Viñas[b], J. Usall[a], R. Torres[a], C. Solsona[a], N. Teixidó[a]. ([a]IRTA, XaRTA-Postharvest, 191 Rovira Roure, 25198 Lleida, Catalonia, Spain, [b] University of Lleida, XaRTA-Postharvest, 191 Rovira Roure, 25198 Lleida, Catalonia, Spain). **Production of the postharvest biocontrol agent *Bacillus subtilis* CPA-8 using low cost commercial products and by-products. Biological Control, Vol.60(3) (2012): 280–289**

The aim of this research was to identify a low cost medium based on commercial products and by-products that provided maximum *Bacillus subtilis* CPA-8 growth and maintained biocontrol efficacy. Low cost media combining economical nitrogen and carbon sources such as yeast extract, peptone, soy products, sucrose, maltose and molasses were tested. Tests were carried out in 250-ml flasks containing 50 ml of each tested medium. Maximum cell growth ($>3 \times 10^9$ CFU ml^{-1}) was obtained in defatted soy flour 44% combined with sucrose or molasses media. Second, CPA-8 production was scaled up in a 5-l fermenter and CPA-8 population dynamics, pH and oxygen consumption in the optimized medium (defatted soy flour 44% – molasses) was recorded. In these tests, there was a 5-h lag phase before growth, after which

exponential growth occurred and maximum production was 3×10^9 CFU ml^{-1} after 20 h. Fruit trials with cells and cell free supernatants from CPA-8 grown in optimized medium maintained biocontrol efficacy against *Monilinia fructicola* on peaches, resulting in disease reductions up to 95%. CPA-8 populations survived in wounds on inoculated peaches, regardless of the culture media used. The results show that *B. subtilis* CPA-8 can be produced in a low cost medium combining inexpensive nitrogen and carbon sources (40 g l^{-1} defatted soy flour 44%, 5 g l^{-1} molasses plus mineral trace supplements) in shake flasks and a laboratory fermenter (5 l). The results could be used to provide a reliable basis for scaling up the fermentation process to an industrial level.

Graphical abstract



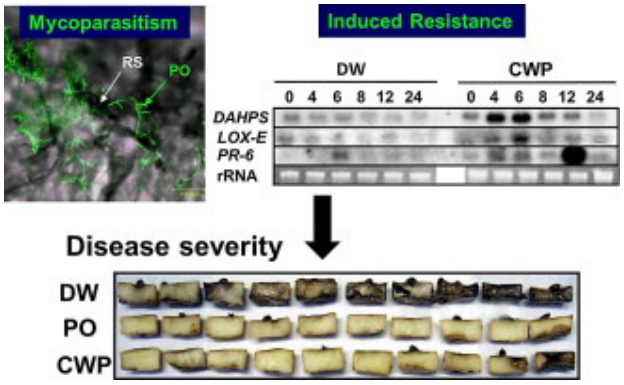
Keywords: Biocontrol agent production; Defatted soy flour; Molasses; Postharvest diseases; Stone fruit; *Monilinia fructicola*

Sachiko Ikeda[a], Ayano Shimizu[b], Motoshige Shimizu[a, 1], Hideki Takahashi[b], Shigehito Takenaka[c]. ([a] Hokkaido, Research Organization, Tokachi Agricultural Experiment Station, Memuro, Hokkaido 082-0081, Japan, [b] Department of Life Science, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan, [c] Memuro Research Station, Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization (NARO), Shinsei, Memuro, Kasaigun, Hokkaido 082-0081, Japan).
Biocontrol of black scurf on potato by seed tuber treatment with *Pythium oligandrum*. Biological Control, Vol.60(3) (2012): 297–304

The biological control activity of *Pythium oligandrum* against black scurf of potato caused by *Rhizoctonia solani* AG-3 was evaluated in field experiments after treatment of potato seed tubers with *P. oligandrum*. Seed tubers infected with black scurf sclerotia were dipped for a few seconds in a suspension of 10^3, 10^4 or 10^5 mL^{-1} *P. oligandrum* oospores and were then air-dried. Each level of *P. oligandrum*-treatment significantly reduced the disease rates of stolon at a level similar to that achieved by chemical control. When *P. oligandrum* populations adherent to the surface of seed tubers were determined, oospore counts on tubers treated with 10^4 or 10^5 oospores mL^{-1} were about 540/cm^2 or about 22,000/cm^2 just after dipping and decreased to about 170/cm^2 or 2900/cm^2 after a 3-week incubation, respectively. Confocal laser scanning microscopic observation with an immuno-enzymatic staining procedure showed that *P. oligandrum* hyphae had colonized the sclerotia and established close contact by coiling around the *R. solani* hyphae present on the surface of seed tubers, in a manner similar to that observed in the dual-culture test. Quantification of *R. solani* DNA by PCR indicated that the *R. solani*

population was reduced on the seed tubers treated with *P. oligandrum* compared to untreated tubers. Furthermore, the ability of *P. oligandrum* to induce resistance against black scurf was determined using a potato tuber disk assay. Treatment of tuber disks with the cell wall protein fraction of *P. oligandrum* enhanced the expression of defense-related genes such as 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase, lipoxygenase and basic *PR-6* genes, and reduced disease severity upon challenge with *R. solani* compared with untreated controls. These results suggest that biocontrol mechanisms employed by *P. oligandrum* against black scurf involve both mycoparasitism and induced resistance.

Graphical abstract



Keywords: Biological control; Black scurf; *Pythium oligandrum*; Colonization; Mycoparasitism; Induced resistance

William G. Meikle, Guy Mercadier, Fatiha Guermache, Marie-Claude Bon. (European Biological Control Laboratory, USDA-ARS, Campus International de Baillarguet, CS 90013 Montferrier sur Lez, 34988 St. Gely du Fesc, France). *Pseudomonas* contamination of a fungus-based biopesticide: Implications for honey bee (Hymenoptera: Apidae) health and Varroa mite (Acari: Varroidae) control. Biological Control, Vol. 60(3) (2012): 312–320

The ectoparasitic mite *Varroa destructor* is a major honey bee pest, and its control using pathogen-based biopesticides would resolve many of the problems, such as contamination and pesticide resistance, experienced with chemical control. A biopesticide, formulated with commercially-prepared conidia of a strain of *Beauveria bassiana* isolated from *V. destructor* was tested against the mites in bee colonies in southern France. The impact of treatment on hive survivorship, weight and mite infestation levels were very different from those of previous experiments using laboratory-prepared conidia: bee hives treated with the biopesticide died at a higher rate, lost more weight, and had higher mite densities at the end of the study than control hives. The biopesticide was subsequently found to be contaminated with bacteria. Two strains of bacteria were identified, by biotyping and sequencing data of the 16S rRNA and *rpoB* regions, and while the strains were distinct both were *Pseudomonas* sp. belonging to the *P. fluorescens* group. In dual cultures *B. bassiana* growth was slowed or suppressed when bacterial cfu density was about equal or greater than that of *B. bassiana*. Experiments using caged adult bees showed that bees ingesting diet and sugar solution treated with *B. bassiana* and kept at 30 °C had significantly lower survival times than those treated with one of the bacterial strains, but the

opposite was true at 33 °C. Because one arthropod (honey bees) was treated for infestation by another (*V. destructor*), the impact of bacterial contamination was likely more noticeable than in most uses of biopesticides, such as treating plants against phytophagous insects. To reduce such risk in biopesticide development, a systematic screening for bacterial contamination prior to field application is recommended.

Graphical abstract

Keywords: *Pseudomonas fluorescens*; *Apis mellifera*; *Varroa destructor*; *Beauveria bassiana*; Biological control; Contamination; 16S rRNA sequencing

Marta Mari, Camilla Martini, Michela Guidarelli, Fiorella Neri. (CRIOF – Diproval, University of Bologna, Via Gandolfi, 19, 40057 Cadriano, Bologna, Italy). Postharvest biocontrol of *Monilinia laxa*, *Monilinia fructicola* and *Monilinia fructigena* on stone fruit by two *Aureobasidium pullulans* strains. Biological Control, Vol. 60(2) (2012): 132–140

The antagonistic effects of yeasts, L1 and L8, isolated from carposphere of 'Redhaven' peaches were tested for the first time in the same experiment against three *Monilinia* species (*Monilinia laxa*, *Monilinia fructicola* and *Monilinia fructigena*) in *in vitro* and *in vivo* trials. The two antagonists were selected after preliminary assays for their ability to reduce brown rot in peaches and nectarines, and both were identified by molecular and morphological tools as *Aureobasidium pullulans*. In *in vivo* trials, neither the autoclaved cells, nor the sterile culture filtrates of either antagonist showed any significant reduction of rot incidence produced by inocula of the three *Monilinia* species, while the washed cells of L1 and L8 completely inhibited *M. laxa* and *M.fructicola* rots and reduced *M. fructigena* infections by 70% and 90%, respectively. In other trials, nectarines treated with antagonist cells and inoculated with the pathogens were stored at 0 °C for 21 days, plus 7 days at 20 °C. The low temperature reduced brown rot development, since all fruit were free from disease symptoms on removal from cold storage. However after 7 d at 20 °C, untreated fruit were rotted over 45% depending on the *Monilinia* species but the antagonists completely inhibited *M. laxa* and *M. fructicola*, while *M. fructigena* infections were reduced by 89.8% and 91.2% by L1 and L8, respectively. For both strains, 10^8 CFU ml^{-1} was the most active concentration, although L1 showed good activity at a concentration of 10^7 CFU ml^{-1}. Isolate L8 at the concentration of 10^7 CFU ml^{-1} was ineffective against *M. fructicola* and *M. fructigena*, showing no difference between treated fruit and the control, excepting the case of nectarines inoculated with *M. laxa*, where L8 at the concentration of 10^7 CFU ml^{-1} reduced the brown rot infections with respect to the control. The increase in population density of *A. pullulans* strains L1 and L8 in the wounds of nectarines stored at 0° or 20 °C was low but sufficient to control brown rot. In conclusion, the present preliminary study identified two antagonistic strains of *A. pullulans* as active ingredients for the development of biofungicides for

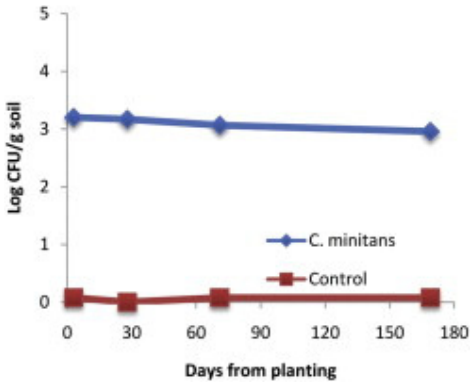
postharvest application against three *Monilinia* species that are responsible for high economic losses in stone fruit crops.

Keywords: Peach; Nectarine; Brown rot; Yeasts; Postharvest disease

Wenting Zeng, William Kirk, Jianjun Hao. (Department of Plant Pathology, Michigan State University, East Lansing, MI 48824, USA). Field management of Sclerotinia stem rot of soybean using biological control agents. Biological Control, Vol. 60(2) (2012): 141–147

Biological control agents (BCAs) were evaluated for their efficacy on reducing the number of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary in the soil and on Sclerotinia stem rot in soybean production systems in Michigan. BCAs included *Coniothyrium minitans* CON/M/91–08 (Product name: Contans®WG), *Streptomyces lydicus* WYEC 108 (Actinovate®AG), *Trichoderma harzianum* T-22 (PlantShield®HC), and *Bacillus subtilis* QST 713 (Serenade®MAX). At two field locations, soil artificially infested with *S. sclerotiorum* sclerotia, was treated by incorporating the above BCAs in the topsoil before planting and boscalid was applied as a foliar fungicide at growth stage R1 as a positive control. *C. minitans* was the most effective BCA and reduced the disease severity index (DSI) by 68.5% and the number of sclerotia of *S. sclerotiorum* in the soil by 95.3%. *S. lydicus* and *T. harzianum* reduced DSI by 43.1% and 38.5% and sclerotia in soil by 90.6% and 70.8%, respectively. *B. subtilis* only had a marginal effect on *S. sclerotiorum*. Populations of *Bacillus*, *Streptomyces*, *Trichoderma* spp., and *C. minitans* collected from soil samples and at 3, 28, 71, and 169 days after BCA application indicated that the population of *Streptomyces*, *Trichoderma* spp., and *C. minitans* did not change significantly throughout the season, which may be the reason for their effectiveness.

Graphical abstract



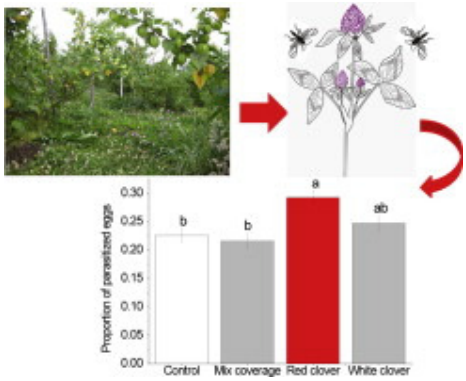
Keywords: *Sclerotinia sclerotiorum*; *Coniothyrium minitans*; *Streptomyces lydicus*; *Trichoderma harzianum*; *Bacillus subtilis*

María Fernanda Díaz[a], Augusto Ramírez[a], Katja Poveda[b]. ([a] Facultad de Agronomía, Universidad Nacional de Colombia, Ciudad Universitaria, Bogotá, Colombia, [b] Entomology, Cornell University, 4142 Comstock Hall, Ithaca, NY 14853, USA). Efficiency of different egg parasitoids and increased floral diversity for the biological control of noctuid pests. Biological Control, Vol. 60(2) (2012): 182–191

Augmentative biological control of insects is a tool of Integrated Pest Management programs. In many agroecosystems, biological control is exercised largely by parasitoids, and it is found that the presence of food resources, as provided by flowering plants, can have a positive effect on survival, search ability and parasitism rate of parasitoid species. In Colombia, a recently established export crop, the cape gooseberry, is under continuous attack by a Lepidopteran species complex in the family Noctuidae. Our first objective was to test the longevity and parasitism rates of three species of egg parasitoids in the genus *Trichogramma* (*Trichogramma atopovirilia* Oatman & Platner, *Trichogramma exiguum* Pinto & Platner, and *Trichogramma pretiosum* Riley) for *Spodoptera frugiperda* Smith and *Copitarsia decolora* Gueené. Our results suggest that *T. atopovirilia* and *T. pretiosum* could be promising parasitoids for the control of *S. frugiperda* and *C. decolora*, with a percentage parasitism of between 30% and 60%, respectively. For our second objective, we selected *T. atopovirilia* as a model species to evaluate the effect of the presence of flowering plants on the longevity and parasitism rate in both, no choice and multiple choice experiments, under laboratory and field conditions. Our results consistently showed that the presence of red clover (*Trifolium pratense* L.) can increase the longevity and parasitism rate of *T. atopovirilia*, suggesting that providing food resources to parasitoids in cape gooseberry fields should be part of a habitat diversification strategy to control noctuid pests.

Graphical abstract

Importance of flowering plants for the parasitism efficiency of *Trichogramma atopovirilia*.



Keywords: Cape gooseberry; Flowering plants; Noctuids pests; Parasitoid wasp; Red clover; *Physalis peruviana*; *Trichogramma atopovirilia*; Conservation biological control

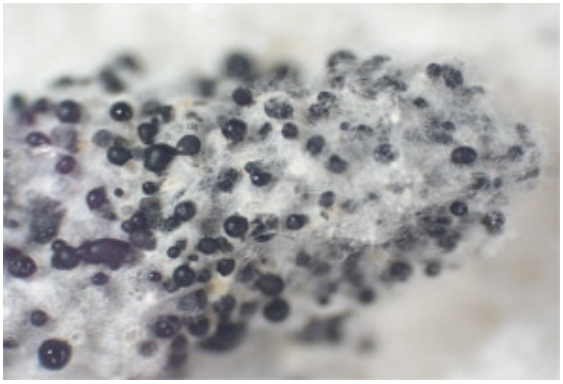
Wenting Zeng, Dechun Wang, William Kirk, Jianjun Hao. (Department of Plant Pathology, 62 Plant Biology Building, Michigan State University, East Lansing, Michigan 48824 USA). Use of *Coniothyrium minitans* and other microorganisms for reducing *Sclerotinia sclerotiorum*. Biological Control, Vol.60(2)(2012): 225–232

Biological control agents (BCAs) *Bacillus subtilis* QST 713, *Coniothyrium minitans* CON/M/91-08, *Streptomyces lydicus* WYEC 108, and *Trichoderma harzianum* T-22 were evaluated for their efficacy in the reduction of survival of sclerotia and production of apothecia of *Sclerotinia sclerotiorum* under controlled environments. A growth chamber assay was conducted where 25 sclerotia were buried in pots containing potting soil, and BCAs were drenched into the soil at various concentrations, and five soybean seeds were planted in each pot. The presence and

number of *S. sclerotiorum* apothecia were recorded daily. *Sclerotinia sclerotiorum* sclerotia were retrieved six weeks after seeding and viability was assessed on water agar plates. All BCAs were effective in reducing *S. sclerotiorum* inoculum at various efficacies. In general, efficacy was positively correlated with the rate of application. At the rate of application when the efficacy did not change significantly by increasing the rate, the BCAs had various reductions of apothecia and sclerotia. *B. subtilis* reduced apothecia and sclerotia by 91.2 and 29.6%, respectively; *C. minitans* reduced apothecia and sclerotia by 81.2 and 50%, respectively; *Streptomyces lydicus* reduced apothecia and sclerotia by 100 and 29.6%, respectively; *Trichoderma harzianum* reduced apothecia and sclerotia by 80.5 and 31.7%, respectively. In addition, the commercial strain of *C. minitans* CON/M/91-08, and a wild Michigan strain of *C. minitans* W09 were compared for their growth and sclerotial reduction. W09 had faster growth rate than the commercial strain, indicating potential diversities of biological control strains to be studied.

Graphical abstract

Colonization of *Sclerotinia sclerotiorum* sclerotia by *Coniothyrium minitans* strain W09. Pycnidia (black spherical bodies) and conidia of *C. minitans* oozing out of sclerotial surface (arrows) of *Sclerotinia sclerotiorum* under a dissecting microscope (25 × magnification).



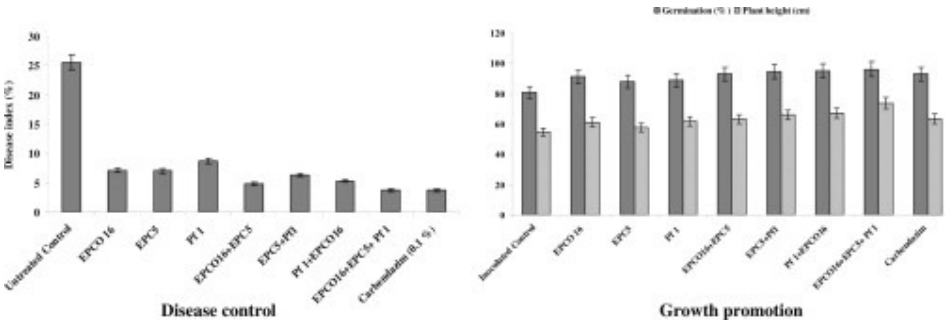
Keywords: *Sclerotinia sclerotiorum*; *Coniothyrium minitans*; *Trichoderma harzianum*; *Bacillus subtilis*; *Streptomyces lydicus*; biological control

S. Sundaramoorthy, T. Raguchander, N. Ragupathi, R. Samiyappan. (Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India). Combinatorial effect of endophytic and plant growth promoting rhizobacteria against wilt disease of *Capsicum annum* L. caused by *Fusarium solani*. Biological Control, Vol.60(1) (2012): 59–67

Combination of biocontrol agents that are compatible with each other is a strategic approach to control the plant disease and pest. The present study was designed to evaluate the protective effects of compatible endophytic bacterial strains (*Bacillus subtilis*; EPCO16 and EPC5) and rhizobacterial strain (*Pseudomonas fluorescens*; Pf1) against chilli wilt disease caused by *Fusarium solani*. Our results showed that *B. subtilis* (EPCO16 and EPC5) and *P. fluorescens* (Pf1) were compatible and effectively inhibited the growth of the *F. solani*. The application of endophytic and rhizobacterial strains, singly and in combination in green house and field

conditions were found to be effective in controlling the chilli *Fusarium* wilt disease by inducing systemic resistance (ISR) as evidenced by enhanced activities of PO, PPO, PAL, β-1,3-glucanase, Chitinase and Phenolic involved in the synthesis of phytolaexins thereby promoting the growth of plants. However, combinations of EPCO16 + EPC5 + Pf1 bacterial strains were more effective than single agents. These findings suggest that synergistic interactions of biocontrol agents may be responsible for the management of chilli wilt disease caused by *F. solani*.

Graphical abstract



Keywords: Biocontrol agents; *Bacillus subtilis*; *Fusarium solani*; ISR; *Pseudomonas fluorescens* consecutively

Ted D. Center, Matthew F. Purcell, Paul D. Pratt, Min B. Rayamajhi and Philip W. Tipping, et al. Biological control of *Melaleuca quinquenervia*: an Everglades invader. BioControl, Vol. 57(2) (2012): 151-165

A massive effort is underway to restore the Florida Everglades, mainly by re-engineering hydrology to supply more water to the system at appropriate times of the year. However, correcting water flow patterns alone will not restore the associated plant communities due to habitat-transforming effects of invasive species, in particular the Australian wetland tree *Melaleuca quinquenervia* (Cav.) S. T. Blake (Myrtales, Myrtaceae), which has invaded vast areas and transformed sawgrass marshes into dense, biologically impoverished, structurally altered forest habitats. To address this threat, an invasive species reduction program was launched that combined mechanical removal and herbicidal control to remove mature trees with the release of specialized insects to suppress seed production and lower seedling survival. Melaleuca has now been removed from most public lands while biological control has limited its ability to regenerate and reinvade from nearby infestations often located on unmanaged privately held lands. This case illustrates how restoration of highly modified ecosystems may require both restoration of physical conditions (water flow), and suppression of high impact or transformative invaders, showing well the need to integrate biological control into conservation biology.

Keywords: Wetlands – Weed biological control – Ecosystem restoration – Transformer species – Herbivory – Florida

J. Hough-Goldstein, E. Lake and R. Reardon. Status of an ongoing biological control program for the invasive vine, *Persicaria perfoliata* in eastern North America. BioControl, Vol. 57(2) (2012): 181-189

Mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross (Polygonaceae), an aggressive annual vine native to Asia, has invaded forest edges, light gaps, open fields, and riparian borders in eastern North America. It was accidentally introduced into Pennsylvania in the 1930s and has since expanded its range north to Massachusetts, south to North Carolina, and west to Ohio. A biological control program was initiated in 1996, and in 2004, a permit was issued for release of *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), a host-specific weevil initially collected in China. Since 2004, the biology of the weevil in its introduced range has been studied, along with its impact on *P. perfoliata*, which can be substantial. Weevils have been released in ten states through 2010, and populations have increased considerably at many sites. Although *P. perfoliata* continues to expand its North American range, natural and human-assisted dispersal of *R. latipes* is reducing its negative effects. Here we review and assess the current status of the biological control program.

Keywords: Weed biocontrol – Polygonaceae – Caryophyllales – Coleoptera – Curculionidae

Andrew Polaszek, Paul F. Rugman-Jones, Richard Stouthamer, Estrella Hernandez-Suarez, Tomás Cabello and Modesto del Pino Pérez. Molecular and morphological diagnoses of five species of *Trichogramma*: biological control agents of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) and *Tuta absoluta* (Lepidoptera: Gelechiidae) in the Canary Islands. BioControl, Vol. 57(1) (2012): 21-35

Prospecting for potential natural enemies of the invasive lepidopteran tomato pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and the banana pest *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) on the Canary Islands archipelago, where no *Trichogramma* species were previously recorded, has led to the discovery of five distinct species. *T. achaeae* Nagaraja & Nagarkatti, *T. bourarachae* Pintureau & Babault, *T. euproctidis* (Girault) and *T. evanescens* Westwood are relatively widespread species. The fifth is close to *T. brassicae* Bezdenko, but differs sufficiently in the sequence of the ITS2 region of ribosomal RNA to warrant further investigation as a species probably new to science. Each species is treated in detail in order to facilitate identification in future using molecular and/or morphological characters, or a combination of both. All species are newly recorded for the Canary Islands, and the distribution of each within the islands and elsewhere is provided. Known host records are given within the Canary Islands and elsewhere. The most common species found, *T. achaeae*, is already being used in biological control programmes against *T. absoluta* in mainland Spain and field trials are ongoing to evaluate its effectiveness as a biological control agent of *C. chalcites* in banana crops.

Keywords: *Trichogramma achaeae – T. bourarachae – T. evanescens – T. euproctidis – Tuta absoluta – Chrysodeixis chalcites – Musa acuminata – Solanum lycopersicum* – Egg parasitoid – Ooparasitoid – Invasive pest – Canary Islands

Justin L. Reeves and Patrick D. Lorch. Biological control of invasive aquatic and wetland plants by arthropods: a meta-analysis of data from the last three decades. BioControl, Vol. 57(1) (2012): 103-116

Results from the last three decades of aquatic and wetland plant biological control by arthropods were combined using meta-analytical techniques to provide an objective, quantitative understanding of control efficacy that cannot be provided by available narrative reviews.

Analyses were performed to determine if differences exist in how well diverse biocontrol agents perform, and if experimental design can impact study results. Across all analyses, biocontrol of the included plants was generally successful. Though little heterogeneity in efficacy was seen between the individual biocontrol agents used, all experimental design analyses showed significant differences between respective study types. Thus, study design can significantly impact the results of biocontrol studies. From these results, we suggest that field studies with controls be performed using subsamples of an area (quadrats, transects, etc.), with biomass or density being the plant variable measured. More long-term (two or more years) and non-target effect studies could also be performed in the future.

Keywords: Meta-analysis – Biological control – Aquatic – Wetland – Plant – Insect

Mark A. Weaver, C. Douglas Boyette and Robert E. Hoagland. Bioherbicidal activity from washed spores of *Myrothecium verrucaria*. World Journal of Microbiology and Biotechnology, Vol. 28(5) (2012): 1941-1946

The fungal plant pathogen, *Myrothecium verrucaria,* is highly virulent to several important weed species and has potential utility as a bioherbicide. However the production of macrocyclic trichothecene mycotoxins by this fungus presents significant safety concerns. It was discovered that trichothecenes are removed from *M. verrucaria* spores by repeated washes with water. These washed spores retained bioherbicidal efficacy against kudzu when tested in field trials and on sicklepod when tested under greenhouse conditions. Changes in the growth medium combined with washing spores with water resulted in greater than 95% reduction in roridin A and verrucarin A. Washing spores reduced trichothecene concentrations in spore preparations with no significant effect on plant biomass reduction, thus demonstrating the possibility of *M. verrucaria* formulations with improved safety to researchers, producers and applicators.

Keywords: Bioherbicide – Sicklepod – *Cassia obtusifolia* – kudzu – *Pueraria lobata* – Roridin – Verrucarin

Biodegradation

Y.H. Chen[1], L.Y. Chai[1], Y.H. Zhu[2], Z.H. Yang[1], Y. Zheng[1], H. Zhang[1]. Biodegradation of kraft lignin by a bacterial strain *Comamonas* sp. B-9 isolated from eroded bamboo slips. Journal of Applied Microbiology, Vol. 112(5) (2012): 900–906

The aim was to obtain evidences for lignin degradation by unicellular bacterium *Comamonas* sp. B-9. *Comamonas* sp. B-9 was inoculated into kraft lignin-mineral salt medium (KL-MSM) at pH 7·0 and 30°C for 7 days of incubation. The bacterial growth, chemical oxygen demand (COD) reduction, secretion of ligninolytic enzymes and productions of low-molecular-weight compounds revealed that *Comamonas* sp. B-9 was able to degrade kraft lignin (KL). COD in KL-MSM reduced by 32% after 7 days of incubation. The maximum activities of manganese peroxidase (MnP) of 2903·2 U l^{-1} and laccase (Lac) of 1250 U l^{-1} were observed at 4th and 6th day, respectively. The low-molecular-weight compounds such as ethanediol, 3, 5-dimethyl-benzaldehyde and phenethyl alcohol were formed in the degradation of KL by *Comamonas* sp. B-9 based on GC-MS analysis.

This study confirmed that *Comamonas* sp. B-9 could utilize KL as a sole carbon source and degrade KL to low-molecular-weight compounds. *Comamonas* sp. B-9 may be useful in the utilization and bioconversion of lignin and lignin-derived aromatic compounds in biotechnological applications. Meanwhile, using *Comamonas* sp. B-9 in treatment of wastewater in pulp and paper industry is a meaningful work.

Keywords: *Comamonas* sp. B-9; kraft lignin; lignin degradation; lignin-degrading bacteria; ligninolytic enzymes activity

Sengadir Chitra, Kanapathy Paramasivan, Mayilsamy Cheralathan and Pradeep Kumar Sinha. Degradation of 1,4-dioxane using advanced oxidation processes. Environmental Science and Pollution Research, Vol. 19(3) (2012): 871-878

In the nuclear industry 1,4-dioxane is used as a solvent in liquid scintillation technique for measuring low-energy beta-emitters such as ^{3}H or C^{14} in aqueous media. Improper disposal of 1,4-dioxane can contaminate the ground and surface waters. Conventional wastewater treatment processes like chemical treatment, air stripping, carbon adsorption, and biological treatment are ineffective for the degradation of 1,4-dioxane.

In the present study, the kinetics of degradation of 1,4-dioxane using advanced oxidation processes viz., H_2O_2 alone, Fe(II)+H_2O_2, UV (15 W)+H_2O_2, UV (15 W)+Fe(II)+H_2O_2, US (130 KHz)+Fe(II) +H_2O_2, and sunlight+Fe(II)+H_2O_2 at pH 3.0 was investigated. The optimization of Fe (II) for the processes using Fe (II)+H_2O_2 was carried out.

The kinetics of degradation using sunlight+Fe (II)+H_2O_2 was found to be fastest when compared to the other processes. The degradation was found to follow first-order kinetics. Formation of acidic intermediates was suspected from the observed pH changes during the degradation processes.

Keywords: Fenton's reagent – Photochemical – Sonochemical – 1,4-Dioxane – Advanced oxidation process – Sunlight-Fenton

James Kanagaraj and Asit Baran Mandal. Combined biodegradation and ozonation for removal of tannins and dyes for the reduction of pollution loads. Environmental Science and Pollution Research, Vol.19(1) (2012): 42-52

Tannins and dyes pose major threat to the environment by generating huge pollution problem. Biodegradation of wattle extract, chrome tannin and dye compounds using suitable fungal culture namely *Aspergillus niger*, *Penicillium* sp. were carried out. In addition to these, ozone treatment was carried out to get higher degradation rate.

The results were monitored by carrying out chemical oxygen demand (COD), total organic carbon (TOC), and UV–Vis analysis. The results showed that wattle extract (vegetable tannin) gave better biodegradation rate than dye and chromium compounds. Biodegradation plus ozone showed degradation rates of 92–95%, 94–95%, and 85–87% for the wattle extract, dyes, chromium compounds, respectively. UV–Vis showed that there were no peaks observed for biodegraded samples indicating better degradation rates as compared to the control samples. FT-

IR spectra analysis suggested that the formation of flavanoid derivatives, chromic oxide and NH_2 compounds during degradation of wattle extract, chromium and dye compounds, respectively, at the peaks of 1,601–1,629 cm^{-1}, 1,647 cm^{-1}, and 1,610–1,680 cm^{-1}.

The present investigation shows that combination of biodegradation with ozone is the effective method for the removal of dyes and tannins. The biodegradation of the said compounds in combination with ozonation showed better rate of degradation than by chemical methods. The combination of biodegradation with ozone helps to reduce pollution problems in terms of COD, TOC, total dissolved solids and total suspended solids.

Keywords: Chromium – Wattle extract – Dye degradation with *Aspergillus niger* and *Penicillium* sp. – Ozone treatment – Pollution reduction

Marlies Bergheim, Reto Gieré and Klaus Kümmerer. Biodegradability and ecotoxicitiy of tramadol, ranitidine, and their photoderivatives in the aquatic environment. Environmental Science and Pollution Research, Vol. 19(1) (2012): 72-85

This study was designed to assess the fate and the overall potential impacts of the widely prescribed drugs ranitidine and tramadol after their introduction into the aquatic environment.

The probability to detect these two drugs in the aquatic environment was studied by analyzing their abiotic and biotic degradation properties. For this purpose, samples were irradiated with different light sources, and three widely used biodegradability tests from the OECD series, the closed bottle test (OECD 301 D), the manometric respirometry test (OECD 301 F) and the Zahn–Wellens test (OECD 302 B), were conducted. The ecotoxicity of the photolytically formed transformation products was assessed by performing the bacterial growth inhibition test (EN ISO 10712). Furthermore, quantitative structure–activity relationship analysis and a risk analysis based on the calculation of the predicted environmental concentrations have also been conducted to assess the environmental risk potential of the transformation products. The possible formation of stable products by microbial or photolytical transformation has been investigated with DOC and LC-MS analytics.

In the present study, neither ranitidine, nor tramadol, nor their photoderivatives were found to be readily or inherently biodegradable according to test guidelines. The photolytic transformation was faster under a UV lamp compared to the reaction under an Xe lamp with a spectrum that mimics sunlight. No chronic toxicity against bacteria was found for ranitidine or its photolytic decomposition products, but a low toxicity was detected for the resulting mixture of the photolytic transformation products of tramadol.

The study demonstrates that transformation products may have a higher environmental risk potential than the respective parent compounds.

Keywords : Aquatic environment – Degradation – Irradiation – Transformation – Ecotoxicology – Ranitidine – Tramadol

M. Nazaré P. F. S. Couto, M. Clara P. Basto and M. Teresa S. D. Vasconcelos. Suitability of *Scirpus maritimus* for petroleum hydrocarbons remediation in a refinery environment. Environmental Science and Pollution Research, Vol.19(1) (2012): 86-95

In the ambit of a project searching for appropriate biological approaches for recovering a refinery soil contaminated with petroleum hydrocarbons (PHC), we compared results obtained in the absence and in the presence of the salt marsh plant *Scirpus maritimus* or *Juncus maritimus* or an association of these two plants, which were tested in the refinery environment. Synergistic effects caused by addition of a non-ionic surfactant and/or a bioaugmentation product were also investigated. Major challenges of this study were: field conditions and weathered contamination.

Transplants of the plants were carried out in individual containers filled with a weathered contaminated soil, which was recontaminated with turbine oil with two purposes: for increasing PHC level and allowing a comparison of the potential of plants for remediation of ancient and recent contamination.

Analysis of total PHC led to the conclusion that, after 24-month exposure, neither *J. maritimus* nor the association caused any improvement in remediation. In contrast, *S. maritimus* revealed potential for PHC remediation, favoring degradation of both recent and older contamination (which was refractory to natural attenuation). About 15% of remediation improvement was found in the soil layer with higher root density (5–10 cm). A more marked improvement in that layer (28%) was observed when non-ionic surfactant amendment and bioaugmentation were used jointly.

The fact that *S. maritimus* has demonstrated capability for PHC remediation, leads to admit that it has potential to be also used for recovering sediments that have suffered accidental oil spills.

Keywords: *Scirpus maritimus* – Petroleum hydrocarbons – Refinery soil – Remediation

Jin Anotai, Pumis Thuptimdang, Chia-Chi Su and Ming-Chun Lu. Degradation of *o*-toluidine by fluidized-bed Fenton process: statistical and kinetic study. Environmental Science and Pollution Research, Vol. 19(1) (2012): 169-176

The optimal conditions of *o*-toluidine degradation by fluidized-bed Fenton process were determined using Box–Behnken designs (BBD). The BBD can be used to find the optimal conditions in multivariable systems. The optimal conditions obtained by the design were further applied in the kinetic analysis of *o*-toluidine oxidation in fluidized-bed Fenton process.

The 1.35-L fluidized-bed reactor used in all experiments was a cylindrical vessel with an inlet, outlet, and recirculation pump. The *o*-toluidine was determined by high-performance liquid chromatography.

Analytical results indicated that pH, Fe^{2+}, and H_2O_2 were significant factors in *o*-toluidine and chemical oxygen demand (COD) removal, but loading carrier was not. The pH significantly affected not only *o*-toluidine degradation, but also total iron removal. The predicted conditions for optimal removal of 1 mM of *o*-toluidine using 100 g of carriers were pH 3±0.5, 1 mM of Fe^{2+}, and 17 mM of H_2O_2. Removal of *o*-toluidine and COD in the actual experiment was higher than predicted, whereas removal of total iron was slightly lower. The kinetic study showed that the initial rate and rate constant (k) of *o*-toluidine degradation in the fluidized-bed Fenton process correlated Fe^{2+} concentration. In the Fe^{2+}/H_2O_2 stage, high concentration of H_2O_2 produced a scavenging effect.

The predicted removal efficiencies of *o*-toluidine and COD were 90.2% and 41.4%, respectively. Moreover, the removals of *o*-toluidine and COD in the actual experiment were 99.8% and 61.8%, respectively.

Keywords: Advanced oxidation process – Fluidized-bed Fenton – *o*-Toluidine – Box–Behnken design – Kinetics – Degradation

Aviraj Datta and Ligy Philip. Biodegradation of Volatile Organic Compounds from Paint Industries. Applied Biochemistry and Biotechnology, Vol. 167 (3) (2012): 564-580

Methyl ethyl ketone (MEK) and methyl iso-butyl ketone (MIBK) constitute significant proportion of the total VOC emissions from manufacturing and application processes of surface coatings. Biodegradation of MEK and MIBK using an acclimatized mixed culture was evaluated, under aerobic condition. Biodegradation studies were carried out using MEK and MIBK as single substrates and in combination. Mixed-pollutant studies were conducted in MEK-dominated system, MIBK-dominated system, and MEK–MIBK equi-concentration systems to understand the concentration-dependent interaction of these compounds in a biosystem. Experimental data obtained from single-pollutant system was used to estimate the biokinetic parameters, viz. μ_{max}, K_s, K_i, and Y_T, for these compounds. Among the several bio-kinetic models tested, Monod inhibition model was best suited for predicting the biodegradation of these two VOCs. Four multiple-substrate models, viz. no-interaction, competitive, un-competitive, and non-competitive were used to study the nature of inhibition for different combinations of these compounds. The biodegradation of MEK and MIBK mixtures was found to be best described by competitive inhibition model. However, the predictions were not very good for systems where MEK concentration was higher than MIBK concentration.

Keywords: Bio-degradation – Inhibition – Rate kinetics – Acclimatized mixed culture – Methyl ethyl ketone – Methyl iso-butyl ketone

Y. S. Salim, A. Sharon, S. Vigneswari, M. N. Mohamad Ibrahim and A. A. Amirul. Environmental Degradation of Microbial Polyhydroxyalkanoates and Oil Palm-Based Composites. Applied Biochemistry and Biotechnology, Vol. 167(2) (2012): 314-326

This paper investigates the degradation of polyhydroxyalkanoates and its biofiber composites in both soil and lake environment. Time-dependent changes in the weight loss of films were monitored. The rate of degradation of poly(3-hydroxybutyrate) [P(3HB)], poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-23 mol% 4HB)] and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-4-hydroxybutyrate) [P(3HB-*co*-9 mol% 3HV-*co*-19 mol% 4HB)] were investigated. The rate of degradation in the lake is higher compared to that in the soil. The highest rate of degradation in lake environment (15.6 % *w*/*w* $week^{-1}$) was observed with P(3HB-*co*-3HV-*co*-4HB) terpolymer. Additionally, the rate of degradation of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-38 mol% 3HV)] was compared to PHBV biofiber composites containing compatibilizers and empty fruit bunch (EFB). Here, composites with 30 % EFB displayed the highest rate of degradation both in the lake (25.6 % *w*/*w* $week^{-1}$) and soil (15.6 % *w*/*w* $week^{-1}$) environment.

Keywords: Polyhydroxyalkanoates (PHAs) – Biodegradation – Biocomposites – Environment

Venkata Nancharaiah Yarlagadda, Renu Kadali, Naresh Sharma, Raju Sekar and Venugopalan Vayalam Purath. Rapid Establishment of *p*-Nitrophenol Biodegradation in Acetate-Fed Aerobic Granular Sludge. Applied Biochemistry and Biotechnology, Volume 166(5) (2012): 1225-1235

The aim of the study was to investigate the acclimation of precultivated acetate-fed aerobic granular sludge to a toxic xenobiotic biodegradation. Establishment of *p*-nitrophenol (PNP) biodegradation in acetate-fed aerobic granular sludge and concomitant changes in the microstructure and bacterial community were determined. Rapid establishment of PNP utilization was observed in the granular sludge when fed with PNP as the sole carbon source. The specific PNP removal was 36-mg h^{-1} g^{-1} granular biomass at an initial PNP concentration of 50 mg L^{-1}. The presence of PNP resulted in significant membrane damage in a subpopulation of the bacterial consortium, as shown by *Bac*Light viability staining. This was coincided with a significant decrease in the culturable bacterial diversity of the granular biomass. PCR-DGGE analysis revealed a shift and decrease in number of bands during the establishment of PNP biodegradation. Scanning electron microscopy showed the dominance of rod-shaped bacteria in the PNP-utilizing microbial granules. Our results suggest that acetate-fed granular sludge could be quickly adapted for PNP biodegradation.

Keywords: Aerobic granules – Aerobic granular sludge – Biodegradation – *p*-Nitrophenol

Yong Jia, Jingjing Du, Fuqiang Song, Guiying Zhao and Xingjun Tian. A Fungus Capable of Degrading Microcystin-LR in the Algal Culture of *Microcystis aeruginosa* PCC7806. Applied Biochemistry and Biotechnology, Vol. 166(4) (2012): 987-996

Microcystins (MCs) are a family of natural toxins produced by cyanobacteria (blue-green algae). Microbial degradation is considered an efficient method for eliminating cyanobacteria and MCs in environmental conditions. This study examines the ability of *Trichaptum abietinum* 1302BG, a white rot fungus, to degrade microcystin-LR in the harmful algal culture of *Microcystis aeruginosa* PCC7806. Results showed that microcystin-LR could not be detected by high-performance liquid chromatography after 12 h in algal culture incubated with the fungus. There were also high activities of catalase and peroxidase in algal culture incubated with the fungus. However, similar to the control, they decreased to normal levels after 72 h. Meanwhile, the micronucleus test in the toxicity studies revealed that the degraded algal culture had low toxicity.

Keywords: Cyanobacteria – *Trichaptum abietinum* 1302BG – Microcystin-LR – Micronucleus

Jijian Yang, Ruihua Liu, Wenli Song, Yao Yang, Feng Cui and Chuanling Qiao. Construction of a Genetically Engineered Microorganism that Simultaneously Degrades Organochlorine and Organophosphate Pesticides. Applied Biochemistry and Biotechnology, Vol. 166(3) (2012): 590-598

Field contamination with pesticide mixtures of organophosphates (OPs) and organochlorines (OCs) is becoming global issues to be solved urgently. The strategy of utilizing engineered microorganisms that have an ability to simultaneously degrade OPs and OCs has increasingly received great interest. In this work, an OP degradation gene (*mpd*) and an OC degradation gene (*linA*) were simultaneously introduced into *Escherichia coli* by using two compatible plasmids,

resulting in strains with both OP degradation and OC degradation capabilities. To overcome the potential substrate uptake limitation, MPH was displayed on the cell surface of *Escherichia coli* using the N- and C-terminal domains of ice nucleation protein (INPNC) as an anchoring motif. The surface localization of INPNC–MPH was verified by cell fractionation, Western blot, proteinase accessibility, and immunofluorescence microscopy. Furthermore, both LinA and green fluorescent protein (GFP) were functionally co-expressed in the MPH-displaying *Escherichia coli.* The engineered *Escherichia coli* degraded OPs as well as OCs rapidly, and it can be easily monitored by GFP fluorescence.

Keywords: Organophosphate – Organochlorine – Co-contamination – Green fluorescent protein – Biodegradation

Kazuhiro Takagi, Kunihiko Fujii, Ken-ichi Yamazaki, Naoki Harada and Akio Iwasaki. Biodegradation of melamine and its hydroxy derivatives by a bacterial consortium containing a novel *Nocardioides* species. Applied Microbiology and Biotechnology, Vol.9 (6) (2012): 1647-1656

Melamine has recently been recognized as a food contaminant with adverse human health effects. Melamine contamination in some crops arises from soil and water pollution from various causes. To remove melamine from the polluted environment, a novel bacterium, *Nocardioides* sp. strain ATD6, capable of degrading melamine was enriched and isolated from a paddy soil sample. The enrichment culture was performed by the soil–charcoal perfusion method in the presence of triazine-degrading bacteria previously obtained. Strain ATD6 degraded melamine and accumulated cyanuric acid and ammonium, via the intermediates ammeline and ammelide. No gene known to encode for triazine-degrading enzymes was detected in strain ATD6. A mixed culture of strain ATD6 and a simazine-degrading *Methyloversatilis* sp. strain CDB21 completely degraded melamine, but the degradation rate of cyanuric acid was slow. The degradation of melamine and its catabolites by the mixed culture was greatly enhanced by including *Bradyrhizobium japonicum* strain CSB1 in the inoculum and adding ethanol to the culture medium. The melamine-degrading consortium consisting of strains ATD6, CDB21, and CSB1 appears to be potentially safer than other known melamine-degrading bacteria for the bioremediation of farmland and other contaminated sites, as no known pathogens were included in the consortium.

Keywords: Melamine – Cyanuric acid – Biodegradation – *Nocardioides sp.* – Bacterial consortium

Won-Jae Chi, Yong-Keun Chang and Soon-Kwang Hong. Agar degradation by microorganisms and agar-degrading enzymes. Applied Microbiology and Biotechnology, Vol. 94(4) (2012): 917-930

Agar is a mixture of heterogeneous galactans, mainly composed of 3,6-anhydro-L-galactoses (or L-galactose-6-sulfates) D-galactoses and L-galactoses (routinely in the forms of 3,6-anhydro-L-galactoses or L-galactose-6-sulfates) alternately linked by β-(1,4) and α-(1,3) linkages. It is a major component of the cell walls of red algae and has been used in a variety of laboratory and industrial applications, owing to its jellifying properties. Many microorganisms that can hydrolyze and metabolize agar as a carbon and energy source have been identified in seawater and marine sediments. Agarolytic microorganisms commonly produce agarases, which catalyze the hydrolysis of agar. Numerous agarases have been identified in microorganisms of various

genera. They are classified according to their cleavage pattern into three types—α-agarase, β-agarase, and β-porphyranase. Although, in a broad sense, many other agarases are involved in complete hydrolysis of agar, most of those identified are β-agarases. In this article we review agarolytic microorganisms and their agar-hydrolyzing systems, covering β-agarases as well as α-agarases, α-neoagarobiose hydrolases, and β-porphyranases, with emphasis on the recent discoveries. We also present an overview of the biochemical and structural characteristics of the various types of agarases. Further, we summarize and compare the agar-hydrolyzing systems of two specific microorganisms: Gram-negative *Saccharophagus degradans* 2–40 and Gram-positive *Streptomyces coelicolor* A3(2). We conclude with a brief discussion of the importance of agarases and their possible future application in producing oligosaccharides with various nutraceutical activities and in sustainably generating stock chemicals for biorefinement and bioenergy.

Keywords: Agar – Agarose – Porphyran – Agarase – Porphyranase – Agar degradation

Ning Yan, Siqing Xia, Linke Xu, Jun Zhu, Yongming Zhang and Bruce E. Rittmann. Internal loop photobiodegradation reactor (ILPBR) for accelerated degradation of sulfamethoxazole (SMX). Applied Microbiology and Biotechnology, Vol.94(2) (2012): 527-535

The internal loop photobiodegradation reactor (ILPBR) was evaluated for the degradation of the pharmaceutical sulfamethoxazole (SMX) using batch experiments following three protocols: photolysis alone (P), biodegradation alone (B), and intimately coupled photolysis and biodegradation (P&B). SMX was removed more rapidly by P&B than by either P or B alone, and the corresponding dissolved organic carbon (DOC) removals by P&B also were higher. The faster SMX removal probably was due to a synergy between photolysis and the rapid biodegradation of SMX by the biofilm. The greater DOC removal was brought about by the presence of biofilm bacteria able to biodegrade photolysis products. Ammonium N released during photolysis of SMX gave more evidence for the formation of intermediates and was enough in P&B experiments to support bioactivity when no other N was supplied. Clone libraries performed on the biofilms before and after the P&B experiments showed profound changes in the microbial community. Whereas *Rhodopirellula baltica* and *Methylibium petroleiphilum* PM1 dominated the biofilm after the B experiments, they were replaced by *Micrococcus luteus*, *Delftia acidovorans*, and *Oligotropha carboxidovorans* after the P&B experiments. The changes in microbial community structure mirrored the change in function in the P&B experiments: SMX biodegradation (presumably the roles of *R. baltica* and *M. petroleiphilum*) was out-competed by SMX photolysis, but biodegradation of photolysis products (most likely by *M. luteus* and *D. acidovorans*) became important. The higher removal rates of SMX and DOC, as well as the changes in microbial community structure, confirm the value of intimately coupling photolysis with biodegradation in the ILPBR.

Keywords: Biodegradation – Biofilm – Microbial community structure – Photolysis – Sulfamethoxazole

Pankaj Kumar Arora, Ch. Sasikala and Ch. Venkata Ramana. Degradation of chlorinated nitroaromatic compounds. Applied Microbiology and Biotechnology, Vol. 93(6) (2012): 2265-2277

Chlorinated nitroaromatic compounds (CNAs) are persistent environmental pollutants that have been introduced into the environment due to the anthropogenic activities. Bacteria that utilize CNAs as the sole sources of carbon and energy have been isolated from different contaminated and non-contaminated sites. Microbial metabolism of CNAs has been studied, and several metabolic pathways for degradation of CNAs have been proposed. Detoxification and biotransformation of CNAs have also been studied in various fungi, actinomycetes and bacteria. Several physicochemical methods have been used for treatment of wastewater containing CNAs; however, these methods are not suitable for in situ bioremediation. This review describes the current scenario of the degradation of CNAs.

Keywords: Chloronitrophenol – Chloronitrobenzene – Biodegradation – Microbial metabolism

Ignacio Poblete-Castro, Judith Becker, Katrin Dohnt, Vitor Martins dos Santos and Christoph Wittmann. Industrial biotechnology of *Pseudomonas putida* and related species. Applied Microbiology and Biotechnology, Vol. 93(6) (2012): 2279-2290

Since their discovery many decades ago, *Pseudomonas putida* and related subspecies have been intensively studied with regard to their potential application in industrial biotechnology. Today, these Gram-negative soil bacteria, traditionally known as well-performing xenobiotic degraders, are becoming efficient cell factories for various products of industrial relevance including a full range of unnatural chemicals. This development is strongly driven by systems biotechnology, integrating systems metabolic engineering approaches with novel concepts from bioprocess engineering, including novel reactor designs and renewable feedstocks.

Keywords: *Pseudomonas putida* – Cell factory – Bio-catalysis – Biofilm – Systems metabolic engineering – Synthetic biology – Bioeconomy

R. W. Nicol, K. Marchand and W. D. Lubitz. Bioconversion of crude glycerol by fungi. Applied Microbiology and Biotechnology, Vol. 93(5) (2012): 1865-1875

The production of synthetic glycerol from petrochemical feedstocks has been decreasing in recent years. This is largely due to increasing supplies of crude glycerol derived as a co-product from the oleochemical industry, especially biodiesel production. The price of glycerol is at historic lows, and the supply of crude glycerol is projected to grow faster than its industrial uses. This oversupply is driving the transition from glycerol as a product to glycerol as a precursor for new industrial applications, including its use as a substrate for bioconversion. This article reviews the use of fungi for the bioconversion of crude glycerol to the value-added products 1,2-propanediol, ethanol, single cell oil, specialty polyunsaturated fatty acids, biosurfactants, and organic acids. Information on the impurities of crude glycerol from different industrial processes is also included.

Keywords: Fungi – Crude glycerol – Single cell oil – Polyunsaturated fatty acids – Glycolipids – Citric acid

Daniel Dobslaw and Karl-Heinrich Engesser. Degradation of 2-chlorotoluene by *Rhodococcus* sp. OCT 10. Applied Microbiology and Biotechnology, Vol. 93(5) (2012): 2205-2214

A strain *Rhodococcus* sp. OCT 10 DSM 45596^T, exhibiting 99.9% of 16S rDNA identity with *Rhodococcus wratislaviensis* NCIMB 13082, was isolated from a soil sample. The strain completely mineralised 2-chlorotoluene, 2-bromotoluene, *o*-xylene, benzyl alcohol and benzoate. In contrast, 2-fluorotoluene was only partially mineralised. By GC-MS and ^{1}H-NMR analyses, 4-chloro-3-methylcatechol was identified as the central intermediate in the degradation pathway of 2-chlorotoluene. It was further degraded by enzymes of the *meta* cleavage pathway. Catechol 1,2-dioxygenase and chlorocatechol 1,2-dioxygenase as the initial enzymes of the *ortho* cleavage pathways were not detectable under these conditions. Furthermore, neither formation nor oxidation of 2-chlorobenzylic alcohol, 2-chlorobenzaldehyde, or 2-chlorobenzoate was observed, thereby excluding side chain oxidation activity.

Keywords: Degradation – 2-Chlorotoluene – 2-Halotoluene – 4-Chloro-3-methylcatechol – Mineralisation – 2-Chloromethylbenzene

Jai S. Ghosh and Kedar B. Rokade. Biodegradation of 2-mercaptobenzothiazolyl-(Z)-(2-aminothiazol-4-yl)-2-(*tert*-butoxycarbonyl) isopropoxyiminoacetate by *Pseudomonas desmolyticum* NCIM 2112. Applied Microbiology and Biotechnology, Vol.93(2) (2012): 753-761

2-Mercaptobenzothiazolyl-(Z)-(2-aminothiazol-4-yl)-2-(*tert*-butoxycarbonyl) isopropoxyiminoacetate is used as supplementary additives in commercial-grade insecticides to compensate for the time factor needed for the actual pesticide chemical to start its action. This investigation describes the biodegradation of 2-mercaptobenzothiazolyl-(Z)-(2-aminothiazol-4-yl)-2-(*tert*-butoxycarbonyl) isopropoxyiminoacetate by *Pseudomonas desmolyticum* NCIM 2112. The biodegradation is influenced by other carbon and nitrogen sources and indicates that glucose and lactose are effective at 0.5% concentration whereas $NaNO_3$ and $NaNO_2$ at 0.05%. The percent degradation of 2-mercaptobenzothiazolyl-(Z)-(2-aminothiazol-4-yl)-2-(*tert*-butoxycarbonyl) isopropoxyiminoacetate was found to be 40%.The pH and temperature optima were found to be 7.0°C and 30°C, respectively. The effect on soil parameters was observed in treated soil and indicates remarkable decrease in soil fertility; the phytotoxicity indicates retarded growth and germination inhibition of treated seeds of *Sorghum bicolor* and *Triticum aestivum*. In paddy field the inhibition of germination of *Oryza sativa* was observed.

Keywords: Insecticide – 2-Mercaptobenzothiazolyl-(Z)-(2-aminothiazol-4-yl)-2-(*tert*-butoxycarbonyl) isopropoxyiminoacetate – *Pseudomonas* – Phytotoxicity

Tomofumi Nakamura, Hirofumi Ichinose and Hiroyuki Wariishi. Flavin-containing monooxygenases from *Phanerochaete chrysosporium* responsible for fungal metabolism of phenolic compounds. Biodegradation, Vol.23(3) (2012): 343-350

We investigated the cellular responses of the white-rot basidiomycete *Phanerochaete chrysosporium* against vanillin. Based upon a proteomic survey, it was demonstrated that two flavin-containing monooxygenases (PcFMO1 and PcFMO2) are translationally up-regulated in response to exogenous addition of vanillin. To elucidate their catalytic functions, we cloned cDNAs and heterologously expressed them in *Escherichia coli*. The recombinant PcFMO1 showed catalytic activities against monocyclic phenols such as phenol, hydroquinone, and 4-chlorophenol. In addition, the product from hydroquinone was identified as 1,2,4-

trihydroxybenzene, an important intermediate in a metabolic pathway of aromatic compounds in which the aromatic ring of 1,2,4-trihydroxybenzene can be further cleaved by fungal dioxygenases for mineralization. Thus, the ortho-cleavage pathway of phenolic compounds would presumably be associated with PcFMO1.

Keywords: White-rot basidiomycete – Proteomics – Flavin-containing monooxygenase – Phenol hydoxylase – Lignin degradation

Weiwei Zhang, Dongxue Xu, Zongliang Niu, Kun Yin and Ping Liu, et al. Isolation and characterization of *Pseudomonas* sp. DX7 capable of degrading sulfadoxine. Biodegradation, Vol. 23(3) (2012): 431-439

Given that the intensive application of sulfonamides in aquaculture, animal husbandry and malaria treatment has lead to an increase in sulfonamide discharge into the environment, there is an increasing need to find a way to remediate sulfonamide-contaminated sites. The bacterial strain DX7 was isolated from a marine environment and is capable of degrading sulfadoxine. DX7 was identified as a *Pseudomonas* sp. based on 16S rRNA gene sequencing. Approximately 30% of sulfadoxine was degraded after *Pseudomonas* sp. DX7 was inoculated into mineral salt plus tryptone media containing 10 mg l^{-1} sulfadoxine for 2 days. The degradation efficiency under different environmental conditions was characterized using HPLC. The optimal temperature and pH for sulfadoxine biodegradation were around 30°C and 6.0, respectively. The optimal concentrations of sulfadoxine and tryptone for sulfadoxine biodegradation were determined to be approximately 30 mg l^{-1} and between 2.0 and 8.0 g l^{-1}, respectively. Cytotoxicity analysis indicated that the metabolites of sulfadoxine generated by *Pseudomonas* sp. DX7 showed significantly reduced cytotoxicity to Hela cells. These results suggest that *Pseudomonas* sp. DX7 is a new bacterial resource for degrading sulfadoxine and indicate the potential of the isolated strain in the bioremediation of sulfadoxine-contaminated environments.

Keywords: Sulfadoxine – *Pseudomonas* sp. – Degradation – Cytotoxicity

P. M. Kulkarni. Isolation, identification and removal of filamentous organism from SND based SBR degrading nitrophenols. Biodegradation, Vol. 23(3) (2012): 455-463

Four identical lab scale sequencing batch reactors R, R1, R2, and R3, were used to assess nitrophenol biodegradation using a single sludge biomass containing *Thiosphaera pantotropha.* Nitrophenols [4-Nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP) and 2,4,6-trinitrophenol (2,4,6-TNP)] were biotransformed by heterotrophic nitrification and aerobic denitrification (SND). Reactor R was used as background control, whereas R1, R2, and R3 were fed with 4-NP, 2,4-DNP, and 2,4,6-TNP, respectively. The concentration of each nitrophenol was gradually increased from 2.5 to 200 mg/l along with increase in COD, during acclimation studies**.** The final COD maintained was 4,500 mg/l with each nitrophenolic loading of 200 mg/l. During late phase of acclimation and HRT study, a filamentous organism started appearing in 2,4-DNP and 2,4,6-TNP bioreactors. Filaments were never found in 4-NP and background control reactor. Biochemistry and physiology behind filamentous organism development, was studied to obtain permanent solution for its removal. The effect of different input parameters such as COD loading, DO levels, SVI etc. were analyzed. The morphology and development of filamentous organism were examined extensively using microscopic techniques involving ESEM, oil immersion, phase contrast, and dark field microscopy. The organism was grown and isolated on selective agar plates and was identified as member of *Streptomyses* species.

Keywords: Nitrophenols – *Thiosphaera pantotropha* – Heterotrophic nitrification – Aerobic denitrification – SND – SBR

Ola M. Gomaa, Hussein Abd El Kareem and Reham Fatahy. Assessment of the efficacy of *Aspergillus* sp. EL-2 in textile waste water treatment. Biodegradation, Vol. 23(2) (2012): 243-251

Fungal biomass has the ability to decolorize a wide variety of dyes successfully through a number of mechanisms. A brown rot isolate, previously identified as *Aspergillus* sp. EL-2, was used in the aerobic treatment of textile waste water efficiently. In the current work, the treated waste water was tested chemically using more than one combined treatment. Microbial toxicity, phytotoxicity, genotoxicity and cytotoxicity were also studied to assess the toxicity level for each treatment. The obtained data suggest that the contribution of more than one mode of treatment is essential to ensure complete destruction of the by-products. The use of gamma irradiation (25 kGy) after the bioremediation step led to the decrease of the by-products of biodegradation as observed by visible spectrum and Fourier transfer infra red spectroscopy (FT-IR). The toxicity assessment presented variable results indicating the need for more than one toxicity test to confirm the presence or absence of hazardous compounds. Brown rot fungus could be used efficiently in the treatment of textile waste water without the risk of obtaining high carcinogenic or genotoxic compounds, especially if combined treatment is employed.

Keywords: Textile waste water – Assessment – Brown rot fungi – Gamma radiation – Combined treatment – Toxicity tests

Charalampos K. Myresiotis, Zisis Vryzas and Euphemia Papadopoulou-Mourkidou. Biodegradation of soil-applied pesticides by selected strains of plant growth-promoting rhizobacteria (PGPR) and their effects on bacterial growth. Biodegradation, Vol. 23(2) (2012): 297-310

A laboratory study was conducted to investigate the influence of four PGPR strains on the degradation of five soil applied pesticides and their effects on bacterial growth. Interactions of *Bacillus subtilis* GB03, *Bacillus subtilis* FZB24, *Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* SE34 with two concentrations of acibenzolar-*S*-methyl, metribuzin, napropamide, propamocarb hydrochloride and thiamethoxam in liquid culture and soil microcosm were studied. The degradation of acibenzolar-*S*-methyl by all PGPR tested in low and high concentration, was 5.4 and 5.7 times, respectively, faster than that in non-inoculated liquid culture medium. At the end of the 72-h liquid cultured experiments, 8–18, 9–11, 15–36 and 11–22% of metribuzin, napropamide, propamocarb hydrochloride and thiamethoxam, respectively, had disappeared from PGPR inoculated medium. Under the soil microcosm experimental conditions, the half-lives of acibenzolar-*S*-methyl incubated in the presence of PGPR strains spiked at 1.0 and 10.0 mg kg^{-1} were 10.3–16.4 and 9.2–15.9 days, respectively, markedly lower compared with >34.2 days in the control. From the rest pesticides studied degradation of propamocarb hydrochloride and thiamethoxam was enhanced in the presence of *B. amyloliquefaciens* IN937a and *B. pumilus* SE34. Acibenzolar-*S*-methyl, propamocarb hydrochloride and thiamethoxam significantly increased the PGPR growth. However, the stimulatory effect was related to the level of pesticide spiked.

Keywords: Pesticides – Biodegradation – Plant growth-promoting rhizobacteria (PGPR) – *Bacillus* sp. – LC–MS/MS

K. Tihomirova, A. Briedis, J. Rubulis and T. Juhna. Effect of biomass adaptation to biodegradation of dissolved organic carbon in water. Biodegradation, Vol. 23(2) (2012): 319-324

In the present study the time of adaptation of fixed biomass for biodegradation of natural organic matter was investigated. The experiments were done in columns that are usually used for rapid determination of biodegradable dissolved organic carbon (BDOC). The biomass was adapted to samples with different concentrations of organic substances before measurements by pumping water to be investigated through the columns for several days. The time of adaptation was dependent on the initial concentration of the organic matter in the water sample. The adaptation time increased from 6 to 24 h with increase of concentration of acetate solution from 2 to 10 mg/l, thus adaptation rate decreased simultaneously from 0.28 to 0.11 min^{-1}. In natural water samples with the initial concentration in the range from 4.61–10.82 mg/l of dissolved organic carbon (DOC) the maximal adaptation time was less than 24 h. During the adaptation period the increase in reproducibility and decrease in the standard deviation was observed. The study showed that adaptation of column to the different concentration of organic matter in water sample is necessary in order to decrease the bias in BDOC measurements when using columns tests.

Keywords: Adaptation – Biodegradable dissolved organic carbon – Biomass – Dissolved organic carbon

Marcel Vilaplana, Ana Belén García, Gloria Caminal, Francisco Guillén and Montserrat Sarrà. Optimisation of the operational conditions of trichloroethylene degradation using *Trametes versicolor* under quinone redox cycling conditions using central composite design methodology. Biodegradation, Vol. 23(2) (2012): 333-341

Extracellular radicals produced by *Trametes versicolor* under quinone redox cycling conditions can degrade a large variety of pollutant compounds, including trichloroethylene (TCE). This study investigated the effect of the agitation speed and the gas–liquid phase volume ratio on TCE degradation using central composite design (CCD) methodology for a future scale-up to a reactor system. The agitation speed ranged from 90 to 200 rpm, and the volume ratio ranged from 0.5 to 4.4. The results demonstrated the important and positive effect of the agitation speed and an interaction between the two factors on TCE degradation. Although the volume ratio did not have a significant effect if the agitation speed value was between 160 and 200 rpm, at lower speed values, the specific pollutant degradation was clearly more extensive at low volume ratios than at high volume ratios. The fitted response surface was validated by performing an experiment using the parameter combination in the model that maximised TCE degradation. The results of the experiments carried out using different biomass concentrations demonstrated that the biomass concentration had a positive effect on pollutant degradation if the amount of biomass present was lower than 1.6 g dry weight l^{-1}. The results show that the maximum TCE degradation was obtained at the highest speed (200 rpm), gas–liquid phase volume ratio (4.4), and a biomass concentration of 1.6 g dry weight l^{-1}.

Keywords: White-rot fungi – Hydroxyl radicals – Surface response methodology – Scale-up

G. D. Gojgic-Cvijovic, J. S. Milic, T. M. Solevic, V. P. Beskoski, M. V. Ilic, L. S. Djokic, T. M. Narancic and M. M. Vrvic. Biodegradation of petroleum sludge and petroleum polluted soil by a bacterial consortium: a laboratory study. Biodegradation, Vol 23(1) (2012): 1-14

This article presents a study of the efficiency and degradation pattern of samples of petroleum sludge and polluted sandy soil from an oil refinery. A bacterial consortium, consisting of strains from the genera *Pseudomonas*, *Achromobacter*, *Bacillus* and *Micromonospora*, was isolated from a petroleum sludge sample and characterized. The addition of nitrogen and phosphorus nutrients and a chemical surfactant to both the samples and bioaugmentation to the soil sample were applied under laboratory conditions. The extent of biodegradation was monitored by the gravimetric method and analysis of the residual oil by gas chromatography. Over a 12-week experiment, the achieved degree of TPH (total petroleum hydrocarbon) degradation amounted to 82–88% in the petroleum sludge and 86–91% in the polluted soil. Gas chromatography–mass spectrometry was utilized to determine the biodegradability and degradation rates of *n*-alkanes, isoprenoids, steranes, diasteranes and terpanes. Complete degradation of the *n*-alkanes and isoprenoids fractions occurred in both the samples. In addition, the intensities of the peaks corresponding to tricyclic terpenes and homohopanes were decreased, while significant changes were also observed in the distribution of diasteranes and steranes.

Keywords: Isoprenoids – Mixed culture – *n*-alkanes – Petroleum sludge – Steranes – Surfactant – Terpanes

Meeta Lavania, Simrita Cheema, Priyangshu Manab Sarma, Ajoy Kumar Mandal and Banwari Lal. Biodegradation of asphalt by *Garciaella petrolearia* TERIG02 for viscosity reduction of heavy oil. Biodegradation, Vol. 23(1) (2012): 15-24

Petroleum hydrocarbon is an important energy resource, but it is difficult to exploit due to the presence of dominated heavy constituents such as asphaltenes. In this study, viscosity reduction of Jodhpur heavy oil (2,637 cP at 50°C) has been carried out by the biodegradation of asphalt using a bacterial strain TERIG02. TERIG02 was isolated from sea buried oil pipeline known as Mumbai Uran trunk line (MUT) located on western coast of India and identified as *Garciaella petrolearia* by 16S rRNA full gene sequencing. TERIG02 showed 42% viscosity reduction when asphalt along with molasses was used as a sole carbon source compared to only asphalt (37%). The viscosity reduction by asphaltene degradation has been structurally characterized by Fourier transform infrared spectroscopy (FTIR). This strain also shows an additional preference to degrade toxic asphalt and aromatics compounds first unlike the other known strains. All these characteristics makes TERIG02 a potential candidate for enhanced oil recovery and a solution to degrading toxic aromatic compounds.

Keywords: Asphaltene degradation – Viscosity reduction – Fourier transform infrared spectroscopy – Gases and volatile fatty acids

Filomena Costa, Cristina Quintelas and Teresa Tavares. Kinetics of biodegradation of diethylketone by *Arthrobacter viscosus.* Biodegradation, Vol. 23(1) (2012): 81-92

The performance of an *Arthrobacter viscosus* culture to remove diethylketone from aqueous solutions was evaluated. The effect of initial concentration of diethylketone on the growth of the

bacteria was evaluated for the range of concentration between 0 and 4.8 g/l, aiming to evaluate a possible toxicological effect. The maximum specific growth rate achieved is 0.221 h^{-1} at 1.6 g/l of initial diethylketone concentration, suggesting that for higher concentrations an inhibitory effect on the growth occurs. The removal percentages obtained were approximately 88%, for all the initial concentrations tested. The kinetic parameters were estimated using four growth kinetic models for biodegradation of organic compounds available in the literature. The experimental data found is well fitted by the Haldane model ($R^2 = 1$) as compared to Monod model ($R^2 = 0.99$), Powell ($R^2 = 0.82$) and Loung model ($R^2 = 0.95$). The biodegradation of diethylketone using concentrated biomass was studied for an initial diethylketone concentration ranging from 0.8–3.9 g/l in a batch with recirculation mode of operation. The biodegradation rate found followed the pseudo-second order kinetics and the resulting kinetic parameters are reported. The removal percentages obtained were approximately 100%, for all the initial concentrations tested, suggesting that the increment on the biomass concentration allows better results in terms of removal of diethylketone. This study showed that these bacteria are very effective for the removal of diethylketone from aqueous solutions.

Keywords: *Arthrobacter viscosus* – Biodegradation kinetics – Degradation – Diethylketone – Growth kinetics

Shenghui Wang, Chen Zhang and Yanchun Yan. Biodegradation of methyl parathion and *p*-nitrophenol by a newly isolated *Agrobacterium* sp. strain Yw12. Biodegradation, Vol. 23(1) (2012): 107-116

Strain Yw12, isolated from activated sludge, could completely degrade and utilize methyl parathion as the sole carbon, phosphorus and energy sources for growth in the basic salt media. It could also completely degrade and utilize *p*-nitrophenol as the sole carbon and energy sources for growth in the minimal salt media. Phenotypic features, physiological and biochemical characteristics, and phylogenetic analysis of 16S rRNA sequence showed that this strain belongs to the genus of *Agrobacterium* sp. Response surface methodology was used to optimize degradation conditions. Under its optimal degradation conditions, 50 mg l^{-1} MP was completely degraded within 2 h by strain Yw12 and the degradation product PNP was also completely degraded within 6 h. Furthermore, strain Yw12 could also degrade phoxim, methamidophos, chlorpyrifos, carbofuran, deltamethrin and atrazine when provided as the sole carbon and energy sources. Enzymatic analysis revealed that the MP degrading enzyme of strain Yw12 is an intracellular enzyme and is expressed constitutively. These results indicated that strain Yw12 might be used as a potential and effective organophosphate pesticides degrader for bioremediation of contaminated sites.

Keywords: Methyl parathion – *p*-Nitrophenol – Degradation – *Agrobacterium* sp. – Response surface methodology

Syed A. Hasan, Piet Wietzes and Dick B. Janssen. Biodegradation kinetics of 4-fluorocinnamic acid by a consortium of *Arthrobacter* and *Ralstonia* strains. Biodegradation, Vol. 23(1) (2012): 117-125

Arthrobacter sp. strain G1 is able to grow on 4-fluorocinnamic acid (4-FCA) as sole carbon source. The organism converts 4-FCA into 4-fluorobenzoic acid (4-FBA) and utilizes the two-carbon side-chain for growth with some formation of 4-fluoroacetophenone as a dead-end side product. We also have isolated *Ralstonia* sp. strain H1, an organism that degrades 4-FBA. A

consortium of strains G1 and H1 degraded 4-FCA with Monod kinetics during growth in batch and continuous cultures. Specific growth rates of strain G1 and specific degradation rates of 4-FCA were observed to follow substrate inhibition kinetics, which could be modeled using the kinetic models of Haldane–Andrew and Luong–Levenspiel. The mixed culture showed complete mineralization of 4-FCA with quantitative release of fluoride, both in batch and continuous cultures. Steady-state chemostat cultures that were exposed to shock loadings of substrate responded with rapid degradation and returned to steady-state in 10–15 h, indicating that the mixed culture provided a robust system for continuous 4-FCA degradation.

Keywords: Organofluorine compounds – *Arthrobacter* – *Ralstonia* – Biodegradation – Defluorination – 4-Fluorocinnamic acid

Mingliang Ding, Min Zhang, Jinming Yang and Jian-hui Qiu. Study on the enzymatic degradation of PBS and its alcohol acid modified copolymer. Biodegradation, Vol. 23(1) (2012): 127-132

Enzymatic hydrolytic degradation of polybutylene succinate (PBS), poly(polybutylenesuccinate-co-1,4-cyclohexane dimethanol) (PBS/CHDM) and poly(polybutylene succinate-co-diglycolic acid) (PBS/DGA) in mixed solvent of tetrahydrofuran (THF) and toluene was examined. Lipase was used as catalyst to degrade polymers with molecular weight of more than 100,000, and the molecular weight of products ranged from hundreds to thousands. Thermal decomposition temperatures of all products were below 250°C. The degradation products of both PBS/CHDM and PBS/DGA showed two melting points at about 85 and 99°C. Mass spectrometry (MS) was employed to obtain the molecular weight of oligomers extracted from the products, which proved to be low-polyesters with the molecular weight of less 1,000. The butanediol (BDO) monomer was found in PBS/CHDM degradation product for the first time.

Keywords: Lipase – Degradation – PBS – Cyclic oligomer – Linear oligomer

Kedar C. Ahire, Balu P. Kapadnis, Girish J. Kulkarni, Yogesh S. Shouche and Rajendra L. Deopurkar. Biodegradation of tributyl phosphate by novel bacteria isolated from enrichment cultures. Biodegradation, Vol. 23(1) (2012): 165-176

Tributyl phosphate (TBP) is an organophosphorous compound, used extensively (3000–5000 tonnes/annum) as a solvent for nuclear fuel processing and as a base stock in the formulation of fire-resistant aircraft hydraulic fluids and other applications. Because of its wide applications and relative stability in the natural environment TBP poses the problem of pollution and health hazards. In the present study, fifteen potent bacterial strains capable of using tributyl phosphate (TBP) as sole carbon and phosphorus source were isolated from enrichment cultures. These isolates were identified on the basis of biochemical and morphological characteristics and 16S rRNA gene sequence analysis. Phylogenetic analysis of 16S rRNA gene sequences revealed that two isolates belonged to class Bacilli and thirteen to β and γ-Proteobacteria. All these isolates were found to be members of genera *Alcaligenes, Providencia, Delftia, Ralstonia,* and *Bacillus.* These isolates were able to tolerate and degrade up to 5 mM TBP, the highest concentration reported to date. The GC–MS method was developed to monitor TBP degradation. Two strains, *Providencia* sp. BGW4 and *Delftia* sp. BGW1 showed respectively, 61.0 ± 2.8% and 57.0 ± 2.0% TBP degradation within 4 days. The degradation rate constants, calculated by

first order kinetic model were between 0.0024 and 0.0099 h^{-1}. These bacterial strains are novel for TBP degradation and could be used as an important bioresource for efficient decontamination of TBP polluted waste streams.

Keywords: Tributyl phosphate – Enrichment – GC–MS – Biodegradation – Phylogenetic analysis

Yongming Zhang, Xia Sun, Lujun Chen and Bruce E. Rittmann. Integrated photocatalytic-biological reactor for accelerated 2,4,6-trichlorophenol degradation and mineralization. Biodegradation, Vol. 23(1) (2012): 189-198

An integrated photocatalytic-biological reactor (IPBR) was used for accelerated degradation and mineralization of 2,4,6-trichlorophenol (TCP) through simultaneous, intimate coupling of photocatalysis and biodegradation in one reactor. Intimate coupling was realized by circulating the IPBR's liquid contents between a TiO_2 film on mat glass illuminated by UV light and honeycomb ceramics as biofilm carriers. Three protocols—photocatalysis alone (P), biodegradation alone (B), and integrated photocatalysis and biodegradation (photobiodegradation, P&B)—were used for degradation of different initial TCP concentrations. Intimately coupled P&B also was compared with sequential P and B. TCP removal by intimately coupled P&B was faster than that by P and B alone or sequentially coupled P and B. Because photocatalysis relieved TCP inhibition to biodegradation by decreasing its concentration, TCP biodegradation could become more important over the full batch P&B experiments. When phenol, an easy biodegradable compounds, was added to TCP in order to promote TCP mineralization by means of secondary utilization, P&B was superior to P and B in terms of mineralization of TCP, giving 95% removal of chemical oxygen demand. Cl^- was only partially released during P experiments (24%), and this corresponded to its poor mineralization in P experiments (32%). Thus, intimately coupled P&B in the IPBR made it possible obtain the best features of each: rapid photocatalytic transformation in parallel with mineralization of photocatalytic products.

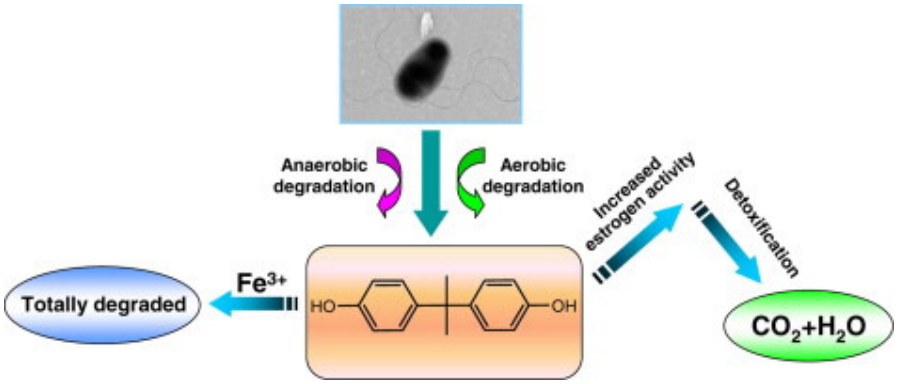
Keywords: Photocatalysis – Biofilm – Reactor – Trichlorophenol – Biodegradation

Guiying Li[a], Lei Zu[a, d], Po-Keung Wong[b], Xinping Hui[c], Yu Lu[a, c, d], Jukun Xiong[a, d], Taicheng An[a]. ([a] The State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Protection and Resources Utilization, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China, [b] School of Life Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China, [c] State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, China, [d] Graduate School of Chinese Academy of Sciences, Beijing 100049, China). **Biodegradation and detoxification of bisphenol A with one newly-isolated strain *Bacillus* sp. GZB: Kinetics, mechanism and estrogenic transition. Bioresource Technology, Vol.114(2012) : 224–230**

A facultative anaerobic bacterial strain, *Bacillus* sp. GZB, was isolated and identified to effectively degrade bisphenol A (BPA) under anaerobic and aerobic conditions. Under anaerobic condition, Fe^{3+} can be used as an electron acceptor for *Bacillus* sp. GZB, while 5 mg L^{-1} BPA can be fully removed and 51% was mineralized under optimal aerobic conditions. Additionally, seven metabolites were identified by GC–MS, four of which were doubly confirmed by authentic standards (two synthesized) and three of four initial degradation intermediates were

also quantified during BPA aerobic degradation. The evolution of 1-(4-hydroxyphenyl)ethanone showed a similar tendency with estrogenic activity changing during BPA biodegradation course, indicating its potential estrogenicity. The estrogenicity temporary increase first and decline ultimately during BPA degradation revealing the GZB can effectively detoxify BPA as well as its estrogenic intermediates. This was the first study to report a facultative anaerobic strain can degrade BPA with or without of oxygen.

Graphical abstract



Keywords: Biodegradation; Bisphenol A; Estrogen disruptor; *Bacillus* sp.; Degradation mechanisms

Varinthorn Boonyaroj[a], Chart Chiemchaisri[b], Wilai Chiemchaisri[b], Suthida Theepharaksapan[b], Kazuo Yamamoto[c]. ([a] International Postgraduate Programs in Environmental Management/Center of Excellence for Environmental and Hazardous Waste Management, Chulalongkorn University, Bangkok, Thailand, [b] Department of Environmental Engineering/National Center of Excellence for Environmental and Hazardous Waste Management, Faculty of Engineering, Kasetsart University, Bangkok 10900, Thailand, [c] Environmental Science Center, University of Tokyo, Tokyo 113, Japan).
Toxic organic micro-pollutants removal mechanisms in long-term operated membrane bioreactor treating municipal solid waste leachate. Bioresource Technology, Vol. 113(2012): 174–180

The performance of two-stage membrane bioreactor (MBR) in term of toxic organic micro-pollutants removal was continuously monitored for 300 days under long sludge age condition. The phenolic compounds and phthalic acid esters (PAEs) in landfill leachate and treated water from MBR unit were quantified by solid phase extraction and gas chromatography–mass spectrometry. Priority pollutants in landfill leachate were phenolics and their degradation products i.e. 4-methyl-2,6-di-tert-butylphenol, bisphenol A at higher concentrations above 100 µg/l, PAEs i.e. dimethyl phthalate, diethyl phthalate, di-n-butyl phthalate, di-n-octyl phthalate, and di (2-ethylhexyl) phthalate. It was found that MBR could remove phenolic compounds and PAEs by 77–96%. Biodegradation and adsorption mechanisms were responsible for their removals in MBR. Additionally, the retention of compounds during filtration through the fouled membrane was also found significant. This research shows that the removal of organic

micro-pollutants in landfill leachate was improved under higher biomass concentration and longer sludge age conditions.

Keywords: Landfill leachate; Membrane bioreactor; Phenolic compounds; Phthalic acid esters; Toxic micro-pollutants

M. M. Santos, C. Piccirillo, P. M. L. Castro, N. Kalogerakis and M. E. Pintado. Bioconversion of oleuropein to hydroxytyrosol by lactic acid bacteria. World Journal of Microbiology and Biotechnology, Vol. 28(6) (2012): 2435-2440

The aim of this work is to study the conversion of oleuropein—a polyphenol present in olives and olive oil by-products—into hydroxytyrosol, a polyphenol with antioxidant and antibacterial properties. The hydrolysis reaction is performed by lactic acid bacteria. Six bacterial strains (*Lactobacillus plantarum 6907, Lactobacillus paracasei 9192, Lactobacillus casei, Bifidobacterium lactis BO, Enterococcus faecium 32, Lactobacillus LAFTI 10*) were tested under aerobic and anaerobic conditions. The oleuropein degradation and hydroxytyrosol formation were monitored by HPLC. Results showed that oleuropein could be successfully converted into hydroxytyrosol. The most effective strain was *Lactobacillus plantarum 6907*, with a reaction yield of hydroxytyrosol of about 30 %. Different reaction mechanisms were observed for different microorganisms; a different yield was observed for *Lactobacillus paracasei* 9192 under aerobic or anaerobic conditions and an intermediate metabolite (oleuropein aglycone) was detected for *Lactobacillus paracasei 9192* and *Lactobacillus plantarum 6907* only. This study could have significant applications, as this reaction can be used to increase the value of olive oil by-products and/or to improve the taste of unripe olives.

Keywords: Oleuropein – Hydroxytyrosol – Lactic acid bacteria – β-Glucosidase – Enzymatic hydrolysis – Olives

Alfredo Gallego, Virginia L. Gemini, Ariana A. Rossen, Susana L. Rossi and Valeria Trípodi, et al. Aerobic degradation of 3-chlorobenzoic acid by an indigenous strain isolated from a polluted river. World Journal of Microbiology and Biotechnology, Vol. 28(3) (2012): 1245-1252

An indigenous strain of *Pseudomonas putida* capable of degrading 3-chlorobenzoic acid as the sole carbon source was isolated from the Riachuelo, a polluted river in Buenos Aires. Aerobic biodegradation assays were performed using a 2-l microfermentor. Biodegradation was evaluated by spectrophotometry, chloride release, gas chromatography and microbial growth. Detoxification was evaluated by using *Vibrio fischeri*, *Pseudokirchneriella subcapitata* and *Lactuca sativa* as test organisms. The indigenous bacterial strain degrades 100 mg l^{-1} 3-chlorobenzoic acid in 14 h with a removal efficiency of 92.0 and 86.1% expressed as compound and chemical oxygen demand removal, respectively. The strain was capable of degrading up to 1,000 mg of the compound l^{-1}. Toxicity was not detected at the end of the biodegradation process. Besides initial concentration, the effect of different factors, such as initial pH, initial inoculum, adaptation to the compound and presence of other substrates and toxic related compounds, was studied.

Keywords: 3-Chlorobenzoic acid – Biodegradation – Detoxification – Indigenous strain

Chitrambalam Sasikala, Sonia Jiwal, Pallabi Rout and Mohandass Ramya. Biodegradation of chlorpyrifos by bacterial consortium isolated from agriculture soil. World Journal of Microbiology and Biotechnology, Vol. 28(3) (2012):1301-1308

Organophosphorous pesticides are widely used in agriculture to control major insect pests. Chlorpyrifos is one of the major organophosphorous pesticides which is used to control insects including termites, beetles. The widespread use of these pesticides is hazardous to the environment and also toxic to mammals, thus it is essential to remove the same from the environment. From the chlorpyrifos contaminated soil nine morphologically different bacterial strains, one actinomycete and two fungal strains were isolated. Among those isolates four bacterial strains which were more efficient were developed as consortium. The four bacterial isolates namely *Pseudomonas putida* (NII 1117), *Klebsiella sp*., (NII 1118), *Pseudomonas stutzeri* (NII 1119), *Pseudomonas aeruginosa* (NII 1120) present in the consortia were identified on the basis of 16S rDNA analysis. The intracellular fractions of the consortium exhibited more organophosphorus hydrolase activity (0.171 ± 0.003 U/mL/min). The degradation studies were carried out at neutral pH and temperature 37°C with chlorpyrifos concentration 500 mg L^{-1}. LC-mass spectral analysis showed the presence of metabolites chlopyrifos-oxon and Diethylphosphorothioate. These results highlight an important potential use of this consortium for the cleanup of chlorpyrifos contaminated pesticide waste in the environment.

Keywords: Biodegradation – Organophosphorous pesticides – Chlorpyrifos – Chlorpyrifos oxon

Biosensor

Zhang, M., Yuan, R. , Chai, Y., Chen, S., Zhong, H., Wang, C., Cheng, Y. (Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China). A biosensor for cholesterol based on gold nanoparticles-catalyzed luminol electrogenerated chemiluminescence. Biosensors and Bioelectronics, Vol. 32(1) (2012): 288-292

A novel cholesterol biosensor was prepared based on gold nanoparticles-catalyzed luminol electrogenerated chemiluminescence (ECL). Firstly, l-cysteine-reduced graphene oxide composites were modified on the surface of a glassy carbon electrode. Then, gold nanoparticles (AuNPs) were self-assembled on it. Subsequently, cholesterol oxidase (ChOx) was adsorbed on the surface of AuNPs to construct a cholesterol biosensor. The stepwise fabrication processes were characterized with cyclic voltammetry and atomic force microscopy. The ECL behaviors of the biosensor were also investigated. It was found that AuNPs not only provided larger surface area for higher ChOx loading but also formed the nano-structured interface on the electrode surface to improve the analytical performance of the ECL biosensor for cholesterol. Besides, based on the efficient catalytic ability of AuNPs to luminol ECL, the response of the biosensor to cholesterol was linear range from 3.3μM to 1.0. mM with a detection limit of 1.1 μM (S/N = 3). In addition, the prepared ECL biosensor exhibited satisfying reproducibility, stability and selectivity. Taking into account the advantages of ECL, we confidently expect that ECL would have potential applications in biotechnology and clinical diagnosis.

Keywords: Cholesterol biosensor; Gold nanoparticles; L-cysteine; Luminol; Reduced graphene oxide

Kun Zhang, Kai Yuan, Hongyan Wu, Qing Li, Yulong Wang, Shouhua Chen, Lili Zhang, He Gu and Rongzhan Fu. Identification of Potential Markers Related to Neoadjuvant Chemotherapy Sensitivity of Breast Cancer by SELDI-TOF MS. Applied Biochemistry and Biotechnology, Vol. 166(3) (2012): 753-763

Neoadjuvant chemotherapy (NACT) is known to be beneficial for patients with locally advanced breast cancer. However, there is still no unified standard on the evaluation of NACT. To identify the potential markers related to NACT sensitivity of breast cancer, in the present study, we examined the protein spectrum of breast cancer tissues before and after NACT using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). Totally, 87 protein samples were extracted from tissues of breast cancer, with 30 from patients before NACT, 30 from patients after NACT, and 27 from patients without any treatment. To eliminate confounding factors a couple of tissue samples from the same patient were mixed. SELDI-TOF MS analysis demonstrated that the intensities of eight different protein peaks, i.e., 26,055.46, 17,898.94, 8,949.50, 11,652.02, 11,053.48, 38,546.56, 5,825.89, and 22,250.63 Da, were higher in samples after NACT than those before NACT. Although further experiments are needed to prove the reliability of the proteins identified in this study, our results will help the establishment of protein model based on drug resistance-related protein peaks to screen whether a patient is suitable for adopting NACT and to improve cancer treatment.

Keywords: Breast cancer – Tissue – Neoadjuvant chemotherapy – Biomarker – Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry

Aili Sun, Qinglin Sheng and Jianbin Zheng. A Hydrogen Peroxide Biosensor Based on Direct Electrochemistry of Hemoglobin in Palladium Nanoparticles/Graphene–Chitosan Nanocomposite Film. Applied Biochemistry and Biotechnology, Vol. 166(3) (2012): 764-773

Thermally two-dimensional lattice graphene (GR) and biocompatibility chitosan (CS) act as a suitable support for the deposition of palladium nanoparticles (PdNPs). A novel hydrogen peroxide (H_2O_2) biosensor based on immobilization of hemoglobin (Hb) in thin film of CS containing GR and PdNPs was developed. The surface morphologies of a set of representative membranes were characterized by means of scanning electron microscopy and showed that the PdNPs are of a sphere shape and an average diameter of 50 nm. Under the optimal conditions, the immobilized Hb showed fast and excellent electrocatalytic activity to H_2O_2 with a small Michaelis–Menten constant of 16 $\mu mol\,L^{-1}$, a linear range from 2.0×10^{-6} to 1.1×10^{-3} $mol\,L^{-1}$, and a detection limit of 6.6×10^{-7} $mol\,L^{-1}$. The biosensor also exhibited other advantages, good reproducibility, and long-term stability, and PdNPs/GR–CS nanocomposites film would be a promising material in the preparation of third generation biosensor.

Keywords: Direct electrochemistry – Nanocomposite – Hemoglobin – Hydrogen peroxide – Graphene

Linfen Yu, Qun Li, Hongwei Gai and Zhanhui Wang. Chemiluminescence Response of Murine Macrophages on Multilayer Microfluidic Chips. Applied Biochemistry and Biotechnology, Vol. 166(3) (2012): 786-795

We have demonstrated an integrated platform for microfluidics and chemiluminescence (CL) detection that is capable of parallel cell culture, convenient liquid manipulation, and sensitive chemiluminescent detection. Luminol-dependent CL responses induced by three different stimuli, phytohemagglutinin (PHA), concanavalin A (ConA), and lipopolysaccharides (LPS), which can evoke a CL response in macrophages, were evaluated on this microfluidic chip. We studied the dose-dependent effect of these three stimuli on CL response in murine macrophages. PHA produced the highest CL response compared to LPS and ConA. The CL intensity produced by PHA was 6.85 and four times higher than that by LPS and ConA, respectively, at the low concentration of 100 μg/ml. We have found microfluidic based CL to be a very useful screening tool, which is less laborious and more sensitive. This microfluidic system is disposable and capable of rapid device prototyping; it may prove to be very useful in clinical and pharmaceutical applications.

Keywords: Microfluidic chip – Macrophage – Cell chemiluminescence

Vanessa Passos Brustein, Carmelita Lima Bezerra Cavalcanti, Mario Ribeiro de Melo-Junior, Maria Tereza Santos Correia, Eduardo Isidoro Carneiro Beltrão and Luiz Bezerra Carvalho. Chemiluminescent Detection of Carbohydrates in the Tumoral Breast Diseases. Applied Biochemistry and Biotechnology, Vol. 166(2) (2012): 268-275

Nowadays, there is an increase of investigations into the fibroadenoma, mainly because some studies have shown that the occurrence of fibroadenoma is linked to an increased risk of developing breast carcinoma. Currently, the chemiluminescence biomarkers are applied for validation methods and screening. Here, a lectin chemiluminescence is proposed as new histochemistry method to identify carbohydrates in mammary tumoral tissues. The lectins concanavalin A (Con A) and peanut agglutinin (PNA) conjugated to acridinium ester were used to characterize the glycocode of breast tissues: normal, fibroadenoma, and invasive duct carcinoma (IDC). The lectin chemiluminescence expressed in relative light units (RLU) was higher in fibroadenoma and IDC than in normal tissue for both lectins tested. The relationship RLU emission versus tissue area described a linear and hyperbolic curve for IDC and fibroadenoma, respectively, using Con A whereas hyperbolic curves for both transformed tissues using PNA. RLU was abolished by inhibiting the interaction between tissues and lectins using their specific carbohydrates: methyl-α-D-mannoside (Con A) and galactose (PNA). The intrinsic fluorescence emission did not change with combination of the lectins (Con A/PNA) to the acridinium ester for hydrophobic residues. These results represent the lectin chemiluminescence as an alternative of histochemistry method for tumoral diagnosis in the breast.

Keywords: Chemiluminescence – Concanavalin A (Con A) – Peanut agglutinin (PNA) – Human mammary tissues – Histochemistry

Se Hoon Jeong[a], Dong Woo Lee[a], Sanghyo Kim[b], Jhingook Kim[c], Bosung Ku[a]. ([a]Advanced Materials & Devices Lab, Corporate R&D Institute, Samsung Electro-Mechanics Co., Ltd., Suwon 443-743, Republic of Korea, [b] College of Bionano technology, Gachon University, Seongnam 461-701, Republic of Korea, [c] Samsung Medical Center, School of Medicine, Sungkyunkwan University, Seoul 135-230, Republic of Korea). A study of electrochemical biosensor for analysis of three-dimensional (3D) cell culture. Biosensors and Bioelectronics, Vol. 35(1) (2012):128–133

Cell culture has a fundamental role not only in regenerative medicine but also in biotechnology, pharmacology, impacting both drug discovery and manufacturing. Although cell culture has been generally developed for only two-dimensional (2D) culture systems, three-dimensional (3D) culture is being spotlighted as the means to mimic in vivo cellular conditions. In this study, a method for cytotoxicity assay using an electrochemical biosensor applying 3D cell culture is presented. In order to strengthen the advantage of a 3D cell culture, the experimental condition of gelation between several types of sol–gels (alginate, collagen, matrigel) and cancer cells can be optimized to make a 3D cell structure on the electrode, which will show the reproducibility of electrical measurement for long-term monitoring. Moreover, cytotoxicity test results applying this method showed IC_{50} value of A549 lung cancer cells to erlotinib. Thus, this study evaluates the feasibility of application of the electrochemical biosensor for 3D cell culture to cytotoxicity assay for investigation of 3D cell response to drug compounds.

Keywords: Cell chip; Three-dimensional (3D) cell culture; Electrochemical biosensor; Electrical conductivity; Cytotoxicity assay

Lidong Wu[1], Dehui Deng[1], Jing Jin, Xianbo Lu, Jiping Chen. (Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China). Nanographene-based tyrosinase biosensor for rapid detection of bisphenol A. Biosensors and Bioelectronics, Vol. 35 (1) (2012): 193–199

Hydrophilic nanographene (NGP) prepared by ball milling of graphite was used as the support to construct a novel tyrosinase biosensor for determination of bisphenol A (BPA). The performances of the nanographene-based tyrosinase biosensor were systematically compared with those of multiwall carbon nanotubes (MWNTs) modified tyrosinase biosensors. The results indicated that the nanographene-based tyrosinase biosensor provided significant advantages over MWNTs-based tyrosinase biosensor in term of response, repeatability, background current and limit of detection (LOD), which could be attributed to its larger specific surface area and unique hierarchical tyrosinase-NGP nanostructures. The nanographene-based tyrosinase biosensor displayed superior analytical performance over a linear range from 100 nmol L^{-1} to 2000 nmol L^{-1}, with LOD of 33 nmol L^{-1} and sensitivity of 3108.4 mA cm^{-2} M^{-1}. The biosensor was further used for detecting BPA (leaching from different vessels) in tap water, and the accuracy of the results was validated by high performance liquid chromatography (HPLC). The nanographene-based tyrosinase biosensor proved to be a promising and reliable tool for rapid detection of BPA leached from polycarbonate plastic products and for on-site rapid analysis of emergency pollution affairs of BPA.

Keywords: Bisphenol A; Nanographene; Carbon nanotubes; Tyrosinase biosensor

Faheem Ahmad[a], Mansoor A. Siddiqui[b], Olubukola O. Babalola[a], Hui-Fen Wu[c, d, e]. ([a]Department of Biological Sciences, Faculty of Agriculture, Science and Technology, North-West University, Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa, [b] Department of Botany, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India, [c] Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, 70, Lien-Hai Road, Kaohsiung, 80424, Taiwan, [d] Center for Nanoscience and Nanotechnology, National Sun Yat-Sen University, 70, Lien-Hai Road, Kaohsiung, 80424, Taiwan, [e] Doctoral Degree Program in Marine Biotechnology, National Sun Yat - Sen University, Kaohsiung, 80424, Taiwan). Biofunctionalization of nanoparticle assisted mass

spectrometry as biosensors for rapid detection of plant associated bacteria. Biosensors and Bioelectronics, Vol. 35 (1) (2012): 235–242

This study is based on the application of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) as biosensor to detect the plant associated bacteria (PAB) isolates from rhizospheric soil and root. The rapid bacterial detection via on particle ionization/enrichment technique using IgG functionalized Pt NPs (IgG-Pt NPs) assisted MALDI-TOF MS was successfully used to explore two PAB isolates, namely, *Bacillus thuringiensis* and *B. subtilis* from rhizospheric soil and roots of carrot plant. When these bacteria are used as bioformulations in agricultural as well as biotechnological applications, the plant growth promotion of economic crops was observed especially when the crops grow in less fertilize soil regions. This study proved that even at low concentrations, bacteria can also be directly detected without morphological, molecular and biochemical test. The current applied technique is simple, rapid and highly sensitive. Besides, it could be widely used for the detection of beneficially important PAB isolates in environmental samples.

Keywords: Rhizospheric soil; Root; PAB; Nanoparticle; MALDI-TOF MS; Detection

Saurabh Mani Tripathi[a], Wojtek J. Bock[a], Predrag Mikulic[a], Raja Chinnappan[b], Andy Ng[b], Mona Tolba[b], Mohammed Zourob[b]. ([a] Centre de Recherche en Photonique, Département d'informatique et d'ingénierie, Université du Québec en Outaouais, Gatineau, QC, J8Y 3G5, Canada, [b] Institut National de la Recherche Scientifique - Énergie, Matériaux et Télécommunications, Varennes, QC, J3X 1S2, Canada). Long period grating based biosensor for the detection of *Escherichia coli* bacteria. Biosensors and Bioelectronics, Vol. 35(1) (2012): 308–312

In this paper we report a stable, label-free, bacteriophage-based detection of *Escherichia coli* (*E. coli*) using ultra sensitive long-period fiber gratings (LPFGs). Bacteriophage T4 was covalently immobilized on optical fiber surface and the *E. coli* binding was investigated using the highly accurate spectral interrogation mechanism. In contrast to the widely used surface plasmon resonance (SPR) based sensors, no moving part or metal deposition is required in our sensor, making the present sensor extremely accurate, very compact and cost effective. We demonstrated that our detection mechanism is capable of reliable detection of *E. coli* concentrations as low as 10^3 cfu/ml with an experimental accuracy greater than 99%.

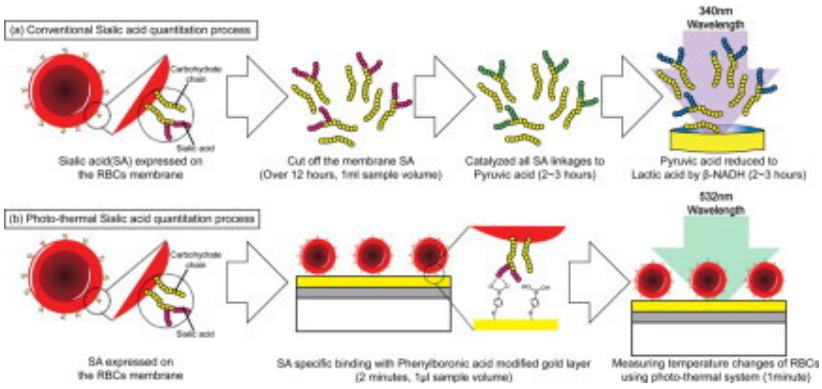
Keywords: Bacteriophages; Long period fiber grating; Spectral interrogation; Pathogen; Covalent binding

Bong Seop Kwak[a], Hyun Ok Kim[b], Jae Hun Kim[c], Seok Lee[c], Hyo-Il Jung[a]. ([a] School of Mechanical Engineering, Yonsei University, Seoul, Republic of Korea, [b] College of Medicine, Yonsei University, Seoul, Republic of Korea, [c] Environmental Sensor System Research Center, Korea Institute of Science and Technology, Seoul, Republic of Korea). Quantitative analysis of sialic acid on erythrocyte membranes using a photothermal biosensor. Biosensors and Bioelectronics, Vol. 35(1) (2012): 484–488

The quantitative analysis of sialic acid (SA) at an erythrocyte membrane is becoming an important clinical parameter in diagnosing cancer and diabetes. In spite of such clinical

importance, there are only a few, very expensive, time consuming and complicated quantifying methods established. To solve this problem, we demonstrate a novel and direct measurement technique for SA exposed to the cell membrane using a photothermal biosensing system in which the hemoglobin molecules in the erythrocyte absorb a specific wavelength of photons (532 nm) and convert it to a temperature change. For measuring the quantity of SA, we first modified the sensor surface of a micro-scaled thermometer using phenylboronic acid (PBA) containing a self-assembled monolayer (SAM) to capture the SA-expressing erythrocytes. Second, the sensor surface was thoroughly washed, and when more SA was expressed, tighter association of erythrocytes to the biosensor was expected. Thirdly, blood sample changes in temperature, heated by the 532 nm wavelength laser, were measured by the bottom layer's micron sized platinum thermometer. The temperature changes from the erythrocytes captured on the sensor surface could be estimated by the amount of SA expressed on the erythrocyte membrane. This novel SA analysis system can solve the problems raised by conventional methods such as multiple enzyme reactions and a time consuming process. We expect that this system will help provide a new tool in the quantitative analysis of SA expression level for the diagnosis of diabetes and cancers.

Graphical abstract



Keywords: Photothermal; Biosensor; Erythrocyte; Sialic acid (SA); Platinum resistance temperature detector (Pt-RTD); Diabetes

Hua Zhang[a], Ying Sun[a], Jing Wang[a], Jia Zhang[a], Hanqi Zhang[a], Hao Zhou[b], Daqian Song[a]. ([a] College of Chemistry, Jilin University, Qianjin Street 2699, Changchun 130012, PR China, [b] Jilin Entry-Exit Inspection and Quarantine Bureau, Changchun 130062, PR China). **Preparation and application of novel nanocomposites of magnetic-Au nanorod in SPR biosensor. Biosensors and Bioelectronics, Vol. 34(1) (2012): 137–143**

A novel nanocomposite Fe_3O_4–Au nanorod (AuNR) was prepared and used as the substrate in the surface plasmon resonance (SPR) biosensor to detect goat IgM. Fe_3O_4–AuNR nanocomposites were synthesized by a method of seed-mediated growth, and further characterized by molecular absorption spectroscopy, transmission electronic microscopy (TEM), energy-dispersive spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS). The nanocomposites exhibit both magnetic property and exceptional optical property, which are beneficial to the antibody immobilization and the sensitivity of detection. The sensing membrane can be regenerated easily and the experimental procedure is simplified. Moreover, the Au nanorods show two plasmon resonance wavelengths defined as transverse mode and longitudinal

mode, and the longitudinal plasmon wavelengths are more sensitive to the changes in the dielectric properties of the surroundings. Fe_3O_4–AuNR nanocomposites got a high sensitivity in detection of antibody–antigen immunoassay. In the optimal conditions, the biosensor based on Fe_3O_4–AuNR nanocomposites exhibits a satisfactory response to goat IgM in the concentration range of 0.15–40.00 μg mL^{-1}. However, the biosensor without Fe_3O_4–AuNR nanocomposites shows a response to goat IgM in the concentration range of 1.25–40.00 μg mL^{-1}. As a result, the sensitivity of the biosensor based on Fe_3O_4–AuNR nanocomposites is enhanced significantly.

Keywords: Nanocomposite; Fe3O4–Au nanorod; Surface plasmon resonance (SPR); Goat IgM

Sahar Moradi-Monfared[a], Vikram Krishnamurthy[a], Bruce Cornell[b]. ([a] Department of Electrical and Computer Engineering, University of British Columbia, 5500 - 2332 Main Mall, Vancouver, BC, V6 T 1Z4, Canada, [b] Surgical Diagnostics Ltd., St Leonards, NSW 2065, Australia). A molecular machine biosensor: Construction, predictive models and experimental studies. Biosensors and Bioelectronics, Vol. 34(1) (2012): 261–266

This paper describes the construction, operation and predictive modeling of a molecular machine, functioning as a high sensitivity biosensor. Embedded gramicidin A (gA) ionchannels in a self-assembled tethered lipid bilayer act as biological switches in response to target molecules and provide a signal amplification mechanism that results in high sensitivity molecular detection. The biosensor can be used as a rapid and sensitive point of care diagnostic device in different media such as human serum, plasma and whole blood without the need for pre and post processing steps required in an enzyme-linked immunosorbent assay. The electrical reader of the device provides the added advantage of objective measurement. Novel ideas in the construction of the molecular machine, including fabrication of biochip arrays, and experimental studies of its ability to detect analyte molecules over a wide range of concentrations are presented. Remarkably, despite the complexity of the device, it is shown that the response can be predicted by modeling the analyte fluid flow and surface chemical reactions. The derived predictive models for the sensing dynamics also facilitate determining important variables in the design of a molecular machine such as the ion channel lifetime and diffusion dynamics within the bilayer lipid membrane as well as the bio-molecular interaction rate constants.

Keywords: Chemically modified electrode; Ion channel biosensor; Molecular detection; Surface based chemical reactions; Fluid flow dynamics; Compartment modeling

Pie Pichetsurnthorn[a, 1], Krishna Vattipalli[b, 1], Shalini Prasad[b]. ([a] Bioengineering Program, College of Engineering, 1745 N. Fairmount St., Wichita State University, Wichita, KS 67260, USA, [b] Department of Bioengineering, The University of Texas at Dallas, 800 W. Campbell Rd., Richardson, TX 75080, USA). Nanoporous impedemetric biosensor for detection of trace atrazine from water samples. Biosensors and Bioelectronics, Vol. 32(1) (2012): 155–162

Trace contamination of ground water sources has been a problem ever since the introduction of high-soil-mobility pesticides, one such example is atrazine. In this paper we present a novel nanoporous portable bio-sensing device that can identify trace contamination of atrazine through a label-free assay. We have designed a pesticide sensor comprising of a nanoporous alumina membrane integrated with printed circuit board platform. Nanoporous alumina in the biosensor

device generates a high density array of nanoscale confined spaces. By leveraging the size based immobilization of atrazine small molecules we have designed electrochemical impedance spectroscopy based biosensor to detect trace amounts of atrazine. We have calibrated the sensor using phosphate buffered saline and demonstrated trace detection from river and bottled drinking water samples. The limit of detection in all the three cases was in the femtogram/mL (fg/mL) (parts-per-trillion) regime with a dynamic range of detection spanning from 10 fg/mL to 1 ng/mL (0.01 ppt to 1 ppm). The selectivity of the device was tested using a competing pesticide; malathion and selectivity in detection was observed in the fg/mL regime in all the three cases.

Keywords: Trace atrazine detection; River water; Drinking water; Electrochemical impedance spectroscopy; Nanoporous alumina; Label-free detection

Meihe Zhang, Ruo Yuan, Yaqin Chai, Shihong Chen, Huaan Zhong, Cun Wang, Yinfeng Cheng. (Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China). A biosensor for cholesterol based on gold nanoparticles-catalyzed luminol electrogenerated chemiluminescence. Biosensors and Bioelectronics, Vol.32(1) (2012): 288–292

A novel cholesterol biosensor was prepared based on gold nanoparticles-catalyzed luminol electrogenerated chemiluminescence (ECL). Firstly, l-cysteine-reduced graphene oxide composites were modified on the surface of a glassy carbon electrode. Then, gold nanoparticles (AuNPs) were self-assembled on it. Subsequently, cholesterol oxidase (ChOx) was adsorbed on the surface of AuNPs to construct a cholesterol biosensor. The stepwise fabrication processes were characterized with cyclic voltammetry and atomic force microscopy. The ECL behaviors of the biosensor were also investigated. It was found that AuNPs not only provided larger surface area for higher ChOx loading but also formed the nano-structured interface on the electrode surface to improve the analytical performance of the ECL biosensor for cholesterol. Besides, based on the efficient catalytic ability of AuNPs to luminol ECL, the response of the biosensor to cholesterol was linear range from 3.3 μM to 1.0 mM with a detection limit of 1.1 μM (S/N = 3). In addition, the prepared ECL biosensor exhibited satisfying reproducibility, stability and selectivity. Taking into account the advantages of ECL, we confidently expect that ECL would have potential applications in biotechnology and clinical diagnosis.

Keywords: Cholesterol biosensor; Reduced graphene oxide; Luminol; Gold nanoparticles; l-cysteine

Lucia Santorufo[a], Cornelis A.M. Van Gestel[b], Annamaria Rocco[a], Giulia Maisto[a]. ([a]Department of Structural and Functional Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia, 80126 Naples, Italy, [b] Department Animal Ecology, Faculty of Earth and Life Sciences, VU University Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands). Soil invertebrates as bioindicators of urban soil quality. Environmental Pollution, Vol. 161(2012) : 57–63

This study aimed at relating the abundance and diversity of invertebrate communities of urban soils to chemical and physical soil characteristics and to identify the taxa most sensitive or tolerant to soil stressors. The invertebrate community of five urban soils in Naples, Italy, was

sampled. To assess soil quality invertebrate community indices (Shannon, Simpson, Menhinick and Pielou indices), Acarina/Collembola ratios, and the soil biological quality index (QBS) were calculated. The chemical and physical characteristics of the soils strongly differed. Abundance rather than taxa richness of invertebrates were more affected by soil characteristics. The community was more abundant and diverse in the soils with high organic matter and water content and low metal (Cu, Pb, Zn) concentrations. The taxa more resistant to the urban environment included Acarina, Enchytraeids, Collembola and Nematoda. Collembolans appeared particularly sensitive to changing soil properties. Among the investigated indices, QBS seems most appropriate for soil quality assessment.

Keywords: Soil metal contamination; Biodiversity indices; Arthropoda; Enchytraeidae; Soil properties

Bioengineering

S. Antony Ceasar and S. Ignacimuthu. Genetic engineering of crop plants for fungal resistance: role of antifungal genes. Biotechnology Letters, Vol.34(6) (2012): 995-1002

Fungal diseases damage crop plants and affect agricultural production. Transgenic plants have been produced by inserting antifungal genes to confer resistance against fungal pathogens. Genes of fungal cell wall-degrading enzymes, such as chitinase and glucanase, are frequently used to produce fungal-resistant transgenic crop plants. In this review, we summarize the details of various transformation studies to develop fungal resistance in crop plants.

Keywords: *Agrobacterium*-mediated – Chitinase – Fungal resistance – Glucanase – Transgenic plants

Pollen Biotechnology

Suzuki, K., Yang, L., Takaiwa, F. (Functional Crop Research and Development Unit, National Institute of Agrobiological Science, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan). Transgenic rice accumulating modified cedar pollen allergen Cry j 2 derivatives. Journal of Bioscience and Bioengineering, Vol. 113(2) (2012): 249-251

In order to create a safe tolerogenic antigen with reduced IgE reactivity, we developed transgenic rice that accumulates in the seed endosperm a sufficient amount of Cry j 2, the cedar pollen allergen, in a restructured form of tail-to-head, providing a feasible mucosal allergy vaccine against cedar pollinosis.

Keywords: Cedar pollen allergen; Cry j 2; Pollinosis; Tolerogen; Transgenic rice

McClure, B. (Department of Biochemistry, University of Missouri, 117 Schweitzer Hall, Columbia, MO 65211, United States). Plant self-incompatibility: Ancient system becomes a new tool. Current Biology, Vol. 22(3) (2012): R86-R87

Expressing a pollen self-incompatibility gene from Papaver rhoeas (poppy) in Arabidopsis thaliana renders the latter sensitive to an exquisitely precise induced cell death response. This simple system may have wide application in biotechnology and research.

Ohkouchi, K.[a], Kawamoto, S.[a] , Tatsugawa, K.[a], Yoshikawa, N.[a], Takaoka, Y.[a], Miyauchi, S.[a], Aki, T.[a], Yamashita, M.[b], Murooka, Y.[c], Ono, K.[a]. ([a] Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8530, Japan, [b] Department of Applied Chemistry, College of Engineering, Shibaura Institute of Technology, 3-7-5 Toyosu, Koto-ku, Tokyo 135-8548, Japan, [c] Department of Health Science, Faculty of Applied Information Science, Hiroshima Institute of Technology, 2-1-1 Miyake, Saiki-ku, Hiroshima 731-5193, Japan). Prophylactic effect of Lactobacillus oral vaccine expressing a Japanese cedar pollen allergen. Journal of Bioscience and Bioengineering, Vol. 113(4) (2012): 536-541

Lactic acid bacteria (LAB) represent an attractive delivery vehicle for oral allergy vaccine because of their safety as a food microorganism as well as their potent adjuvant activity triggering anti-allergic immune response. Here, we report the generation of recombinant LAB expressing a major Japanese cedar pollenallergen Cry j 1 (Cry j 1-LAB), and their prophylactic effect in vivo. To facilitate heterologous expression, the codon usage in the Cry j 1 gene was optimized for the host LAB strain Lactobacillus plantarum by the recursive PCR-based exhaustive site-directed mutagenesis. Use of the codon-optimized Cry j 1 cDNA and a lactate dehydrogenase gene fusion system led to a successful production of recombinant Cry j 1 in L. plantarum NCL21. We also found that oral vaccination with the Cry j 1-LAB suppressed allergen-specific IgE response and nasal symptoms in a murine model of cedar pollinosis.

Keywords: Allergen; Codon optimization; IgE; Japanese cedar pollen; Lactobacillus

Biotechnology Policy Issue

Bahar Celikkol Erbas*[1] · Selin Arslanhan Memis[2]. ([1] TOBB University of Economics and Technology, Department of Economics, Sogutozu, Ankara, Turkey, [2] Economic Policy Research Foundation of Turkey, Sogutozu, Ankara, Turkey. *Corresponding author: bcelikkol@etu.edu.tr). An economic valuation of a biotechnology R&D project in a developing economy. Electronic Journal of Biotechnology, Vol. 15(3) (2012): 1-1

Biotechnology complements technological developments in main sectors of economies, such as health, energy, and agriculture, and thus contributes to economic development. It provides solutions to the problems that are frequently faced in developing economies, such as resource constraints, lower productivity and environmental concerns. In order to benefit from biotechnology, its associated markets need to develop and function well to support the developments and transactions of intangible assets, such as technology transfers, license agreements and research and development joint ventures. Economic valuation of the intangible assets is necessary for the development and functioning of these markets. It provides better

understanding of value creation at micro scales and its economic and financial dynamics. The literature lacks valuation studies in biotechnology sectors in developing economies. This study performs economic valuation analysis of a research and development project of a Turkish biotechnology company operating in health sector. Turkey, as a developing economy, has slow progress in biotechnology despite its wealth of biological resources and genetic variety. Thus, the study provides an excellent case to analyze valuation issues in developing economies. It uses data from in-depth interviews from the company and employs real options and discounted cash flow (DCF) methods.

Developing countries and biotechnology sector introduce additional risks that need to be accounted for in valuation. These risks reduce the value of the project under real options and discounted cash flow methods. Since real options method permits the valuation of options that might arise during the R&D process and provides flexibility to managers to act, it results in higher values compared to discounted cash flow method. The grant from a public institution that partially financed the Project reduces the discount factor and thus increases the value of it. Economic values of biotechnology intangibles in developing countries are affected by country and sector risks and public financing. Thus, both microeconomic and macroeconomic policy interventions are important for the development of biotechnology in these economies. While public financing enables the risky R&D projects to take place, it makes them more valuable than they would be under no intervention. Long run effects of these interventions require diligent analyses.

Keywords: biotechnology sector, developing economies, real options, R&D project valuation, Turkey.

Agricultural Biotechnology

Parvaiz Ahmad[a], Muhammad Ashraf[b, f], Muhammad Younis[c], Xiangyang Hu[d], Ashwani Kumar[e], Nudrat Aisha Akram[b], F. Al-Qurainy[f]. ([a] Department of Botany, A.S. College, 190008, University of Kashmir, Srinagar, India, [b] Department of Botany, University of Agriculture, Faisalabad, Pakistan, [c] Department of Biotechnology, IIT, Delhi, Hauz Khas, New Delhi 110016, India, [d] Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Institute of Tibet Plateau Research at Kunming, Chinese Academy of Sciences, Kunming 650204, China, [e] Biochemistry Lab, CRDT, IIT, Delhi, Hauz Khas, New Delhi 110016, India, [f] Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia). **Role of transgenic plants in agriculture and biopharming. Biotechnology Advances, Vol. 30(3) (2012): 524–540**

At present, environmental degradation and the consistently growing population are two main problems on the planet earth. Fulfilling the needs of this growing population is quite difficult from the limited arable land available on the globe. Although there are legal, social and political barriers to the utilization of biotechnology, advances in this field have substantially improved agriculture and human life to a great extent. One of the vital tools of biotechnology is genetic engineering (GE) which is used to modify plants, animals and microorganisms according to desired needs. In fact, genetic engineering facilitates the transfer of desired characteristics into other plants which is not possible through conventional plant breeding. A variety of crops have

been engineered for enhanced resistance to a multitude of stresses such as herbicides, insecticides, viruses and a combination of biotic and abiotic stresses in different crops including rice, mustard, maize, potato, tomato, etc. Apart from the use of GE in agriculture, it is being extensively employed to modify the plants for enhanced production of vaccines, hormones, etc. Vaccines against certain diseases are certainly available in the market, but most of them are very costly. Developing countries cannot afford the disease control through such cost-intensive vaccines. Alternatively, efforts are being made to produce edible vaccines which are cheap and have many advantages over the commercialized vaccines. Transgenic plants generated for this purpose are capable of expressing recombinant proteins including viral and bacterial antigens and antibodies. Common food plants like banana, tomato, rice, carrot, etc. have been used to produce vaccines against certain diseases like hepatitis B, cholera, HIV, etc. Thus, the up- and down-regulation of desired genes which are used for the modification of plants have a marked role in the improvement of genetic crops. In this review, we have comprehensively discussed the role of genetic engineering in generating transgenic lines/cultivars of different crops with improved nutrient quality, biofuel production, enhanced production of vaccines and antibodies, increased resistance against insects, herbicides, diseases and abiotic stresses as well as the safety measures for their commercialization.

Keywords: Biopharming; Transgenics; Stress tolerance; Antibodies; Vaccines

Bioenergy

Torbjørn Ølshøj Jensen, Thomas Kvist, Marie Just Mikkelsen, Peter Vittrup Christensen and Peter Westermann. Fermentation of crude glycerol from biodiesel production by *Clostridium pasteurianum*. Journal of Industrial Microbiology & Biotechnology, Vol. 39(5) (2012): 709-717

Clostridium pasteurianum can utilize glycerol as the sole carbon source for the production of butanol and 1,3-propanediol. Crude glycerol derived from biodiesel production has been shown to be toxic to the organism even in low concentrations. By examination of different pretreatments we found that storage combined with activated stone carbon addition facilitated the utilization of crude glycerol. A pH-controlled reactor with in situ removal of butanol by gas stripping was used to evaluate the performance. The fermentation pattern on pretreated crude glycerol was quite similar to that on technical grade glycerol. *C. pasteurianum* was able to utilize 111 g/l crude glycerol. The average consumption rate was 2.49 g/l/h and maximum consumption rate was 4.08 g/l/h. At the maximal glycerol consumption rate butanol was produced at 1.3 g/l/h. These rates are higher than those previously reported for fermentations on technical grade glycerol by the same strain. A process including pretreatment and subsequent fermentation of the crude glycerol could be usable for industrial production of butanol by *C. pasteurianum.*

Keywords: Glycerol – Pretreatment – Biofuel – Butanol – Anaerobic fermentation

Harinder Singh Oberoi, Neha Babbar, Simranjeet Kaur Sandhu, Sandeep Singh Dhaliwal and Ujjal Kaur, et al. Ethanol production from alkali-treated rice straw via simultaneous saccharification and fermentation using newly isolated thermotolerant *Pichia kudriavzevii* HOP-1. Journal of Industrial Microbiology & Biotechnology, Vol.39(4) (2012): 557-566

In this study, simultaneous saccharification and fermentation (SSF) was employed to produce ethanol from 1% sodium hydroxide-treated rice straw in a thermostatically controlled glass

reactor using 20 FPU gds^{-1} cellulase, 50 IU gds^{-1} β-glucosidase, 15 IU gds^{-1} pectinase and a newly isolated thermotolerant *Pichia kudriavzevii* HOP-1 strain. Scanning electron micrograph images showed that the size of the *P. kudriavzevii* cells ranged from 2.48 to 6.93 μm in diameter while the shape of the cells varied from oval, ellipsoidal to elongate. *Pichia kudriavzevii* cells showed extensive pseudohyphae formation after 5 days of growth and could assimilate sugars like glucose, sucrose, galactose, fructose, and mannose but the cells could not assimilate xylose, arabinose, cellobiose, raffinose, or trehalose. In addition, the yeast cells could tolerate up to 40% glucose and 5% NaCl concentrations but their growth was inhibited at 1% acetic acid and 0.01% cyclohexamide concentrations. *Pichia kudriavzevii* produced about 35 and 200% more ethanol than the conventional *Saccharomyces cerevisiae* cells at 40 and 45°C, respectively. About 94% glucan in alkali-treated rice straw was converted to glucose through enzymatic hydrolysis within 36 h. Ethanol concentration of 24.25 g l^{-1} corresponding to 82% theoretical yield on glucan basis and ethanol productivity of 1.10 g l^{-1} h^{-1} achieved using *P. kudriavzevii* during SSF hold promise for scale-up studies. An insignificant amount of glycerol and no xylitol was produced during SSF. To the best of our knowledge, this is the first study reporting ethanol production from any lignocellulosic biomass using *P. kudriavzevii*.

Keywords: Ethanol productivity – *Pichia kudriavzevii* – Rice straw – Simultaneous saccharification and fermentation – Sodium hydroxide

Yuan Lu, Chong Zhang, Hongxin Zhao and Xin-Hui Xing. Improvement of Hydrogen Productivity by Introduction of NADH Regeneration Pathway in *Clostridium paraputrificum.* Applied Biochemistry and Biotechnology, Vol.167(4) (2012): 732-742

To improve the hydrogen productivity and examine the hydrogen evolution mechanism of *Clostridium paraputrificum*, roles of formate in hydrogen evolution and effects of introducing formate-originated NADH regeneration were explored. The formate-decomposing pathway for hydrogen production was verified to exist in *C. paraputrificum*. Then NAD^+-dependent formate dehydrogenase FDH1 gene (*fdh1*) from *Candida boidinii* was overexpressed, which regenerate more NADH from formate to form hydrogen by NADH-mediated pathway. With *fdh1* overexpression, the hydrogen yield via NADH-involving pathway increased by at least 59 % compared with the control. Accompanied by the change of hydrogen metabolism, the whole cellular metabolism was redistributed greatly.

Keywords: Anaerobic fermentation – *Clostridium paraputrificum* – Formate dehydrogenase – Hydrogen production – NADH regeneration

Rajni Kumari and K. Pramanik. Improved Bioethanol Production Using Fusants of *Saccharomyces cerevisiae* and Xylose-Fermenting Yeasts. Applied Biochemistry and Biotechnology, Vol. 167(4) (2012): 873-884

The present research deals with the development of a hybrid yeast strain with the aim of converting pentose and hexose sugar components of lignocellulosic substrate to bioethanol by fermentation. Different fusant strains were obtained by fusing protoplasts of *Saccharomyces cerevisiae* and xylose-fermenting yeasts such as *Pachysolen tannophilus*, *Candida shehatae* and *Pichia stipitis*. The fusants were sorted by fluorescent-activated cell sorter and further confirmed by molecular characterization. The fusants were evaluated by fermentation of glucose–xylose

mixture and the highest ethanol producing fusant was used for further study to ferment hydrolysates produced by acid pretreatment and enzymatic hydrolysis of cotton gin waste. Among the various fusant and parental strains used under present study, RPR39 was found to be stable and most efficient strain giving maximum ethanol concentration (76.8±0.31 g L^{-1}), ethanol productivity (1.06 g L^{-1} h^{-1}) and ethanol yield (0.458 g g^{-1}) by fermentation of glucose–xylose mixture under test conditions. The fusant has also shown encouraging result in fermenting hydrolysates of cotton gin waste with ethanol concentration of 7.08±0.142 g L^{-1}, ethanol yield of 0.44 g g^{-1}, productivity of 0.45 g L^{-1} h^{-1} and biomass yield of 0.40 g g^{-1}.

Keywords: *S. cerevisiae* – Protoplast – Fusant – Ethanol fermentation – Cotton gin waste – FACS – RAPD

Ye Ni, Yun Wang and Zhihao Sun. Butanol Production from Cane Molasses by *Clostridium saccharobutylicum* DSM 13864: Batch and Semicontinuous Fermentation. Applied Biochemistry and Biotechnology, Vol.166(8) (2012): 1896-1907

Clostridium acetobutylicum strains used in most Chinese ABE (acetone–butanol–ethanol) plants favorably ferment starchy materials like corn, cassava, etc., rather than sugar materials. This is one major problem of ABE industry in China and significantly limits the exploitation of cheap waste sugar materials. In this work, cane molasses were utilized as substrate in ABE production by *Clostridium saccharobutylicum* DSM 13864. Under optimum conditions, total solvent of 19.80 g/L (13.40 g/L butanol) was reached after 72 h of fermentation in an Erlenmeyer flask. In a 5-L bioreactor, total solvent of 17.88 g/L was attained after 36 h of fermentation, and the productivity and yield were 0.50 g/L/h and 0.33 g ABE/g sugar consumption, respectively. To further enhance the productivity, a two-stage semicontinuous fermentation process was steadily operated for over 8 days (205 h, 26 cycles) with average productivity (stage II) of 1.05 g/L/h and cell concentration (stage I) of 7.43 OD_{660}, respectively. The average batch fermentation time (stage I and II) was reduced to 21−25 h with average solvent of 15.27 g/L. This study provides valuable process data for the development of industrial ABE fermentation process using cane molasses as substrate.

Keywords: Acetone–butanol–ethanol – Fermentation – Cane molasses – *Clostridium saccharobutylicum* – Semicontinuous

Lianhua Li, Xiaoying Kong, Fuyu Yang, Dong Li and Zhenhong Yuan, et al. Biogas Production Potential and Kinetics of Microwave and Conventional Thermal Pretreatment of Grass. Applied Biochemistry and Biotechnology, Vol. 166(5) (2012): 183-1191

Pretreatment methods play an important role in the improvement of biogas production from the anaerobic digestion of energy grass. In this study, conventional thermal and microwave methods were performed on raw material, namely, *Pennisetum* hybrid, to analyze the effect of pretreatment on anaerobic digestion by the calculation of performance parameters using Logistic function, modified Gompertz equation, and transference function. Results indicated that thermal pretreatment improved the biogas production of *Pennisetum* hybrid, whereas microwave method had an adverse effect on the performance. All the models fit the experimental data with R^2>0.980, and the Reaction Curve presented the best agreement in the fitting process. Conventional thermal pretreatment showed an increasing effect on maximum production rate and total methane produced, with an improvement of around 7% and 8%, respectively. With

regard to microwave pretreatment, maximum production rate and total methane produced decreased by 18% and 12%, respectively.

Keywords: *Pennisetum* hybrid – Anaerobic fermentation – Mathematical model – Pretreatment

Xin Wang, Zhang Cai, Qixing Zhou*, Zhineng Zhang, Cuihong Chen. Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells. Biotechnology and Bioengineering, Vol. 109(2) (2012): 426–433

Bioremediation is a cost-effective and eco-friendly approach to decontaminate soils polluted by petroleum hydrocarbons. However, this technique usually requires a long time due to the slow degradation rate by bacteria. By applying U-tube microbial fuel cells (MFCs) designed here, the degradation rate of petroleum hydrocarbons close to the anode (<1 cm) was enhanced by 120% from 6.9±2.5% to 15.2±0.6% with simultaneous 125±7C of charge output (0.85±0.05mW/m^2, 1kΩ) in the tested period (25 days). Hydrocarbon fingerprint analysis showed that the degradation rate of both alkanes and polycyclic aromatic hydrocarbons (PAHs) was accelerated. The decrease of initial water content from 33% to 28% and 23% resulted in a decrease on charge output and hydrocarbon degradation rate, which could be attributed to the increase of internal resistance. A salt accumulation was observed in each reactor due to the evaporation of water from the air-cathode, possibly inhibited the activity of exoelectrogenic bacteria (EB) and resulted in the elimination of the current at the end of the tested period. The number of hydrocarbon degradation bacteria (HDB) in soil close to the anode increased by nearly two orders of magnitude in the MFC assisted system ($373\pm56\times10^3$CFU/g-soil) than that in the disconnected control ($8\pm2\times10^3$ CFU/g-soil), providing a solid evidence for in situ biostimulation of HDB growth by colonization of EB in the same system.

Keywords: petroleum hydrocarbon; biostimulation; microbial fuel cells (MFCs); saline soil; polycyclic aromatic hydrocarbons (PAHs)

Fabien Durand[a], Christian Hauge Kjaergaard[b], Emmanuel Suraniti[a], Sébastien Gounel[a], Ryan G. Hadt[b], Edward I. Solomon[b], Nicolas Mano[a]. ([a] CRPP-UPR 8641, Univ. Bordeaux, F-33600, Pessac, France, [b] Department of Chemistry, Stanford University, Stanford, CA, 94305, USA). Bilirubin oxidase from *Bacillus pumilus*: A promising enzyme for the elaboration of efficient cathodes in biofuel cells. Biosensors and Bioelectronics, Vol. 35(1) (2012): 140–146

A CotA multicopper oxidase (MCO) from *Bacillus pumilus*, previously identified as a laccase, has been studied and characterized as a new bacterial bilirubin oxidase (BOD). The 59 kDa protein containing four coppers, was successfully over-expressed in *Escherichia coli* and purified to homogeneity in one step. This 509 amino-acid enzyme, having 67% and 26% sequence identity with CotA from *Bacillus subtilis* and BOD from *Myrothecium verrucaria*, respectively, shows higher turnover activity towards bilirubin compared to other bacterial MCOs. The current density for O_2 reduction, when immobilized in a redox hydrogel, is only 12% smaller than the current obtained with *Trachyderma tsunodae* BOD. Under continuous electrocatalysis, an electrode modified with the new BOD is more stable, and has a higher tolerance towards NaCl, than a *T. tsunodae* BOD modified electrode. This makes BOD from *B. pumilus* an attractive new candidate for application in biofuel cells (BFCs) and biosensors.

Keywords: Biofuel cells; Bilirubin oxidase; Oxygen reduction; Bacillus pumilus; Osmium polymer

Lingling Zhang[1], Ming Zhou[1], Dan Wen, Lu Bai, Baohua Lou, Shaojun Dong. (State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China). Small-size biofuel cell on paper. Biosensors and Bioelectronics, Vol. 35(1) (2012): 155–159

In this work, we demonstrated a novel paper-based mediator-less and compartment-less biofuel cell (BFC) with small size (1.5 cm × 1.5 cm). Ionic liquid functionalized carbon nanotubes (CNTs-IL) nanocomposite was used as support for both stably confining the anodic biocatalyst (i.e., NAD^+-dependent glucose dehydrogenase, GDH) for glucose electrooxidation and for facilitating direct electrochemistry of the cathodic biocatalyst (i.e., bilirubin oxidase, BOD) for O_2 electroreduction. Such BFC provided a simple approach to fabricate low-cost and portable power devices on small-size paper, which can harvest energy from a wide range of commercial beverages containing glucose (e.g., Nescafe instant coffee, Maidong vitamin water, Watermelon fresh juice, and Minute Maid grape juice). These made the low-cost paper-based biodevice potential for broad energy applications.

Keywords: Biofuel cells; Paper; Carbon nanotubes; Ionic liquid; Enzyme

Shoubao Yan, Xiangsong Chen, Jingyong Wu and Pingchao Wang. Ethanol production from concentrated food waste hydrolysates with yeast cells immobilized on corn. stalk Applied Microbiology and Biotechnology, Vol. 94(3) (2012): 829-838

The aim of the present study was to examine ethanol production from concentrated food waste hydrolysates using whole cells of *S. cerevisiae* immobilized on corn stalks. In order to improve cell immobilization efficiency, biological modification of the carrier was carried out by cellulase hydrolysis. The results show that proper modification of the carrier with cellulase hydrolysis was suitable for cell immobilization. The mechanism proposed, cellulase hydrolysis, not only increased the immobilized cell concentration, but also disrupted the sleek surface to become rough and porous, which enhanced ethanol production. In batch fermentation with an initial reducing sugar concentration of 202.64±1.86 g/l, an optimal ethanol concentration of 87.91±1.98 g/l was obtained using a modified corn stalk-immobilized cell system. The ethanol concentration produced by the immobilized cells was 6.9% higher than that produced by the free cells. Ethanol production in the 14th cycle repeated batch fermentation demonstrated the enhanced stability of the immobilized yeast cells. Under continuous fermentation in an immobilized cell reactor, the maximum ethanol concentration of 84.85 g/l, and the highest ethanol yield of 0.43 g/g (of reducing sugar) were achieved at hydraulic retention time (HRT) of 3.10 h, whereas the maximum volumetric ethanol productivity of 43.54 g/l/h was observed at a HRT of 1.55 h.

Keywords: Corn stalk – Food waste – Immobilization – Ethanol fermentation – Immobilized cell reactor

Baohua Zhang, Yanqing Weng, Hong Xu and Zhiping Mao. Enzyme immobilization for biodiesel production. Applied Microbiology and Biotechnology, Vol. 93(1) (2012): 61-70

Biodiesel has attracted more and more attention in recent years because of its biodegradability, environmentally friendliness, and renewability. Contrary to the conventional chemical catalysis

method to produce biodiesel, the biochemical catalysis method developed quickly in the past decade and many immobilized enzymes are commercially available to meet the large-scale industrialization of biodiesel. This review is focusing on the current status of biodiesel production by biochemical catalysis method, especially the commercial enzyme and its immobilization for biodiesel production. Consequently, we believe that biochemical catalysis with immobilized enzymes is bound to be an alternative method instead of chemical catalysis in biodiesel production in the near future.

Keywords: Biochemical catalysis – Biodiesel production – Enzyme – Immobilization – Review

Stijn Cornelissen[1], Michèle Koper, Yvonne Y. Deng[2]. (Ecofys bv, P.O. Box 8408, 3503 RK Utrecht, The Netherlands). The role of bioenergy in a fully sustainable global energy system. Biomass and Bioenergy, Vol. 41(2012):21–33

We present a detailed analysis of the supply potential and use of biomass in the context of a transition to a fully renewable global energy system by 2050. We investigate bioenergy potential within a framework of technological choices and sustainability criteria, including criteria on land use and food security, agricultural and processing inputs, complementary fellings, residues and waste. This makes our approach more comprehensive, more stringent in the applied sustainability criteria and more detailed on both the supply potential and the demand side use of biomass than that of most other studies.

We find that the potential for sustainable bioenergy from residues and waste, complementary fellings, energy crops and algae oil in 2050 is 340 EJ a^{-1} of primary energy. This potential is then compared to the demand for biomass-based energy in the demand scenario related to this study, the Ecofys Energy Scenario. This scenario, after applying energy efficiency and non-bioenergy renewable options, requires a significant contribution of bioenergy to meet the remaining energy demand; 185 EJ a^{-1} of the 340 EJ a^{-1} potential supply. For land use for energy crops, we find that a maximum of 2,500,000 km^2 is needed of a 6,730,000 km^2 sustainable potential. For greenhouse gas emissions from bioenergy, a 75%–85% reduction can be achieved compared to fossil references. We conclude that bioenergy can meet residual demand in the Ecofys Energy Scenario sustainably with low associated greenhouse gas emissions. It thus contributes to its achievement of a 95% renewable energy system globally by 2050.

Keywords: Bioenergy; Potential; Sustainability; Land use; Biofuel

Hifjur Raheman, Subhrajit Mondal. (Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur 721302, India). Biogas production potential of jatropha seed cake. Biomass and Bioenergy, Vol. 37(2012): 25–30

Jatropha seed cake (JSC) was anaerobically digested at different total solid contents (TS) and carbon to nitrogen (C:N) ratios in batch type digesters with 40 days hydraulic retention time (HRT). Biogas production from kg of TS was found to be maximum i.e. 0.17 m^3 at 20% TS of JSC slurry followed by 15%, 25% and 10% TS in that order as compared to 0.166 m^3 in case of cow dung (CD) slurry alone. Higher gas production from JSC slurry was observed when the carbon to nitrogen ratio (C:N) ratio was between 22:1 to 27:1 (by adding different quantity of paddy straw). Further gas production from kg of TS of the mixture of JSC and CD was higher than that produced from JSC/CD slurry alone and was maximum when JSC percentage in the

mixture was within 25%. The Nitrogen content in the biodigested JSC slurry was increased by 5.9% as compared to JSC alone and its use as fertilizer produced better growth of maize and tomato crops.

Keywords: Jatropha seed cake; Cow dung; Total solid content; C:N ratio; Biogas production; Fertilizer value

Poritosh Roy[a], Ken Tokuyasu[b], Takahiro Orikasa[c], Nobutaka Nakamura[b], Takeo Shiina[b]. ([a] School of Engineering, University of Guelph, 50 Stone Road East Guelph, Ontario N1G 2W1, Canada, [b] National Food Research Institute, National Agriculture and Food Research Organization, Japan, [c] School of Food, Agriculture and Environmental Sciences, Miyagi University, Japan). A techno-economic and environmental evaluation of the life cycle of bioethanol produced from rice straw by RT-CaCCO process. Biomass and Bioenergy, Vol. 37(2012): 188–195

Japan has set an ambitious goal to produce bioethanol from abundant biomass in views to offset some of her greenhouse gas (GHG) emissions. This study attempts to evaluate the life cycle of bioethanol produced from the most common variety of rice straw in Japan (*cv. Koshihikari*) by enzymatic hydrolysis. Three scenarios are established in the evaluation process. The net energy consumption, CO_2 emission and production costs are estimated to determine if environmentally friendly and economically viable bioethanol can be produced from rice straw in Japan. The net energy consumption, CO_2 emission and production costs are estimated to be 10.43–11.56 GJ/m^3, 1106.34–1144.94 kg/L and 88.54–137.55 k¥/m^3 (1 US$≈100¥), respectively depending on the scenarios of this study. This study reveals that despite a bit of environmental benefits, the economic viability is doubtful unless innovative technologies along with the renewable energy policy and stakeholders participation are considered. A shift in scenarios not only reduces the production cost, but may also minimize the risk of soil degradation and productivity loss and encourage more stakeholder participation and investment in the bioethanol industry in Japan.

Keywords: Rice straw; RT-CaCCO process; Bioethanol; Net energy consumption; CO2 emission; Production cost.

Mustafa Acaroğlu, Hasan Aydoğan. (Selçuk University, Technology Faculty, Mechanical Engineering Department, 42031 Kampus, Konya, Turkey). Biofuels energy sources and future of biofuels energy in Turkey. Biomass and Bioenergy, Vol. 36 (2012): 69–76

Today, in Turkey, the most commonly used renewable resources are classical biomass energy and hydraulic energy. Although geothermal energy ranks third, its usage is limited. The usage of solar energy is at a symbolic level, that of wind energy is in its initial stages and the idea of tidal energy is out of concern. In spite of the important potential of modern biomass energy, agriculture of energy plants is not widely recognized, and the establishment of energy forests is realized in a limited fashion. This study is based on two essential considerations about biofuels: agricultural potential of Turkey for biofuels production and governmental policies about environmental friendly alternative fuels in Turkey. A policy to increase biodiesel production in Turkey by importing biofuels or shifting to oilseed- biomass production from another plant does not seem reliable or practical.

As far as environmental pollution is concerned, the utilization of all fuels produces air pollutants, causing local, trans-boundary air pollution, and acid rain problems. Biofuel is a clean renewable fuel due to its properties, which is similar to diesel but generated from renewable resources such as vegetable oils, animal fats and energy crops. Although researchers developed the technology

of biofuel some two to three decades ago, its use is not widespread, mainly due to the higher production cost involved. Due to the increasing concern on environmental protection, the number of research studies conducted on the usage of this fuel has increased especially in recent years.

Keywords: Biomass; Biodiesel; Bioethanol; Energy potential; Turkey

Polycarpos Polycarpou. (Agricultural Research Institute, P.O. Box 22016, 1516 Lefkosia, Cyprus). Ethanol production from *Ferula communis*. Biomass and Bioenergy, Vol. 36(2012): 289–292

Mediterranean countries are faced with severe water shortage and unavailability of agricultural land that limit the cultivation of energy crops that supply the feedstock for biofuel production. A possibility would be to use *Ferula communis* that is encountered in Cyprus and other Mediterranean countries, growing wild in pastures. Its flower stalks contain sugars and starch that were measured to be 0.50–0.55 kg kg^{-1}, based on dry material. The ethanol is produced by fermentation of the juice extracted by crashing and pressing the flower stalks of the plant. The first stage of the process was cooking the juice at a temperature of 95 °C, combined by liquefaction and saccharification of the starch using enzymes, like alpha amylase and glucoamylase. The process was followed by fermentation of the juice for three days and finally distillation of ethanol. The alcohol yield per kilogram dry stalks was 55.8 cm^3 kg^{-1}, compared to the theoretical value of 57.3 cm^3 kg^{-1}, mainly due to the incomplete fermentation of the sugars. The plant seems to be a potential energy plant for ethanol production in arid regions cultivated on degraded land with minimal attention.

Keywords: Ferula communis; Bioethanol production; Energy plants; Ethanol; Biofuels from biomass

Elena Comino[a], Vincenzo A. Riggio[a], Maurizio Rosso[b]. ([a] Politecnico di Torino, Dipartimento di Ingegneria del Territorio dell'Ambiente e delle Geotecnologie, C.so Duca degli Abruzzi, 24, 10129 Turin, Italy, [b] Politecnico di Torino, Dipartimento di Idraulica, Trasporti e Infrastrutture Civili, C.so Duca degli Abruzzi, 24, 10129 Turin, Italy). Biogas production by anaerobic co-digestion of cattle slurry and cheese whey. Bioresource Technology, Vol.114(2012) : 46–53

Biogas yield of mixtures of cattle slurry and cheese whey, rates of production of methane, removal efficiencies of chemical oxygen demand (COD) and biological oxygen demand (BOD) were investigated at 35 °C. Stable biogas production of 621 l/kg volatile solids at a hydraulic retention time of 42 days in a mixture containing 50% slurry and whey was obtained. The concentration of methane in the biogas was around 55%. Maximum removal efficiencies for COD and BOD5 were 82% and 90%, respectively. A maximum biogas production increase of 79% with respect to the start-up phase was achieved. The result of this study show that co-digestion of a high volume of whey (up to 65% in volume) is possible without the use of chemicals for pH correction, but also that this kind of mix has a similar energetic potential for anaerobic digestion as energy crops such as maize.

Keywords: Anaerobic digestion; Methane yield; COD reduction; Digestate yield test; Energy production

Weizhang Zhong, Zhongzhi Zhang, Yijing Luo, Wei Qiao, Meng Xiao, Min Zhang. (State Key Laboratory of Heavy Oil Processing, College of Chemical Engineering, China University of Petroleum, Beijing 102249, PR China). Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. Bioresource Technology, Vol. 114(2012): 281–286

A batch anaerobic test was conducted to evaluate the effects of adding high carbon content of corn straw to the digestion of Taihu blue algae to attain an optimal C/N ratio for higher methane yield. The addition of corn straw in algae at a C/N ratio of 20/1 increased methane yield by 61.69% at 325 mL g^{-1} VS^{-1} (compared with 201 mL g^{-1} VS^{-1} of algae digestion alone), followed by C/N ratios of 16/1 and 25/1, all operated at 20 g VS L^{-1} and 35 °C. The results suggest the optimal C/N ratio for co-digestion of algae with corn straw is 20/1. The findings could offer options for efficient methane production and waste treatment.

Keywords: Anaerobic co-digestion; Biogas production; Taihu blue algae; Corn straw; C/N ratio

Yalini Arudchelvam, Nagamany Nirmalakhandan. (Civil Engineering Department, New Mexico State University, Las Cruces, NM 88003, United States). Optimizing net energy gain in algal cultivation for biodiesel production. Bioresource Technology, Vol. 114(2012) : 294–302

An approach based on energy gain was utilized to optimize algal cultivation in bubble columns. Net energy gain was estimated considering the energy input for mixing and providing carbon dioxide, and the energy that can be generated from the lipids extracted from the algal biomass. Energy input for sparging was minimized based on the gas-to-culture volume ratio and energy output from lipid production was maximized based on nitrate and CO_2 levels. Sparging at a gas-to-culture volume ratio of 0.18 min^{-1} with CO_2-enrichment of 0.5% and initial nitrate concentration of 1 mM was optimal for improving net energy gain with *Nannochloropsis salina*. Sparging with CO_2-enriched air of 0.5% along with nitrogen starvation resulted in 50% more lipid productivity than sparging with ambient air.

Keywords: Net energy gain; Gas-culture volume ratio; CO_2-air ratio; Nitrate starvation; Lipid productivity

J.C. Costa, P.R. Gonçalves, A. Nobre[1], M.M. Alves. (IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, 4710-057 Braga, Portugal). Biomethanation potential of macroalgae *Ulva* spp. and *Gracilaria* spp. and in co-digestion with waste activated sludge. Bioresource Technology, Vol. 114(2012) : 320–326

Biochemical methane potential of four species of *Ulva* and *Gracilaria* genus was assessed in batch assays at mesophilic temperature. The results indicate a higher specific methane production (per volatile solids) for one of the *Ulva* sp. compared with other macroalgae and for tests running with 2.5% of total solids (196 ± 9 L CH_4 kg^{-1} VS). Considering that macroalgae can potentially be a post treatment of municipal wastewater for nutrients removal, co-digestion of macroalgae with waste activated sludge (WAS) was assessed. The co-digestion of macroalgae (15%) with WAS (85%) is feasible at a rate of methane production 26% higher than WAS alone without decreasing the overall biodegradability of the substrate (42–45% methane yield). The use of anoxic marine sediment as inoculum had no positive effect on the methane production in batch assays. The limiting step of the overall anaerobic digestion process was the hydrolysis.

Keywords: Anaerobic digestion; Biochemical methane potential; Macroalgae; UIva sp; Waste activated sludge

E.N. Efremenko, A.B. Nikolskaya, I.V. Lyagin, O.V. Senko, T.A. Makhlis, N.A. Stepanov, O.V. Maslova, F. Mamedova, S.D. Varfolomeev. (Institute of Biochemical Physics, RAS, Kosygin Str. 4, Moscow 119334, Russia, Department of Chemistry, Lomonosov Moscow State University, Lenin Hills 1/3, Moscow 119991, Russia). Production of biofuels from pretreated microalgae biomass by anaerobic fermentation with immobilized *Clostridium acetobutylicum* cells. Bioresource Technology, Vol. 114(2012) : 342–348

The purpose of this work was to study the possible use of pretreated biomass of various microalgae and cyanobacteria as substrates for acetone–butanol–ethanol (ABE) fermentation by *Clostridium acetobutylicum* cells immobilized into poly(vinyl alcohol) cryogel. To this end, the biochemical composition of photosynthetic microorganisms cultivated under various conditions was studied. The most efficient technique for pretreating microalgal biomass for its subsequent conversion into biofuels appeared to be thermal decomposition at 108 °C. For the first time the maximum productivity of the ABE fermentation in terms of hydrogen (8.5 mmol/L medium/day) was obtained using pretreated biomass of *Nannochloropsis* sp. Maximum yields of butanol and ethanol were observed with *Arthrospira platensis* biomass used as the substrate. Immobilized *Clostridium* cells were demonstrated to be suitable for multiple reuses (for a minimum of five cycles) in ABE fermentation for producing biofuels from pretreated microalgal biomass.

Abbreviations: ABE fermentation, acetone–butanol–ethanol fermentation; PVA, poly(vinyl alcohol); DMSO, dimethyl sulfoxide; SDS, sodium dodecyl sulfate
Keywords: Biofuel; Microalgae; Anaerobic fermentation; Biochemical composition; Immobilized cells

Yinguang Chen, Naidong Xiao, Yuxiao Zhao, Hui Mu. (State Key Laboratory of Pollution Control and Resources Reuse, School of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China). Enhancement of hydrogen production during waste activated sludge anaerobic fermentation by carbohydrate substrate addition and pH control. Bioresource Technology, Vol. 114(2012): 349–356

The effects of carbohydrate/protein ratio (CH/Pr) and pH on hydrogen production from waste activated sludge (WAS) were investigated. Firstly, the optimal pH value for hydrogen production was influenced by the CH/Pr ratio, which was pH 10, 9, 8, 8, 8 and 6 at the CH/Pr ratio (COD based) of 0.2 (sole sludge), 1, 2.4, 3.8, 5 and 6.6, respectively. The maximal hydrogen production (100.6 mL/g-COD) was achieved at CH/Pr of 5 and pH 8, which was due to the synergistic effect of carbohydrate addition on hydrogen production, the enhancement of sludge protein degradation and protease and amylase activities, and the suitable fermentation pathway for hydrogen production. As hydrogen consumption was observed at pH 8, in order to further increase hydrogen production a two-step pH control strategy (pH 8 + pH 10) was developed and the hydrogen production was further improved by 17.6%.

Keywords: Carbohydrate/protein ratio (CH/Pr); pH; Hydrogen production; Two-step pH control

Haris Nalakath Abubackar, María C. Veiga, Christian Kennes. (Chemical Engineering Laboratory, Faculty of Sciences, University of La Coruña, Rúa da Fraga 10, 15008 La Coruña, Spain). Biological conversion of carbon monoxide to ethanol: Effect of pH, gas

pressure, reducing agent and yeast extract. Bioresource Technology, Vol. 114(2012) :518–522

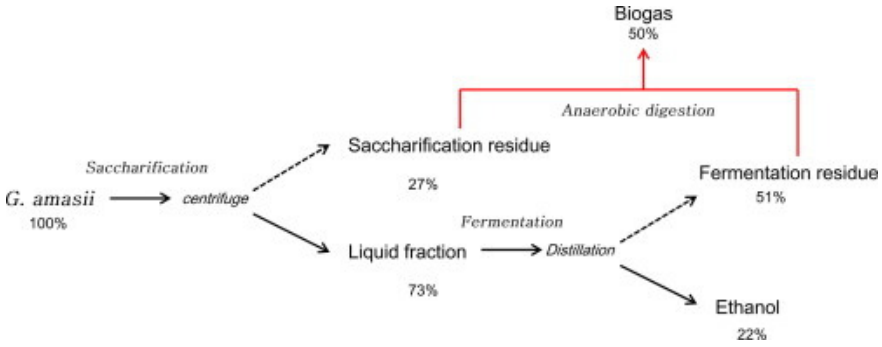
A two-level full factorial design was carried out in order to investigate the effect of four factors on the bioconversion of carbon monoxide to ethanol and acetic acid by *Clostridium autoethanogenum*: initial pH (4.75–5.75), initial total pressure (0.8–1.6 bar), cysteine–HCl·H_2O concentration (0.5–1.2 g/L) and yeast extract concentration (0.6–1.6 g/L). The maximum ethanol production was enhanced up to 200% when lowering the pH and amount yeast extract from 5.75 to 4.75 g/L and 1.6 to 0.6 g/L, respectively. The regression coefficient, regression model and analysis of variance (ANOVA) were obtained using MINITAB 16 software for ethanol, acetic acid and biomass. For ethanol, it was observed that all the main effects and the interaction effects were found statistically significant ($p < 0.05$). The comparison between the experimental and the predicted values was found to be very satisfactory, indicating the suitability of the predicted model.

Keywords: CO–bioconversion; Clostridium autoethanogenum; Factorial design; Medium optimization; Waste gas

Jeong-Hoon Park[a, b], Jeong-Jun Yoon[b], Hee-Deung Park[a], Dong Jung Lim[c], Sang-Hyoun Kim[d]. ([a] Department of Civil, Environmental and Architectural Engineering Korea University, Anam-Dong, Seongbuk-gu, Seoul 136-714, Republic of Korea, [b] Green Materials Technology Center, Korea Institute of Industrial Technology (KITECH), 35-3 Hongcheon-ri, Ipjang-myeon, Cheonan, Chungnam 330-825, Republic of Korea, [c] Biolsystems Co. Ltd., Joong Pyung B/D 6F 64-1, Umyeon-dong, Seocho-gu, Seoul 137-900, Republic of Korea, [d] Department of Environmental Engineering, Daegu University, Jillyang, Gyeongsan, Gyeongbuk 712-714, South Korea). Anaerobic digestibility of algal bioethanol residue. Bioresource Technology, Vol. 113(2012) : 78–82

The aim of this work was to investigate anaerobic digestibility of algal bioethanol residue from saccharification and fermentation processes. A series of batch anaerobic digestion tests using saccharification and fermentation residue showed that the maximum methane yields of saccharification residue and fermentation residue were 239 L/kg VS (Volatile Solids) and 283 L/kg VS (Volatile Solids), respectively. Energy recovered by anaerobic digestion of the residue was 2.24 times higher than that from the ethanol produced in the main process. 5-HMF (5-hydroxymethylfurfural), a saccharification byproduct, could retard methanogenesis at over 3 g/L however, the inhibition was prevented by increasing cell biomass concentration. Anaerobic digestion of residue has the potential to enhance bioenergy recovery and environmental sustainability of algal bioethanol production.

Graphical abstract



Keywords: Biogas; Macroalgal biomass; Saccharification; Fermentation; 5-Hydroxymethylfurfural (5-HMF)

Xue-fang Chen, Chao Huang, Lian Xiong, Xin-de Chen and Long-long Ma. Microbial oil production from corncob acid hydrolysate by *Trichosporon cutaneum.* Biotechnology Letters, Vol. 34(6) (2012): 1025-1028

Corncob was treated by dilute H_2SO_4. The hydrolysate contained 45.7 g sugar/l. Without concentration or adding other nutrients, the hydrolysate, after being detoxified by overliming and adsorption with activated charcoal, was used for oil production using *Trichosporon cutaneum.* After 8 days' growth in shake-flasks, the biomass was 22.1 g/l with a lipid content of 36%. The lipid yield per mass of sugar was 17.4% (w/w). Corncob thus is a promising raw material for microbial oil production by this yeast.

Keywords: Biodiesel – Corncob acid hydrolysate – Microbial oil – *Trichosporon cutaneum*

Hongyan Liu and Guangce Wang. Hydrogen production of a salt tolerant strain *Bacillus* sp. B2 from marine intertidal sludge. World Journal of Microbiology and Biotechnology, Vol. 28(1) (2012): 31-37

To isolate a salt tolerant hydrogen-producing bacterium, we used the sludge from the intertidal zone of a bathing beach in Tianjin as inoculum to enrich hydrogen-producing bacteria. The sludge was treated by heat-shock pretreatment with three different temperature (80, 100 and 121°C) respectively. A hydrogen-producing bacterium was isolated from the sludge pretreated at 80°C by sandwich plate technique and identified using microscopic examination and 16S rDNA gene sequence analysis. The isolated bacterium was named as *Bacillus* sp. B2. The present study examined the hydrogen-producing ability of *Bacillus* sp. B2. The strain was able to produce hydrogen over a wide range of initial pH from 5.0 to 10.0, with an optimum at pH 7.0. The level of hydrogen production was also affected by the salt concentration. Strain B2 has unique capability to adapt high salt concentration. It could produce hydrogen at the salt concentration from 4 to 60‰. The maximum of hydrogen-producing yield of strain B2 was 1.65 ± 0.04 mol H_2/mol glucose (mean ± SE) at an initial pH value of 7.0 in marine culture conditions. Hydrogen production under fresh culture conditions reached a higher level than that in marine ones. As a

result, it is likely that *Bacillus* sp. B2 could be applied to biohydrogen production using both marine and fresh organic waste.

Keywords: Intertidal zone sludge – Denaturing gradient gel electrophoresis (DGGE) – *Bacillus* sp. – Hydrogen production

Nano Biotechnology

M.K. Rai, S.D. Deshmukh, A.P. Ingle, A.K. Gade. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. Journal of Applied Microbiology. Volume 112, Issue 5, pages 841–852, May 2012

In the present scenario, pharmaceutical and biomedical sectors are facing the challenges of continuous increase in the multidrug-resistant (MDR) human pathogenic microbes. Re-emergence of MDR microbes is facilitated by drug and/or antibiotic resistance, which is acquired way of microbes for their survival and multiplication in uncomfortable environments. MDR bacterial infections lead to significant increase in mortality, morbidity and cost of prolonged treatments. Therefore, development, modification or searching the antimicrobial compounds having bactericidal potential against MDR bacteria is a priority area of research. Silver in the form of various compounds and bhasmas have been used in Ayurveda to treat several bacterial infections since time immemorial. As several pathogenic bacteria are developing antibiotic resistance, silver nanoparticles are the new hope to treat them. This review discusses the bactericidal potential of silver nanoparticles against the MDR bacteria. This multiactional nanoweapon can be used for the treatment and prevention of drug-resistant microbes.

Keywords: antimicrobial; methicillin-resistant Staphylococcus aureus; multidrug resistance; nanoweapon; silver nanoparticles; vancomycin-resistant Staphylococcus aureus

Guillaume P. Gruère. (International Food Policy Research Institute, 2033 K Street NW, Washington, DC 20006-1002, USA). Implications of nanotechnology growth in food and agriculture in OECD countries. Food Policy, Vol. 37(2) (2012): 191–198

This article provides an analysis of the implications of the growth of nanotechnology in the agriculture and food sector in OECD countries. Three main policy challenges are identified related to funding and investment, risk governance, and public acceptance. Each of these interconnected challenges underlines a number of ethical questions that need to be addressed. Several recommendations are laid out to move forward and adapt to these emerging policy issues.

Keywords: Nanotechnology; Governance; Ethical considerations; OECD

Karthik, R. , Harish Nagarajan, R. , Raja, B. , Damodharan, P. (Indian Institute of Information Technology, Design and Manufacturing (IIITD and M), Kancheepuram, Chennai 600 048, India). Thermal conductivity of CuO-DI water nanofluids using 3-ω measurement technique in a suspended micro-wire. Experimental Thermal and Fluid Science, Vol. 40(2012): 1-9

There are growing needs to measure the thermal properties using low volume fluid samples in various fields such as biotechnology and nanofluids and there has been significant research toward miniaturization of these measurement device. In this paper, a device that uses 3- ω method for the purpose of thermal conductivity measurement is designed and presented. The 3-ω method requires temperature data in the frequency domain requires relatively smaller sample and power and hence is devoid of transient errors. The sensor is a platinum wire of 50. μm in diameter and 30. mm in length, which is immersed in a cylindrical bore that can accommodate a sample size of 25. μl. The device is validated with de-ionized water with the accuracy between ±0.2% and ±1.2%. Using the device, the thermal conductivity of CuO-deionized water nanofluids is measured for volume fractions of nanoparticles namely 0.025%, 0.05% and 0.1% for temperatures between 15 and 35. °C. An enhancement in thermal conductivity over the base fluid is witnessed for the tested temperature and volume fraction. Finally, the influence of pH ranging including the iso-electric point on the thermal conductivity is also studied and presented.

Keywords: 3-ω method; De-ionized water; Frequency domain; Nanofluid; Thermal conductivity

Kundu, S.C. , Kundu, B., Talukdar, S., Bano, S., Nayak, S., Kundu, J., Mandal, B.B., Bhardwaj, N., Botlagunta, M., Dash, B.C., Acharya, C., Ghosh, A.K. (Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur 721302, India). Invited review: Nonmulberry silk biopolymers (Review). Biopolymers, Vol. 97(6) (2012): 455-467

The silk produced by silkworms are biopolymers and can be classified into two types-mulberry and nonmulberry. Mulberry silk of silkworm Bombyx mori has been extensively explored and used for century old textiles and sutures. But for the last few decades it is being extensively exploited for biomedical applications. However, the transformation of nonmulberry silk from being a textile commodity to biomaterials is relatively new. Within a very short period of time, the combination of load bearing capability and tensile strength of nonmulberry silk has been equally envisioned for bone, cartilage, adipose, and other tissue regeneration. Adding to its advantage is its diverse morphology, including macro to nano architectures with controllable degradation and biocompatibility yields novel natural material systems in vitro. Its follow on applications involve sustained release of model compounds and anticancer drugs. Its 3D cancer models provide compatible microenvironment systems for better understanding of the cancer progression mechanism and screening of anticancer compounds. Diversely designed nonmulberry matrices thus provide an array of new cutting age technologies, which is unattainable with the current synthetic materials that lack biodegradability and biocompatibility. Scientific exploration of nonmulberry silk in tissue engineering, regenerative medicine, and biotechnological applications promises advancement of sericulture industries in India and China, largest nonmulberry silk producers of the world. This review discusses the prospective biomedical applications of nonmulberry silk proteins as natural biomaterials.

Keywords: biomaterial; fibroin; nonmulberry silk; regenerative medicine; sericin; tissue engineering

Yurt, A.[a] , Daaboul, G.G.[b] , Connor, J.H.[c] , Goldberg, B.B.[abde] , Selim Ünlü, M.[abde] . ([a]Division of Materials Science and Engineering, Boston University, Boston, MA 02215, United States, [b] Biomedical Engineering Department, Boston University, Boston, MA

02215, United States, [c] Physics Department, Boston University, Boston, MA 02215, United States, [d] Department of Microbiology, Boston University, School of Medicine, Boston, MA 02118, United States, [e] Electrical and Computer Engineering Department, Boston University, Boston, MA 02215, United States). Single nanoparticle detectors for biological applications. Nanoscale, Vol. 4(3) (2012): 715-726

Nanoparticle research has become increasingly important in the context of bioscience and biotechnology. Practical use of nanoparticles in biology has significantly advanced our understanding about biological processes in the nanoscale as well as led to many novel diagnostic and therapeutic applications. Besides, synthetic and natural nanoparticles are of concern for their potential adverse effect on human health. Development of novel detection and characterization tools for nanoparticles will impact a broad range of disciplines in biological research from nanomedicine to nanotoxicology. In this article, we discuss the recent progress and future directions in the area of single nanoparticle detectors with an emphasis on their biological applications. A brief critical overview of electrical and mechanical detection techniques is given and a more in-depth discussion of label-free optical detection techniques is presented.

Keywords: Adverse effect; Biological applications; Biological process; Biological research; Future directions; Human health; Label free; Mechanical detection; Nano scale; Nanotoxicology; Novel diagnostics; Optical detection; Recent progress; Single nanoparticle; Therapeutic Application

Satish V. Patil, Hemant P. Borase, Chandrashekhar D. Patil and Bipinchandra K. Salunke. Biosynthesis of Silver Nanoparticles Using Latex from Few *Euphorbian* Plants and Their Antimicrobial Potential. Applied Biochemistry and Biotechnology, Vol. 167(4) (2012): 776-790

The synthesis of well-dispersed and ultrafine metal nanoparticles has great interest due to their distinctive physicochemical properties and biomedical applications. This study is the first report of one-step solvent-free synthesis of AgNPs using Euphorbiaceae plant latex. Among evaluated eight latex-producing plants, four (*Jatropha curcas*, *Jatropha gossypifolia*, *Pedilanthus tithymaloides*, and *Euphorbia milii*) showed high potential to produce physicochemically distinct, small-sized and bactericidal AgNPs. Phytochemical screening showed presence of rich amount of biochemicals in these plants. *J. gossypifolia* showed uniformly dispersed comparatively small-sized AgNPs. Dose-dependent growth inhibition of bacterial pathogens *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermis*, and *Micrococcus luteus* was observed for *J. gossypifolia* latex-synthesized AgNPs with minimum inhibitory concentration values 30, 40, 70, 60, and 60 ppm, respectively, after 24 h. Possible mode of action of AgNPs against pathogens was confirmed by analyzing enzymes and cell leakage.

Keywords: Euphorbiaceae – Latex – Nanoparticles – Antimicrobial – *Jatropha gossypifolia* – Zeta potential

Xiu-Mei Jiang, Li-Ming Wang, Jing Wang and Chun-Ying Chen. Gold Nanomaterials: Preparation, Chemical Modification, Biomedical Applications and Potential Risk Assessment. Applied Biochemistry and Biotechnology, Vol. 166(6) (2012): 1533-1551

Gold nanomaterials (Au NMs) have attracted increasing attention in biomedicine due to their facile preparation, multifunctional modifications, unique optical and electrical properties, and good biocompatibility. The physicochemical properties of Au NMs at nanoscale, like size, shape, surface chemistry, and near field effects, are rendering Au NMs potent candidates in biomedicine. Thus, risk assessment of negative effects of Au NMs on biological systems is becoming urgent and necessary for future applications. In this review, we summarize up-to-date progresses on the preparation and modification of Au NMs and their biomedical applications, including biosensor, bioimaging and phototherapy, gene/drug delivery. Finally, we discuss the potential risk of Au NMs to biological systems, which is instructive for rationally designing and preparing nanomaterials for safe applications in nanomedicine.

Keywords: Preparation – Chemical modification – Biosensor – Imaging – Phototherapy – Gene/drug delivery – Risk assessment

Si Amar Dahoumane[1], Chakib Djediat[2], Claude Yéprémian[2], Alain Couté[2], Fernand Fiévet[1], Thibaud Coradin[3], Roberta Brayner[1,*]. Recycling and adaptation of *Klebsormidium flaccidum* microalgae for the sustained production of gold nanoparticles. Biotechnology and Bioengineering, Vol. 109(1) (2012):284–288

Targeting the development of cell-based bioreactors for the production of metal nanoparticles, the possibility to perform the sustained synthesis of colloidal gold using *Klebsormidium flaccidum* green algae was studied. A first strategy relying on successive growth/reduction/reseeding recycling steps demonstrated maintained biosynthesis capability of the microalgae but limitation in metal content due to toxic effects. An alternative approach consisting of progressive gold salt addition revealed to be suitable to favor cell adaptation to larger metal concentrations and supported particle release over month periods.

Keywords: gold nanoparticles; photosyntetic activity; green microalgae; living materials

Muhammad J.A. Shiddiky[a], Sakandar Rauf[a], Prakash H. Kithva[a], Matt Trau[a, b]. ([a]Australian Institute for Bioengineering & Nanotechnology (AIBN), Centre for Biomarker Research and Development, The University of Queensland, QLD 4072, Australia, [b] Australian Institute for Bioengineering & Nanotechnology (AIBN), School of Chemistry & Molecular Biosciences, Centre for Biomarker Research and Development, The University of Queensland, QLD 4072, Australia). Graphene/quantum dot bionanoconjugates as signal amplifiers in stripping voltammetric detection of EpCAM biomarkers. Biosensors and Bioelectronics, Vol. 35(1) (2012): 251–257

A sensitive electrochemical immunosensor for the detection of epithelial cell adhesion molecule (EpCAM) antigen, a common marker for tumors of epithelial origin, employing bionanoconjugates as signal-transduction labels has been developed. The bionanoconjugates were fabricated by carboxylation of the two-dimensional graphene oxide nanosheets (GRs) and immobilizing streptavidin and amine-functionalized CdSe quantum dots (QDs) on carboxylated GRs *via* carbodiimide coupling chemistry, followed by the immunoreaction with the biotinylated secondary antibodies. Since carboxylated GRs have a higher density of active sites, it allows a large number of CdSe QDs to be immobilized onto the surface of the bionanoconjugates, and hence, enhance the sensitivity of the immunosensor. The method enabled detection limits of

100 fg/mL and 1 pg/mL (based on the S/N = 3) in PBS buffer and serum samples, respectively, using anodic stripping voltammetric readout. The immunosensor showed a good selectivity, reproducibility, and long-storage stability, and may become a promising technique for the early detection of tumor biomarker in clinical/biological samples.

Keywords: Electrochemical immunosensor; Signal amplification; Graphene oxide nanosheet; Quantum dots; Tumor biomarkers

Jing Zhang[a], Muhammad Sajid[a], Na Na[a], Lingyun Huang[b], Dacheng He[b], Jin Ouyang[a]. ([a]College of Chemistry, Beijing Normal University, Beijing 100875, PR China, [b] Key Laboratory for Cell Proliferation and Regulation Biology, Ministry of Education, Beijing Normal University, Beijing 100875, PR China). The application of Au nanoclusters in the fluorescence imaging of human serum proteins after native PAGE: Enhancing detection by low-temperature plasma treatment. Biosensors and Bioelectronics, Vol. 35(1) (2012): 313–318

Proteins in human serum are increasingly being studied for their roles in a wide variety of biochemical interactions. To improve the sensitivity of the detection of human serum proteins after native polyacrylamide gel electrophoresis (PAGE), we have developed a fluorescence imaging detection technique for the detection. BSA (bovine serum albumin)-stabilized Au nanoclusters (NCs) were applied as fluorescent probes for imaging, and low-temperature plasma (LTP) treatment of the Au NCs was introduced to enhance the fluorescence imaging. Here, a series of optimization experiments (e.g. those to optimize for pH) were conducted for protein detection after 1-DE and 2-DE, and several types of discharge gases (He, O_2, and N_2) were selected for the LTP treatment. The possible mechanism of interaction between the proteins and the Au NCs was demonstrated by an isothermal titration calorimetry experiment. Using the present method, a sensitivity of 7–14 times higher than that of traditional staining detection methods was observed in the oxygen LTP-treated Au NCs fluorescence images, and some relatively low abundance proteins (identified by the MS/MS technique) were easily detected. In addition, this fluorescence imaging method was applied to distinguish between the serum samples of patients with liver diseases and those of healthy people. Thus, this fluorescence imaging method is suitable for the highly sensitive detection of various serum proteins, and it shows potential capabilities for clinical diagnosis.

Keywords: Au nanoclusters; Fluorescence imaging; Human serum protein; Low-temperature plasma; Polyacrylamide gel electrophoresis

Huajun Qiu, Feixue Zou. (School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, China). Fabrication of stratified nanoporous gold for enhanced biosensing. Biosensors and Bioelectronics, Vol. 35(1) (2012): 349–354

By a dealloying/annealing/redealloying strategy, nanoporous gold (NPG) with hierarchical microstructure is fabricated for electrochemical biosensing application. The first dealloying and annealing would produce NPG/AuAg alloy composite with a large-pore NPG layer and the second dealloying would further etch the AuAg alloy part in the composite, generating a small-pore NPG layer. By using the large-pore (100 nm) layer as the glucose oxidase (GOx) container, and the small-pore (12 nm) layer as a signal producer, this novel hierarchical NPG is demonstrated to be a good support for enzyme immobilization and fabricating enzyme-based biosensors. The immobilized GOx retains 92% of the initial activity after 7 repeated use. The

GOx-loaded stratified NPG biosensor can detect glucose more sensitively with a wider linear range (up to 22 mM) than normal NPG with a uniform pore size of 30–40 nm (linear range: up to 17 mM).

Keywords: Hierarchical; Dealloying; Enzyme immobilization

Kun Chen, Heyou Han, Zhihui Luo, Yanjun Wang, Xiuping Wang. (State Key Laboratory of Agricultural Microbiology, College of Science. Huazhong Agricultural University, Wuhan 430070, PR China). A practicable detection system for genetically modified rice by SERS-barcoded nanosensors. Biosensors and Bioelectronics, Vol. 34(1) (2012):118–124

Since the global cultivation of genetically modified crops constantly expands, it remains a high demand to establish different ways to sort food and feed that consist or contain genetically modified organisms. Surface-enhanced Raman scattering (SERS) spectroscopy is a flexible tool for biological analysis due to its excellent properties for detecting wide varieties of target biomolecules including nucleic acids. In the present study, a SERS-barcoded nanosensor was developed to detect *Bacillus thuringiensis* (Bt) gene-transformed rice expressing insecticidal proteins. The barcoded sensor was designed by encapsulation of gold nanoparticles with silica and conjugation of oligonucleotide strands for targeting DNA strands. The transition between the *cry1A(b)* and *cry1A(c)* fusion gene sequence was used to construct a specific SERS-based detection method with a detection limit of 0.1 pg/mL. In order to build the determination models to screen transgene, a series mixture of Bt rice and normal rice were prepared for SERS assay, and the limit of detection was 0.1% (w/w) transgenic Bt rice relative to normal rice. The sensitivity and accuracy of the SERS-based assay was comparable with real-time PCR. The SERS-barcoded analytical method would provide precise detection of transgenic rice varieties but also informative supplement to avoid false positive outcomes.

Keywords: *Bacillus thuringiensis*; SERS; Nanosensor; Genetically modified rice; Core–shell nanoparticles

Jianhua Shen, Xiaoling Yang, Yihua Zhu, Haigang Kang, Huimin Cao, Chunzhong Li. (Key Laboratory for Ultrafine Materials of Ministry of Education, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai 200237, China). Gold-coated silica-fiber hybrid materials for application in a novel hydrogen peroxide biosensor. Biosensors and Bioelectronics, Vol. 34(1) (2012):132–136

We describe the preparation and characterization of a novel type of core–shell hybrid material for application in a novel hydrogen peroxide biosensor, where the structure consists of a continuous gold shell that encapsulates the silica fiber. The SiO_2@Au nanofibers had been synthesized by electrospinning silica sol, and then golden seeds were *in situ* grown on the fiber, lastly the gold-seeded silica fibers were further coated by continuous gold shells. The above nanocomposites had satisfactory chemical stability, excellent biocompatibility and efficient electron transfer property, which may have potential application for the highly sensitive chemical or biological sensors. Cyclic voltammetry (CV) was used to evaluate the electrochemical performance of the SiO_2@Au nanocomposites at indium tin oxide (ITO). The biosensor showed high sensitivity and fast response upon the addition of H_2O_2 and the linear range to H_2O_2 was from 5×10^{-6} to 1.0×10^{-3} M with a detection limit of 2 μM (S/N = 3). The

apparent Michaelis–Menten constant of the biosensor was 1.11 mmol L^{-1}. These results indicated that SiO_2@Au nanocomposites have potential for constructing of a variety of electrochemical biosensors.

Keywords: K-gold solution; Gold-coated silica-fiber; Core–shell hybrid nanostructure; Biosensor; Horseradish peroxidase; Hydrogen peroxide

Jing Deng, Yan Jin, Lin Wang, Guozhen Chen, Chengxiao Zhang. (Key Laboratory of Applied Surface and Colloid Chemistry, Ministry of Education, Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710062, China). Sensitive detection of endonuclease activity and inhibition using gold nanorods. Biosensors and Bioelectronics, Vol. 34(1) (2012): 144–150

It is important to develop reliable and sensitive methods for assay of nuclease activity. With this goal in mind, we report a new strategy for nuclease assay by taking advantage of efficient fluorescence resonance energy transfer (FRET) between gold nanorods (GNRs) and fluorescein-tagged single-stranded DNA (FDNA). Upon mixing with GNRs, the FRET between positively charged GNRs and negatively charged FDNA caused a decrease in fluorescence of FDNA. The formation of FDNA/cDNA duplex further improved the FRET efficiency, leading to a significant decrease in fluorescence intensity. However, fluorescence is restored when FDNA1/cDNA1 hybrid was cleaved into small fragments by EcoRI endonucleases, resulting in a decrease in FRET efficiency because of weakened electrostatic interaction between GNRs and the shortened DNA fragments. Activity of EcoRI endonuclease has been real-time studied by monitoring fluorescence change with the prolonging of interaction time. Under optimized conditions, the cleaved fraction is linear with EcoRI concentration over the range of 1.0×10^{-3} to 1.0×10^{-1} U μL^{-1}, with a limit of detection of 6.5×10^{-4} U μL^{-1} which is much better or at least comparable to previous reports. Site-specific DNA cleavage by EcoRI endonuclease has also been verified by gel electrophoresis, fluorescence anisotropy and TEM analysis, which indicated that this method is a feasible and reasonable approach to study sequence-specific protein–DNA interactions. Assay of BamHI activity demonstrated that it is a more universally applied method for studying the activity of endonuclease. Furthermore, this fluorescence assay has been also used for studying the inhibition of EcoRI endonuclease activity. Importantly, experimental results suggested that endonuclease inhibitors can be screened by monitoring the change of fluorescence change. Therefore, this FRET assay is a simple, sensitive and effective approach to study endonuclease activity and inhibition, and as such, it promises to provide a feasible method to screen nuclease inhibitors.

Keywords: EcoRI endonuclease; Gold nanorods; Fluorescence resonance energy transfer; Activity; Inhibition; Endonuclease inhibitor

Fátima Fernández, Francisco Sánchez-Baeza, M.-Pilar Marco. (Applied Molecular Receptors Group (AMRg), CIBER de Bioingeniería, Biomateriales y Nanomedicina, Department of Chemical and Biomolecular Nanotechnology, IQAC-CSIC, Jorge Girona, 18-26, 08034 Barcelona, Spain). Nanogold probe enhanced Surface Plasmon Resonance immunosensor for improved detection of antibiotic residues. Biosensors and Bioelectronics, Vol. 34(1) (2012): 151–158

An exhaustive study is reported on the effect that antibody nanogold probes produce on the performance of a Surface Plasmon Resonance (SPR) immunosensor. The paper studies the improvement that different nanogold probes prepared at different antibody:gold nanoparticle (IgG:AuNP) ratios and AuNP sizes produce on the maximum signal and detectability of a simple SPR immunosensor developed to analyze fluoroquinolone (FQ) antibiotic residues (SPReeta system). The investigation compares the features of sensor enhanced formats using both, *secondary* and *primary nanogold probes* (anti-IgG and IgG coupled to AuNP, on double and single-antibody immunochemical assay steps, respectively), in respect to the unenhanced format. For this purpose, a reproducible bioconjugation procedure for preparing gold biohybrid nanoparticles has been established, involving the formation of a mixed self-assembled monolayer (m-SAM) with PEGylated cross-linkers around the AuNP followed by the covalent attachment of the antibodies. The procedure allows controlling the IgG:AuNP ratio of the nanogold probes on a reproducible manner and the functionalized NPs have been found to be stable during assay and storage. Both formats, using *secondary* and *primary nanogold probes*, are excellent strategies to improve immunosensor detectability. Thus, using anti-IgG-AuNP, the detectability could be improved by a factor of 14 (LOD 0.07 ± 0.01 μg L^{-1} *vs.* 0.98 ± 0.38 μg L^{-1}) reducing at the same time the amount of primary antibody used (30,000 *vs.* 1000 dilution factor). Likewise, the format using IgG-AuNP also allows improving detectability (LOD 0.11 ± 0.01 μg L^{-1}), but reducing the number of needed steps.

Keywords: Surface Plasmon Resonance immunosensor; Fluoroquinolone antibiotics; Gold nanoparticles; Nanogold probes; Signal enhancement; Antibody

Sukunya Oaew[a], Ratthaphol Charlermroj[b], Thitiporn Pattarakankul[b], Nitsara Karoonuthaisiri[b]. ([a] Biochemical Engineering and Pilot Plant Research and Development Unit, National Center for Genetic Engineering and Biotechnology, National Sciences and Technology Development Agency at King Mongkut's University of Technology Thonburi (Bangkhuntien), Bangkok 10150, Thailand, [b] Microarray Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani 12120, Thailand). **Gold nanoparticles/horseradish peroxidase encapsulated polyelectrolyte nanocapsule for signal amplification in *Listeria monocytogenes* detection. Biosensors and Bioelectronics, Vol. 34(1) (2012): 238–243**

Bioconjugate nanocapsules were fabricated by using polystyrene sulfonate (PSS) to encapsulate gold nanoparticles (AuNPs) bearing adsorbed horseradish peroxidase (HRP). The average size of nanocapsule was in a range 150–400 nm. The efficiency of the capsules to enhance signals in an immunoassay was demonstrated by using an enzyme linked immunosorbent assay (ELISA) to detect the food-borne pathogen – *Listeria monocytogenes.* The antibody adsorbed onto the PSS shell of the nanocapsules provided the recognition molecule. For a given quantity of antibody, the bioconjugate nanocapsules showed 30 times greater sensitivity and a shorter assay time (5 min) when compared to conventional ELISA using an HRP labelled antibody. This proof-of-concept encapsulation of HRP through PSS nanocapsules may pave the way for alternative signal enhancement strategies where sensitivity is a priority.

Keywords: Gold nanoparticles; Horseradish peroxidase; Polyelectrolyte; Nanocapsule; Encapsulation; *Listeria monocytogenes*

Natalia Malashikhina, Valeri Pavlov. (Biofunctional Nanomaterials Department, CIC biomaGUNE, Parque tecnológico de San Sebastian, Paseo Miramon 182, Donostia – San Sebastian 20009, Spain). DNA-decorated nanoparticles as nanosensors for rapid detection of ascorbic acid. Biosensors and Bioelectronics, Vol. 33(1) (2012): 241–246

We designed an assay for rapid detection of ascorbic acid (AA) with a DNAzyme cleaving its DNA substrate in the presence of Cu^{2+} and AA. The sensor consists of two DNA strands that form a complex between each other. The 5′-end of the DNAzyme binds the substrate DNA via Watson–Crick bonding and the 3′-end binds through formation of a DNA-triplex via Hoogsteen hydrogen bonding. The substrate DNA was prepared by two different methods. In the first case the nucleic acid was modified with fluorescein/dabcyl FRET pair across the cleavage site. In the second case the nucleic acid modified with fluorescein was immobilised on gold nanoparticles. DNAzyme contains a loop forming a complex with Cu^{2+} ions. The oxidation of ascorbic acid (AA) with oxygen yields hydrogen peroxide. The latter interacts with Cu^{2+} to give hydroxyl radicals. They break substrate DNA in close vicinity to the copper/DNA complex to separate fluorescein from gold nanoparticles leading to the increase in fluorescence intensity. Use of substrate DNA modified with the fluorescein/dabcyl couple allowed to measure AA concentration within 3 min with the detection limit of 2.5 μM. Employment of gold nanoparticles decorated with fluorescein-modified DNA allowed to improve the detection limit of AA quantification by two orders of magnitude due to enhanced cleavage of DNA catalysed by Au clusters. Fructose, sucrose, glucose, urea, and citric acid did not interfere with our assay even at concentration of 1 mM. Good selectivity allowed us to apply our rapid and sensitive assays to detection of AA in vitamin C tablets, urine and orange juice.

Keywords: Ascorbic acid; Gold nanoparticles; DNA cleavage; Nanosensor

Mohammad Azam Ansari, Haris M. Khan, Aijaz A. Khan, Asfia Sultan and Ameer Azam. Synthesis and characterization of the antibacterial potential of ZnO nanoparticles against extended-spectrum β-lactamases-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from a tertiary care hospital of North India. Applied Microbiology and Biotechnology, Vol. 94(2) (2012): 467-477

The reemergence of infectious diseases and the continuous development of multidrug resistance among a variety of disease-causing bacteria in clinical setting pose a serious threat to public health worldwide. Extended-spectrum β-lactamases (ESBLs) that mediate resistance to third-generation cephalosporin are now observed all over the world in all species of Enterobacteriaceae, especially *Escherichia coli* and *Klebsiella pneumoniae*. In this work, ZnO nanoparticles (NPs) were synthesized by the sol–gel method and characterized by powder X-ray diffraction, scanning electron microscopy (SEM) and atomic force microscopy (AFM). The image of synthesized ZnO NPs appeared spherical in SEM with a diameter of ≈19 nm and as hexagonal crystal in AFM. Clinical isolates were assessed for ESBL production and shown to be sensitive to ZnO NPs by different methods such as minimal inhibitory concentration (MIC) and minimal bactericidal concentration, time-dependent growth inhibition assay, well diffusion agar methods and estimation of colony forming units (CFU) of bacteria. The lowest MIC value for *E. coli* and *K. pneumoniae* was found to be 500 μg/ml. The results showed that ZnO NPs at 1,000 μg/ml completely inhibit the bacterial growth. The antibacterial effect of ZnO nanoparticles was gradual, but time- and concentration-dependent. The maximum inhibition zone at100 μg/ml for *E. coli* and *K. pneumoniae* was 22 and 20 mm, respectively. With the increasing ZnO NP loading, there is significant reduction in the numbers of CFU. At the

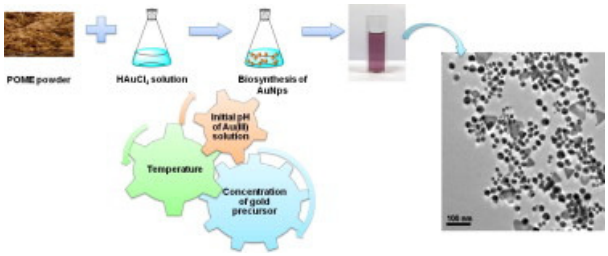
concentration of 1,000 µg/ml, the decline in per cent survival of *E. coli* and *K. pneumoniae* was found to be 99.3% and 98.6%, respectively.

Keyword: ZnO nanoparticles – ESBL – *E. coli* – *K. pneumoniae* – AFM – CFU

Pei Pei Gan, Shi Han Ng, Yan Huang, Sam Fong Yau Li. (Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore). Green synthesis of gold nanoparticles using palm oil mill effluent (POME): A low-cost and eco-friendly viable approach. Bioresource Technology, Vol.113(2012). 132–135

The present study reports the synthesis of gold nanoparticles (AuNps) from gold precursor using palm oil mill effluent (POME) without adding external surfactant, capping agent or template. The biosynthesized AuNps were characterized by using UV–vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). According to the image analysis performed on a representative TEM micrograph by counting 258 particles, the obtained AuNps are predominantly spherical with an average size of 18.75 ± 5.96 nm. In addition, some triangular and hexagonal nanoparticles were also observed. The influence of various reaction parameters such as reaction pH, concentration of gold precursor and interaction time to the morphology and size of biosynthesized AuNps was investigated. This study shows the feasibility of using agro waste material for the biosynthesis of AuNps which is potentially more scalable and economic due to its lower cost.

Graphical abstract



Keywords: Palm oil mill effluent; Biosynthesis; Gold nanoparticles; Bioreduction; Agro waste

Hansen, S.[ab] , Lehr, C.-M.[ab]. ([a] Department of Drug Delivery, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz-Center for Infection Research (HZI), Saarbruecken, Germany, [b] Saarland University, Saarbruecken, Germany). Nanoparticles for transcutaneous vaccination (Short Survey). Microbial Biotechnology, Vol. 5(2) (2012): 156-167

The living epidermis and dermis are rich in antigen presenting cells (APCs). Their activation can elicit a strong humoral and cellular immune response as well as mucosal immunity. Therefore, the skin is a very attractive site for vaccination, and an intradermal application of antigen may be much more effective than a subcutaneous or intramuscular injection. However, the stratum corneum (SC) is a most effective barrier against the invasion of topically applied vaccines. Products which have reached the stage of clinical testing, avoid this problem by injecting the nano-vaccine intradermally or by employing a barrier disrupting method and applying the

vaccine to a relatively large skin area. Needle-free vaccination is desirable from a number of aspects: ease of application, improved patient acceptance and less risk of infection among them. Nanocarriers can be designed in a way that they can overcome the SC. Also incorporation into nanocarriers protects instable antigen from degradation, improves uptake and processing by APCs, and facilitates endosomal escape and nuclear delivery of DNA vaccines. In addition, sustained release systems may build a depot in the tissue gradually releasing antigen which may avoid booster doses. Therefore, nanoformulations of vaccines for transcutaneous immunization are currently a very dynamic field of research. Among the huge variety of nanocarrier systems that are investigated hopes lie on ultra-flexible liposomes, superfine rigid nanoparticles and nanocarriers, which are taken up by hair follicles. The potential and pitfalls associated with these three classes of carriers will be discussed.

Keywords: EMTREE drug terms: 1,2 dioleoyl 3 trimethylammoniopropane; cholera toxin; DNA vaccine; gamma interferon; hepatitis B surface antigen; immunoglobulin G; interleukin 10; interleukin 2; interleukin 4; metal nanoparticle; nanocarrier; pollen antigen; polyglactin; quantum dot; small interfering RNA; virosome

EMTREE medical terms: antibody response; antigen presentation; antigen presenting cell; aqueous solution; biodegradability; cellular immunity; contact dermatitis; cytokine production; degradation; dermis; drug delivery system; drug efficacy; drug formulation; drug penetration; endocytosis; endosome; epidermis; expression vector; hair follicle; Hepatitis A virus; histopathology; human; Human immunodeficiency virus; humoral immunity; hydrophilicity; immune response; infection risk; lipid bilayer; liposomal delivery; malaria; mucosal immunity; multiple myeloma; nanoencapsulation; nanotoxicology; nonhuman; particle size; prostate cancer; Respiratory syncytial pneumovirus; seasonal influenza; short survey; skin penetration; skin toxicity; stratum corneum; sustained drug release; Th1 cell; vaccination; Wart virus; zeta potential

Faiez Alani, Murray Moo-Young and William Anderson. Biosynthesis of silver nanoparticles by a new strain of *Streptomyces* sp. compared with *Aspergillusfumigatus*. World Journal of Microbiology and Biotechnology, Vol. 28(3) (2012): 1081-1086

Locally isolated strains of a thermoalkalotolerant *Streptomyces* sp. and *Aspergillus fumigatus* were used for the in vitro biosynthesis of silver nanoparticles from $AgNO_3$ solutions. An autolysed cell-free culture filtrate from each strain was used, indicating that the formation mechanism depends on intra-cellular components for both organisms, since culture broths had no significant nanoparticle formation potential. Nanoparticle formation was indicated by a change of the solution from colourless or light brown to dark brown after 24 h or more, and UV–visible spectroscopy and x-ray diffraction analysis confirmed the formation by both organisms. The initial formation kinetics were faster with *Aspergillus*, but formation continued for a longer period with *Streptomyces*, resulting in higher concentrations after 48 h. Transmission electron microscope images revealed well dispersed nanoparticles with diameters ranging from 15 to 45 nm from *A. fumigatus*, while those from *Streptomyces* sp. had a narrower size distribution of 15–25 nm. The higher productivity and preferred narrower size distribution of *Streptomyces*, together with its well established industrial use, may make it the preferred choice for further optimization studies.

Keywords: Silver nanoparticles – In vitro biosynthesis – Industrial filamentous organisms – Cell-free biosynthesis

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2. Aerobiologia
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4. Annual Review- Ecology and Systematics
5. Annual Review-Biochemistry
6. Annual Review-Biomedical Engineering
7. Annual Review-Biophysics and Biomolecular Structure
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