ENVIS CENTER
on
ENVIRONMENTAL BIOTECHNOLOGY

Abstract Vol. XIX

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ENVIS CENTRE

on

ENVIRONMENTAL BIOTECHBNOLOGY

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BACKGROUND

Environmental Information System (ENVIS) is established in the year 1984 as a network of Information Centres. It is planned by the Ministry of Environment and Forest. Aim of this centre is to provide descriptive and environmental subject related numerical data. 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 19th publication of Abstract Volume of this ENVIS Centre. This contains the abstracts of research papers collected from the various areas of Environmental Biotechnology from different journals published in December, 2011. 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.

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

Qiong Zhang[a, b], Liuyan Yang[b], Wen-Xiong Wang[a]. ([a]Division of Life Science, Hong Kong University of Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong, [b]School of Environment, State Key Laboratory of Pollutant Control and Resource Reuse, Nanjing University, Nanjing, China). Bioaccumulation and trophic transfer of dioxins in marine copepods and fish. Environmental Pollution, Volume 159(12) (2011) : 3390-3397

Despite the great concerns about dioxins in the marine environments, the biokinetics and bioaccumulation of these compounds in marine organisms remains little known. Using radioactive tracers the aqueous uptake, dietary assimilation efficiency, and elimination of dioxins were measured in marine phytoplankton, copepods and seabream. The calculated uptake rate constant of dioxins decreased with increasing trophic levels, whereas the dietary assimilation efficiency (AE) was 28.5–57.6% in the copepods and 36.6–70.2% in the fish. The dietary AE was highly dependent on the food concentrations and food type. The elimination rate constant of dioxin in the copepods varied with different exposure pathways as well as food concentration and food type. Biokinetic calculation showed that dietary accumulation was the predominant pathway for dioxin accumulation in marine copepods and fish. Aqueous uptake can be an important pathway only when the bioconcentration of dioxins in the phytoplankton was low.

Keywords: Dioxins; Biokinetics; Exposure; Copepods; Fish

Cecilia Easton[a], Andrew Turner[a], Graham Sewell[b]. ([a]School of Geography, Earth and Environmental Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK, [b]School of Health Professions, University of Plymouth, Peninsula Allied Health Centre, Plymouth PL6 8BH, UK). An evaluation of the toxicity and bioaccumulation of cisplatin in the marine environment using the macroalga, *Ulva lactuca.* Environmental Pollution, Volume 159(12) (2011): 3504-3508

The cytotoxic drug, cisplatin (*cis*-$PtCl_2(NH_3)_2$), has been added to cultures of the marine macroalga, *Ulva lactuca*, under various experimental conditions. Both accumulation and internalisation over a 48 h period was greater when cisplatin was added to coastal sea water (salinity = 33) from a distilled water solution than when added to either sea water or estuarine water (salinity = 16.5) from a saline solution. This effect is attributed to the greater abundance of the more reactive monoaqua complex (*cis*-$PtCl(OH_2)(NH_3)_2^+$) in the distilled water solution and kinetic constraints on its conversion back to *cis*-$PtCl_2(NH_3)_2$ in sea water. Despite its mode of action at the cellular level, cisplatin added up to concentrations of 150 nM did not incur a measurable reduction in the efficiency of photochemical energy conversion under any of experimental conditions tested.

Keywords: Cisplatin; Monoaquacisplatin; *Ulva lactuca*; Accumulation; Internalisation; Phytotoxicity

Patrícia Pereira[1,2,*], Hilda de Pablo[1], Maria Dulce Subida[3], Carlos Vale[1], Mário Pacheco[2]. Bioaccumulation and biochemical markers in feral crab (*Carcinus maenas*) exposed to moderate environmental contamination—The impact of non-contamination-related variables. Environmental Toxicology, Volume 26(5) (2011): 524–540

Moderate contamination is a challenging scenario for ecotoxicologists because of the occurrence of subtle biomarker responses and the increased relevance of non-contamination related variables. This investigative biomonitoring study was performed in a moderately contaminated coastal system (Óbidos lagoon, Portugal) to examine winter–summer variations on biochemical responses and accumulated metals in *Carcinus maenas*, searching for associations with environmental and biological factors. Males and females were collected in three sites: Barrosa (BB) and Bom-Sucesso (BS) in upper lagoon, and the middle lagoon (ML), closer to the lagoon inlet. Water and sediment were monitored for metals (Cu, Mn, Ni, Cr, Cd). Catalase (CAT), glutathione peroxidase (GPx), glutathione *S*-transferase (GST), total glutathione content (GSH_t), lipid peroxidation (LPO), and ethoxyresorufin-*O*-deethylase (EROD), as well as Cu, Mn, Ni, Cr and Cd were measured in the crabs' hepatopancreas. Inter-site differences, though infrequent, pointed to the presence of crab stressors at BB. This was particularly obvious in summer when higher GST as well as lower GSH_t and EROD were found in females, and accompanied by higher Ni accumulation. Seasonal differences of biochemical responses superimposed spatial variations in line with the contrasting winter–summer conditions regarding water quality and, to a lesser extent, with metal bioaccumulation. CAT, GSH_t, and LPO were higher in summer, whereas enhancements of GPx and GST were recorded in winter. Winter increases were in agreement with higher availability of metals in water and enhancement of accumulated levels, particularly in females as emphasized by a bioaccumulation index. On the other hand, increases in summer were mainly driven by non-contamination related factors. Males and females exhibited different patterns of metal accumulation and biochemical responses, with females being more responsive, as confirmed by a general stress index (IBR). Results recommend gender separation in biomonitoring programs using crabs. The integration of biochemical responses into IBR substantiated data interpretation. This is particularly relevant under moderate contamination allowing for better site-distinction rather than biochemical responses considered individually.

Keywords: oxidative stress; biotransformation; metals; *Carcinus maenas*; seasonal variability; gender-specific responses

Gabriela Busuioc, Carmen Cristina Elekes, Claudia Stihi, Stefania Iordache and Sorin Constantin Ciulei. The bioaccumulation and translocation of Fe, Zn, and Cu in species of mushrooms from *Russula* genus. Environmental Science and Pollution Research, Volume 18(6) (2011): 890-896

The studied *Russula* species are *Russula virescens*, *Russula cyanoxantha*, *Russula foetens*, and *Russula nigrescens*, which were harvested from forestry ecosystem from South Romania. The metal concentration in mushrooms and their substrate was established by EDXRF method.

The concentrations of iron (Fe), zinc (Zn), and copper (Cu) in the fruiting body depends on species and vary between 58.83–340.34, 19.70–99.62, and 5.03–9.37 □mg/kg for Fe, Zn, and Cu, respectively. The bioaccumulation factor has subunit values for the three studied trace metals, which show the low capacity of these species of mushrooms to accumulate metals if the concentrations in soil increase over the normal threshold for these elements. The high values of translocation factor demonstrate the mobility of Fe, Zn, and Cu in the studied mushrooms.

Keywords: Macromycetes – Heavy metals – Bioaccumulation – Translocation

Abdulali Taweel*, M. Shuhaimi-Othman and A. K. Ahmad. (School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia, Bangi, 43600, Selangor, Malaysia. *Corresponding author. E-mail: aktaweel@yahoo.com). Heavy metals concentration in different organs of tilapia fish (*Oreochromis niloticus*) from selected areas of Bangi, Selangor, Malaysia. African Journal of Biotechnology Vol. 10 (55)(2011): 11562-11566

The present study was aimed at investigating the six heavy metals: Pb, Cd, Cr, Cu, Ni and Zn measured in the liver, gills and muscles of tilapia fish (*Oreochromis niloticus*) which was collected from five locations around Bangi area, Selangor, Malaysia. The sites included Culture Pond A, Culture Pond B, Langat River, Cempaka Lake and Engineering Lake. The results show that in general, the highest heavy metal concentrations were detected in the liver followed by the gill and the muscle. The heavy metal concentration in the tissues varied significantly depending upon the locations from where the fish was collected. In the liver, the highest Pb was detected in the Langat River (4.8 ± 0.84 µg/g dry weight) followed by that of Engineering Lake (3.28 ± 1.15 µg/g). Copper and Ni levels were observed as the highest in the fish collected from Cempaka Lake with value of 449 ± 37.7 and 20.9±5.7 µg/g, respectively. For Cd, the highest level was detected in the fish from Engineering Lake (0.70 ± 0.17 µg/g), while the highest values of Zn was recorded in those from the Langat River (143 ± 9.8 µg/g). The metal accumulation in the liver of fish was found to be quite high in comparison to the gills and muscles (edible part). However, the concentrations of heavy metals in the muscles of fishes collected from all the sites were within the permissible levels and are safe for the human consumption and public health.

Keywords: Heavy metals, tilapia fish organs, Bangi area, five sites.

Bioremediation

K. Rama Krishna and Ligy Philip. Bioremediation of Single and Mixture of Pesticide-Contaminated Soils by Mixed Pesticide-Enriched Cultures. Applied Biochemistry and Biotechnology, Volume 164(8) (2011): 1257-1277

In the present study, degradation efficiencies for individual as well as mixed pesticide in different Indian soils, by mixed pesticide-enriched cultures, were evaluated under submerged and unsaturated conditions, Lindane (L), methyl parathion (MP), carbofuran (C), and a mixture of L, MP, and C were used in the study. For all the various conditions considered, methyl parathion degradation was the maximum and lindane degradation was the minimum. The degradation kinetics of the pesticides in sandy, clayey, compost, and red soils by various microbial isolates were studied. It was observed that adsorption was maximum and degradation of pesticides was minimum in compost soil. The degradation efficiencies of pesticides in liquid phase associated with soil sediment were less than those under the normal liquid phase conditions as leaching of pesticides from soil phase was continuous. Pesticide degradation was more in submerged soils compared to that in unsaturated soils. The degradation by-products of

individual and mixed pesticides in liquid, unsaturated, and submerged soils were identified. Different metabolites were produced under submerged and unsaturated conditions.

Keywords: Lindane – Methyl parathion – Carbofuran – Mixed pesticides – Soil and liquid phase

D. Randall Simpson, Nisha Ravi Natraj, Michael J. McInerney and Kathleen E. Duncan. Biosurfactant-producing *Bacillus* are present in produced brines from Oklahoma oil reservoirs with a wide range of salinities. Applied Microbiology and Biotechnology, Volume 91(4) (2011): 1083-1093

Nine wells producing from six different reservoirs with salinities ranging from 2.1% to 15.9% were surveyed for presence of surface-active compounds and biosurfactant-producing microbes. Degenerate primers were designed to detect the presence of the surfactin/lichenysin (*srfA3/licA3*) gene involved in lipopeptide biosurfactant production in members of *Bacillus subtilis/licheniformis* group and the *rhlR* gene involved in regulation of rhamnolipid production in pseudomonads. Polymerase chain reaction amplification, cloning, and sequencing confirmed the presence of the *srfA3/licA3* genes in brines collected from all nine wells. The presence of *B. subtilis/licheniformis* strains was confirmed by sequencing two other genes commonly used for taxonomic identification of bacteria, *gyrA* (gyrase A) and the 16S rRNA gene. Neither *rhlR* nor 16S rRNA gene related to pseudomonads was detected in any of the brines. Intrinsic levels of surface-active compounds in brines were low or not detected, but biosurfactant production could be stimulated by nutrient addition. Supplementation with a known biosurfactant-producing *Bacillu*s strain together with nutrients increased biosurfactant production. The genetic potential to produce lipopeptide biosurfactants (e.g., *srfA3/licA3* gene) is prevalent, and nutrient addition stimulated biosurfactant production in brines from diverse reservoirs, suggesting that a biostimulation approach for biosurfactant-mediated oil recovery may be technically feasible.

Keywords: Biosurfactant – MEOR – Surfactin – Lichenysin – Rhamnolipid – Biostimulation – Bioaugmentation – Oil reservoir

Luke Beesley[a], Eduardo Moreno-Jiménez[b], Jose L. Gomez-Eyles[c], Eva Harris[d], Brett Robinson[d], Tom Sizmur[e]. ([a]The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH, UK, [b]Departamento de Química Agrícola, Universidad Autónoma de Madrid, 28049 Madrid, Spain, [c]Department of Civil and Environmental Engineering, University of Maryland Baltimore County, Baltimore, MD 21250, United States, [d]Department of Soil and Physical Sciences, Lincoln University, Lincoln 7647, New Zealand, [e]Soil Research Centre, Department of Geography and Environmental Science, University of Reading, Whiteknights, Reading RG6 6DW, UK). A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environmental Pollution, Volume 159(12) (2011): 3269-3282

Biochars are biological residues combusted under low oxygen conditions, resulting in a porous, low density carbon rich material. Their large surface areas and cation exchange capacities, determined to a large extent by source materials and pyrolysis temperatures, enables enhanced sorption of both organic and inorganic contaminants to their surfaces, reducing pollutant mobility when amending contaminated soils. Liming effects or release of carbon into soil solution may increase arsenic mobility, whilst low capital but enhanced retention of plant nutrients can restrict revegetation on degraded soils amended only with biochars; the

combination of composts, manures and other amendments with biochars could be their most effective deployment to soils requiring stabilisation by revegetation. Specific mechanisms of contaminant-biochar retention and release over time and the environmental impact of biochar amendments on soil organisms remain somewhat unclear but must be investigated to ensure that the management of environmental pollution coincides with ecological sustainability.

Keywords: Biochar; PAH; Heavy metals; Soil degradation; Pollution; Environmental clean-up

Manuel A. Caraballo[a], Francisco Macías[a], Tobias S. Rötting[b], José Miguel Nieto[a], Carlos Ayora[c]. ([a]Geology Department, University of Huelva, Avenida 3 de Marzo s/n, Campus "El Carmen", E-21071 Huelva, Spain, [b]Technical University of Catalonia (UPC), Hydrogeology Group, E-08034 Barcelona, Spain, [c]Institute of Environmental Assessment and Water Research, IDÆA – CSIC, Jordi Girona 18, 08034 Barcelona, Spain). **Long term remediation of highly polluted acid mine drainage: A sustainable approach to restore the environmental quality of the Odiel river basin. Environmental Pollution, Volume 159(12) (2011) : 3613-3619**

During 20 months of proper operation the full scale passive treatment in Mina Esperanza (SW Spain) produced around 100 mg/L of ferric iron in the aeration cascades, removing an average net acidity up to 1500 mg/L as $CaCO_3$ and not having any significant clogging problem. Complete Al, As, Cd, Cr, Cu, Ti and V removal from the water was accomplished through almost the entire operation time while Fe removal ranged between 170 and 620 mg/L. The system operated at a mean inflow rate of 43 m^3/day achieving an acid load reduction of 597 $g\cdot(m^2\ day)^{-1}$, more than 10 times higher than the generally accepted 40 $g\cdot(m^2\ day)^{-1}$ value commonly used as a passive treatment system designing criteria. The high performance achieved by the passive treatment system at Mina Esperanza demonstrates that this innovative treatment design is a simple, efficient and long lasting remediation option to treat highly polluted acid mine drainage.

Keywords: Acid mine drainage; Passive treatment system; Iberian Pyrite Belt; Schwertmannite; Hydrobasaluminite

Wen-Ling Ye[a, b], M. Asaduzzaman Khan[a, c], Steve P. McGrath[a], Fang-Jie Zhao[a]. ([a]Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK, [b]School of Earth and Space Sciences, University of Science and Technology of China, Hefei, Anhui 230026, China, [c]Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh). **Phytoremediation of arsenic contaminated paddy soils with *Pteris vittata* markedly reduces arsenic uptake by rice. Environmental Pollution, Volume 159(12) (2011) : 3739-3743**

Arsenic (As) accumulation in food crops such as rice is of major concern. To investigate whether phytoremediation can reduce As uptake by rice, the As hyperaccumulator *Pteris vittata* was grown in five contaminated paddy soils in a pot experiment. Over a 9-month period *P. vittata* removed 3.5–11.4% of the total soil As, and decreased phosphate-extractable As and soil pore water As by 11–38% and 18–77%, respectively. Rice grown following *P. vittata* had significantly lower As concentrations in straw and grain, being 17–82% and 22–58% of those in the control, respectively. Phytoremediation also resulted in significant changes in As speciation

in rice grain by greatly decreasing the concentration of dimethylarsinic acid (DMA). In two soils the concentration of inorganic As in rice grain was decreased by 50–58%. The results demonstrate an effective stripping of bioavailable As from contaminated paddy soils thus reducing As uptake by rice.

Keywords: Arsenic; Arsenic speciation; Rice; Phytoremediation; *Pteris vittata*

Eva M. Seeger[a], Peter Kuschk[a], Helga Fazekas[a], Peter Grathwohl[b], Matthias Kaestner[a]. ([a]Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research – UFZ, Permoserstr. 15, 04318 Leipzig, Germany, [b]Center of Applied Geoscience, University of Tübingen, Hölderlinstr. 12, 72074 Tübingen, Germany). Bioremediation of benzene-, MTBE- and ammonia-contaminated groundwater with pilot-scale constructed wetlands. Environmental Pollution, Volume 159(12) (2011) : 3769-3776

In this pilot-scale constructed wetland (CW) study for treating groundwater contaminated with benzene, MTBE, and ammonia-N, the performance of two types of CWs (a wetland with gravel matrix and a plant root mat) was investigated. Hypothesized stimulative effects of filter material additives (charcoal, iron(III)) on pollutant removal were also tested. Increased contaminant loss was found during summer; the best treatment performance was achieved by the plant root mat. Concentration decrease in the planted gravel filter/plant root mat, respectively, amounted to 81/99% for benzene, 17/82% for MTBE, and 54/41% for ammonia-N at calculated inflow loads of 525/603 mg/m^2/d, 97/112 mg/m^2/d, and 1167/1342 mg/m^2/d for benzene, MTBE, and ammonia-N. Filter additives did not improve contaminant depletion, although sorption processes were observed and elevated iron(II) formation indicated iron reduction. Bacterial and stable isotope analysis provided evidence for microbial benzene degradation in the CW, emphasizing the promising potential of this treatment technique.

Keywords: BTEX; Fuel hydrocarbon; Plant root mat; Phytoremediation

Yang Du, Fei Lian, Lingyan Zhu. (Key Laboratory of Pollution Process and Environmental Criteria, Ministry of Education, Tianjin Key Laboratory of Urban Ecology Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China). Biosorption of divalent Pb, Cd and Zn on aragonite and calcite mollusk shells. Environmental Pollution, Volume 159(7) (2011) : 1763-1768

The potential of using mollusk shell powder in aragonite (razor clam shells, RCS) and calcite phase (oyster shells, OS) to remove Pb^{2+}, Cd^{2+} and Zn^{2+} from contaminated water was investigated. Both biogenic sorbents displayed very high sorption capacities for the three metals except for Cd on OS. XRD, SEM and XPS results demonstrated that surface precipitation leading to crystal growth took place during sorption. Calcite OS displayed a remarkably higher sorption capacity to Pb than aragonite RCS, while the opposite was observed for Cd. However, both sorbents displayed similar sorption capacities to Zn. These could be due to the different extent of matching in crystal lattice between the metal bearing precipitate and the substrates. The initial pH of the solution, sorbent's dosage and grain size affected the removal efficiency of the heavy meals significantly, while the organic matter in mollusk shells affected the removal efficiency to a lesser extent.

Keywords: Biogenic sorbent; Aragonite; Calcite; Heavy metals; Crystal lattice

Q. Wang[1,†], D. Xiong[2,†], P. Zhao[1], X. Yu[1], B. Tu[2], G. Wang[1]. Effect of applying an arsenic-resistant and plant growth–promoting rhizobacterium to enhance soil arsenic phytoremediation by *Populus deltoides* LH05-17. Journal of Applied Microbiology, Volume 111(5) (2011): 1065–1074

A rhizobacterium D14 was isolated and identified within *Agrobacterium radiobacter.* This strain was highly resistant to arsenic and produced indole acetic acid and siderophore. Greenhouse pot bioremediation experiments were performed for 5 months using poplar (*Populus deltoides* LH05-17) grown on As-amended soils, inoculated with strain D14. The results showed that *P. deltoides* was an efficient arsenic accumulator; however, high As concentrations (150 and 300 mg kg^{-1}) inhibited its growth. With the bacterial inoculation, in the 300 mg kg^{-1} As-amended soils, 54% As in the soil was removed, which was higher than the uninoculated treatments (43%), and As concentrations in roots, stems and leaves were significantly increased by 229, 113 and 291%, respectively. In addition, the As translocation ratio [(stems + leaves)/roots = 0·8] was significantly higher than the uninoculated treatments (0·5). About 45% As was translocated from roots to the above-ground tissues. The plant height and dry weight of roots, stems and leaves were all enhanced; the contents of chlorophyll and soluble sugar, and the activities of superoxide dismutase and catalase were all increased; and the content of a toxic compound malondialdehyde was decreased.

The results indicated that the inoculation of strain D14 could contribute to the increase in the As tolerance of *P. deltoides*, promotion of the growth, increase in the uptake efficiency and enhancement of As translocation.

The use of *P. deltoides* in combination with the inoculation of strain D14 provides a potential application for efficient soil arsenic bioremediation.

Keywords: *Agrobacterium radiobacter*; arsenic-contaminated soil; arsenic-resistant bacteria; phytoremediation; plant growth–promoting rhizobacterium; *Populus deltoides*

Mónica Patrón-Prado, Margarita Casas-Valdez, Elisa Serviere-Zaragoza, Tania Zenteno-Savín, Daniel B. Lluch-Cota and Lía Méndez-Rodríguez. Biosorption Capacity for Cadmium of Brown Seaweed *Sargassum sinicola* and *Sargassum lapazeanum* in the Gulf of California. Water, Air, & Soil Pollution, Volume 221(1-4) (2011): 137-144

Brown algae *Sargassum sinicola* and *Sargassum lapazeanum* were tested as cadmium biosorbents in coastal environments close to natural and enriched areas of phosphorite ore. Differences in the concentration of cadmium in these brown algae were found, reflecting the bioavailability of the metal ion in seawater at several sites. In the laboratory, maximum biosorption capacity (q_{max}) of cadmium by these nonliving algae was determined according to the Langmuir adsorption isotherm as 62.42±0.44 mg g^{-1} with the affinity constant (b) of 0.09 and 71.20±0.80 with b of 0.03 for *S. sinicola* and *S. lapazeanum*, respectively. Alginate yield was 19.16±1.52% and 12.7±1.31%, respectively. Although *S. sinicola* had far lower biosorption capacity than *S. lapazeanum*, the affinity for cadmium for *S. sinicola* makes this alga more suitable as a biosorbent because of its high q_{max} and large biomass on the eastern coast of the Baja California Peninsula. *Sargassum* biomass was estimated at 180,000 t, with *S. sinicola* contributing to over 70%.

Keywords: Alginate – Biosorption – Cadmium – Gulf of California – *Sargassum*

Yuanyuan Qu, Ruijie Zhang, Fang Ma, Jiti Zhou and Bin Yan. Bioaugmentation with a novel alkali-tolerant *Pseudomonas* strain for alkaline phenol wastewater treatment in sequencing batch reactor. World Journal of Microbiology and Biotechnology, Volume 27(8) (2011): 1919-1926

A novel alkali-tolerant strain JY-2, which could utilize phenol as sole source of carbon and energy, was isolated from activated sludge. It was identified as *Pseudomonas* sp. by 16S rDNA sequencing analysis. The appropriate conditions for strain growth and phenol biodegradation were as follows: pH 8.0–10.0 and temperature 23–30°C. With initial phenol concentrations of 225, 400, 550 and 750 mg/l, the degradation efficiencies were 94.9, 93.3, 89.3 and 48.2% within 40 h at pH 10.0 and 30°C, respectively. The alkaline phenol-containing wastewater treatment augmented with strain JY-2 in sequencing batch reactor (SBR) system was investigated, which suggested that the bioaugmented (BA) system exhibited the better performance for adjusting high pH to neutral value than the non-bioaugmented (non-BA) one. Also, the BA system showed strong abilities for phenol degradation and maintaining good sedimentation coefficient (SV_{30}). The microbial community dynamics of both sequencing batch reactor (SBR) systems were analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) technique, which showed substantial changes between the two systems. This study suggests that it is feasible to treat alkaline phenol-containing wastewater augmented with strain JY-2.

Keywords: Alkali-tolerance – Phenol degradation – Bioaugmentation – Denaturing gradient gel electrophoresis (DGGE) – Microbial community

Mohammad Hassan Khani. Uranium biosorption by *Padina* sp. algae biomass: kinetics and thermodynamics. Environmental Science and Pollution Research, Volume 18(9) (2011): 1593-1605

The kinetic data were found to follow the pseudo-second-order model. Intraparticle diffusion is not the sole rate-controlling factor. The equilibrium experimental results were analyzed in terms of Langmuir isotherm depending with temperature. Equilibrium data fitted very well to the Langmuir model. The maximum uptakes estimated by using the Langmuir model were 434.8, 416.7, 400.0, and 370.4 mg/g at 10°C, 20°C, 30°C, and 40°C, respectively. Gibbs free energy was spontaneous for all interactions, and the adsorption process exhibited exothermic enthalpy values. *Padina* sp. algae were shown to be a favorable biosorbent for uranium removal from aqueous solutions.

Keywords: Biosorption – Uranium – *Padina* sp. algae biomass – Kinetics – Thermodynamics

Akhil N. Kabra, Rahul V. Khandare, Mayur B. Kurade and Sanjay P. Govindwar. Phytoremediation of a sulphonated azo dye Green HE4B by *Glandularia pulchella* (Sweet) Tronc. (Moss Verbena). Environmental Science and Pollution Research, Volume 18(8) (2011): 1360-1373

The dyes and dye stuffs present in effluents released from textile dyeing industries are potentially mutagenic and carcinogenic. Phytoremediation technology can be used for remediating sites contaminated with such textile dyeing effluents. The purpose of the work was

to explore the potential of *Glandularia pulchella* (Sweet) Tronc. to decolorize different textile dyes, textile dyeing effluent, and synthetic mixture of dyes.

Enzymatic analysis of the plant roots was performed before and after decolorization of dye Green HE4B. Analysis of the metabolites of Green HE4B degradation was done using UV–Vis spectroscopy, high-performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FTIR), and gas chromatography–mass spectroscopy (GC-MS). The ability of the plant to decolorize and detoxify a textile dyeing effluent and a synthetic mixture of dyes was studied by a determination of the American Dye Manufacturer's Institute (ADMI), biological oxygen demand (BOD), and chemical oxygen demand (COD). Phytotoxicity studies were performed.

Induction of the activities of lignin peroxidase, laccase, tyrosinase, and 2,6-dichlorophenol indophenol reductase was obtained, suggesting their involvement in the dye degradation. UV–Vis spectroscopy, HPLC, and FTIR analysis confirmed the degradation of the dye. Three metabolites of the dye degradation were identified, namely, 1-(4-methylphenyl)-2-{7-[(*Z*)-phenyldiazenyl] naphthalen-2-yl} diazene; 7,8-diamino-2-(phenyldiazenyl) naphthalen-1-ol; and (*Z*)-1,1′-naphthalene-2,7-diylbis (phenyldiazene) using GC-MS. ADMI, BOD, and COD values were reduced. The non-toxic nature of the metabolites of Green HE4B degradation was revealed by phytotoxicity studies.

This study explored the phytoremediation ability of *G. pulchella* (Sweet) Tronc. in degrading Green HE4B into non-toxic metabolites.

Keywords: Phytoremediation – *Glandularia pulchella* (Sweet) Tronc. – GreenHE4B – Decolorization – Textile dyeing effluent

Jean-Paul Schwitzguébel, Elena Comino, Nadia Plata and Mohammadali Khalvati. Is phytoremediation a sustainable and reliable approach to clean-up contaminated water and soil in Alpine areas? Environmental Science and Pollution Research, Volume 18(6) (2011): 842-856

Phytoremediation does exploit natural plant physiological processes and can be used to decontaminate agricultural soils, industrial sites, brownfields, sediments and water containing inorganic and organic pollutants or to improve food chain safety by phytostabilisation of toxic elements. It is a low-cost and environment friendly technology targetting removal, degradation or immobilisation of contaminants. The aim of the present review is to highlight some recent advances in phytoremediation in the Alpine context.

Case studies are presented where phytoremediation has been or can be successfully applied in Alpine areas to: (1) clean-up industrial wastewater containing sulphonated aromatic xenobiotics released by dye and textile industries; (2) remediate agricultural soils polluted by petroleum hydrocarbons; (3) improve food chain safety in soils contaminated with toxic trace elements (As, Co, Cr and Pb); and (4) treat soils impacted by modern agricultural activities with a special emphasis on phosphate fertilisation.

Worlwide, including in Alpine areas, the controlled use of appropriate plants is destined to play a major role for remediation and restoration of polluted and degraded ecosystems, monitoring and assessment of environmental quality, prevention of landscape degradation and immobilisation of trace elements. Phytotechnologies do already offer promising approaches towards environmental remediation, human health, food safety and sustainable development for the 21st century in Alpine areas and elsewhere all over the world.

Keywords: Phytoremediation – Alpine regions – Contaminated soils – Industrial wastewater – Petroleum hydrocarbons – Sulphonated aromatic compounds – Trace elements – Mycorrhizal fungi

Diejun Chen, Z. Lewis Liu and Wanye Banwart. Concentration-dependent RDX uptake and remediation by crop plants. Environmental Science and Pollution Research, Volume 18(6) (2011): 908-917

The potential RDX contamination of food chain from polluted soil is a significant concern in regards to both human health and environment. Using a hydroponic system and selected soils spiked with RDX, this study disclosed that four crop plant species maize (*Zea mays*), sorghum (*Sorghum sudanese*), wheat (*Triticum aestivum*), and soybean (*Glycine max*) were capable of RDX uptake with more in aerial parts than roots. The accumulation of RDX in the plant tissue is concentration-dependent up to 21 mg RDX/L solution or 100 mg RDX/kg soil but not proportionally at higher RDX levels from 220 to 903 mg/kg soil. While wheat plant tissue harbored the highest RDX concentration of 2,800 μg per gram dry biomass, maize was able to remove a maximum of 3,267 μg RDX from soil per pot by five 4-week plants at 100 mg/kg of soil. Although RDX is toxic to plants, maize, sorghum, and wheat showed reasonable growth in the presence of the chemical, whereas soybeans were more sensitive to RDX. Results of this study facilitate assessment of the potential invasion of food chain by RDX-contaminated soils.

Keywords: Environmental contamination – Food chain safety – Phytoremediation – Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Tanja Ljubič Mlakar, Milena Horvat, Jože Kotnik, Zvonka Jeran, Tomaž Vuk, Tanja Mrak and Vesna Fajon. Biomonitoring with epiphytic lichens as a complementary method for the study of mercury contamination near a cement plant. Environmental Monitoring and Assessment, Volume 181(1-4) (2011): 225-241

The study was focused on understanding the mercury contamination caused by a cement plant. Active and passive biomonitoring with epiphytic lichens was combined with other instrumental measurements of mercury emissions, mercury concentrations in raw materials, elemental mercury concentrations in air, quantities of dust deposits, temperatures, precipitation and other measurements from the cement plant's regular monitoring programme. Active biomonitoring with transplanted lichens *Pseudevernia furfuracea* (L.) Zopf was performed at seven of the most representative sites around the cement plant and one distant reference site for periods of 3, 6 and 12 months. In situ lichens of different species were collected at the beginning of the monitoring period at the same sites. Mercury speciation of the plant exhaust gas showed that the main form of emitted mercury is reactive gaseous mercury Hg^{2+}, which is specific for cement plants. Elemental mercury in air was measured in different meteorological conditions using a portable mercury detector. Concentrations in air were relatively low (on average below 10 ng m^{-3}). In situ lichens showed Hg concentrations comparable to lichens taken from the background area for

transplantation, indicating that the local pollution is not severe. Transplanted lichens showed an increase of mercury, especially at one site near the cement plant. A correlation between precipitation and Hg uptake was not found probably due to a rather uniform rainfall in individual periods. Dust deposits did not influence Hg uptake significantly. Lichens vitality was affected over longer biomonitoring periods, probably due to some elements in dust particles, their alkalinity and the influence of other emissions. Mercury uptake measured in vital transplanted lichens was in a good correlation with the working hours (i.e. emitted Hg quantity) of the kiln. The study showed that selected lichens could be used to detect low to moderate Hg emissions from a cement plant and that the biomonitoring procedure could be further standardized and used as part of an environmental monitoring programme.

Keywords: Mercury – Emission – Biomonitoring – Epiphytic lichens – Cement plant

Nik M. Majid, M. M. Islam*, Veronica Justin, Arifin Abdu and Parisa Ahmadpour. (Department of Forest Management, Faculty of Forestry, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia, *Corresponding author. E-mail: monirupm.my@gmail.com). **Evaluation of heavy metal uptake and translocation by *Acacia mangium* as a phytoremediator of copper contaminated soil. African Journal of Biotechnology, Vol. 10 (42)(2011): 8373-8379**

Many organic and inorganic pollutants, including heavy metals are being transported and mixed with the cultivated soils and water. Heavy metals are the most dangerous pollutants as they are non-degradable and accumulate and become toxic to plants and animals. An experiment was conducted in the glasshouse to evaluate the potential of *Acacia mangium* as a phytoremediator to absorb heavy metals from contaminated soils. *A. mangium* seedlings were planted in the growth media (soil + different levels of copper). The different levels of Cu were: T_0 (control, soil), T_1 (50 ppm Cu), T_2 (100 ppm Cu), T_3 (200 ppm Cu), T_4 (300 ppm Cu) and T_5 (400 ppm Cu). The highest growth performance such as basal diameter, height and number of leaves was in T_1. The highest biomass was recorded in T_1. Highest accumulation of Cu (93.55 ppm) and Zn (79.13 ppm) were recorded in T_5 while Cd (8.88 ppm) in T_3. Cu was highly concentrated in the roots, Cd was accumulated in the leaves and roots, whereas, Zn was in stems and leaves. *A. mangium* showed high translocation factor (TF) and low bioconcentration factor (BCF) values in soil at higher metal concentrations as well as it was able to tolerate and accumulate high concentrations of Cd, Cu and Zn. It may be concluded that this species can be a good efficient phtyoremediator for heavy metals (Cd, Cu and Zn) contaminated soils to mitigate soil pollution.

Keywords: Heavy metals, phytoremediation, bioaccumulation capacity.

R. Vennila * and V. Kannan. (Centre for Advanced Study in Botany, Guindy Campus, University of Madras, Chennai 600 025, India, Corresponding author. E-mail: venn779@gmail.com). **Bioremediation of petroleum refinery effluent by *Planococcus halophilus.* African Journal of Biotechnology Vol. 10 (44)(2011): 8829-8833**

In the present investigation, *Planococcus halophilus* was screened for hydrocarbon degradation and bioremediation of refinery effluent. The test organism, *P. halophilus*, showed the capability to utilize kerosene as carbon source in minimal medium. Biological treatment of the refinery effluent with *P. halophilus* reduced the oil and grease and sulphide content to about 91.2 and

28%, respectively, on the 4th day of the incubation. The present work defined that the test organism *P. halophilus* can be exploited for bioremediation of sites contaminated with hydrocarbons and industrial effluents polluted with hydrocarbons even under adverse conditions.

Keywords: Kerosene, *Planococcus halophilus*, bioremediation, parameters, hydrocarbon.

Changqing Zhao[1,3*], Qinhuan Yang[2], Wuyong Chen[3], Hui Li[3] and Hao Zhang[3]. ([1]College of Bioengineering, Sichuan University of Science and Engineering, Zigong 643000, P. R. China, [2]College of Material and Chemical Engineering, Sichuan University of Science and Engineering, Zigong 643000, P. R. China, [3]National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu 610065, P. R. China. *Corresponding author. E-mail: zhaocq2010@163.com. Tel/Fax: +86-0813-5505872). Isolation of a sulfate reducing bacterium and its application in sulfate removal from tannery wastewater. African Journal of Biotechnology Vol. 10 (56), pp. 11966-11971

In order to remove sulfate in tannery wastewater efficiently, a sulfate reducing bacterium (SRB) was isolated in tannery anaerobic activated sludge. With biochemical and genetics method, the isolated bacterium was identified as *Citrobacter freundii*. Then, the isolate was inoculated to tubes containing sulfate in simulated tannery wastewater to optimize the process. The results show that the effect of *C. freundii* in removing sulfate was best when the temperature was 32°C, pH was 7.0, COD/SO_4^{2-} was 5.0 and the initial SO_4^{2-} concentration was 1500 mg/L. Also, the SRB was inoculated onto an up-flow anaerobic sludge bed (UASB) to remove sulfate in actual tannery wastewater. It was found that the removal rate of sulfate in actual tannery wastewater reached 89.66% which was 12.13% higher than the treatment without inoculating the isolated SRB when the initial SO_4^{2-} concentration was 1069 mg/L. The experiment demonstrates that *C. freundii* could be selected as a new biomaterial to remove sulfate in tannery wastewater.

Keywords: Tannery wastewater, sulfate, *Citrobacter freundii*.

Kamiyango, M. W.[1], Sajidu, S. M. I.[2]* and Masamba, W. R. L.[3]. ([1]College of Medicine, University of Malawi, P/Bag 360, Chichiri, Blantyre 3, Malawi, [2]Chemistry Department, Chancellor College, P.O. Box 280, Zomba, Malawi, [3]Harry Oppeinheimer, Okavango Research Centre, P/Bag 285, Maun, Botswana. *Corresponding author. Email: sajidu@chanco.unima.mw, ssajidu@yahoo.co.uk. Tel: +265 (0) 8 88 891 714. Fax: +265 (1) 524 046). Removal of phosphate ions from aqueous solutions using bauxite obtained from Mulanje, Malawi. African Journal of Biotechnology Vol. 10 (56) (2011): 11972-11982

Studies on stream water and effluent from selected wastewater treatment plants in Blantyre, Malawi, have reported phosphate concentrations above recommended levels. High phosphate levels in the effluent and streams pose a threat to aquatic life through the stimulation of excessive growth of plants and toxic cyanobacteria. Phosphate removal by bauxite was investigated as a function of pH, contact time, dosage, competing ions and initial phosphate concentration by means of jar tests. Phosphate removal increased with decreasing pH with maximum removal (99.75%) recorded at pH 2.40 ± 0.10. Phosphate removal was attributed to ligand exchange reactions with gibbsite and goethite minerals that are chemically and electrostatically favoured at low pH. Bauxite indicated a high phosphate removal capacity with 98.42% removal recorded for a dosage of 15 g/l. This was attributed to the presence of goethite and gibbsite minerals for which phosphate has a strong affinity. Kinetics studies revealed a fast adsorption reaction with 61 and 65% phosphate removal achieved after 30 min of contact at 20

and 40°C respectively. Phosphate removal was enhanced in the individual presence of calcium and magnesium ions whereas carbonate and sulphate ions reduced it by competing for active sites.

Keywords: Adsorption, bauxite, phosphate, gibbsite, goethite, eutrophication.

Augustine E. Ofomaja[a], Emmanuel E. Ukpebor[b], Stephen A. Uzoekwe[c]. ([a]Department of Chemistry, Vaal University of Technology, P. Bag X021, Vanderbijlpark 1900, South Africa, [d]Department of Chemistry, Faculty of Physical Sciences, University of Benin, Nigeria, [c]Department of Chemistry, Benson Idahosa University, Benin City, Nigeria). Biosorption of Methyl violet onto palm kernel fiber: Diffusion studies and multistage process design to minimize biosorbent mass and contact time. Biomass and Bioenergy, Volume 35(10) (2011): 4112-4123

Determination of the overall rate controlling step in the biosorption of Methyl violet dye onto a new biosorbent, palm kernel fiber has been determined. Pseudo-second-order model described the kinetics over the whole contact time period for the effect of initial concentration and temperature. Using the Wu's approaching equilibrium factor, R_w, it was observed that the time for the switch from initial biosorption to intraparticle diffusion is affected by initial concentration and temperature. A comparison between the activation parameters of film diffusion, pseudo-second order ion exchange and intraparticle diffusion revealed that film diffusion is the overall slowest step in the biosorption process. Temperature increased the biosorption capacity but reduced slightly the rate of intraparticle diffusion, indicating that the biosorbent surface was activated by temperature which limited the diffusion of Methyl violet molecules into the interior of the biosorbent. A multistage process design to minimize mass and contact time was done.

Keywords: Methyl violet; Overall biosorption rate; Approaching equilibrium factor; Multistage optimization; Intraparticle diffusion

U. Adie Gilbert, I. Unuabonah Emmanuel, A. Adeyemo Adebanjo, G. Adeyemi Olalere. (Department of Chemical Sciences, College of Natural Sciences, Redeemer's University, Km 46, Lagos-Ibadan Expressway, Redemption City, Ogun State, Nigeria). Biosorptive removal of $Pb^{2+}$and Cd^{2+} onto novel biosorbent: Defatted *Carica papaya* seeds. Biomass and Bioenergy, Volume 35(7) (2011): 2517-2525

Carica papaya seeds, an agricultural waste in Nigeria, were defatted to obtain defatted *C. papaya* seed biosorbent. The Fourier Transformed Infrared spectrum of defatted *C. papaya* seed biosorbent suggests the presence of C═O, ─OH of carboxylic acid, lactonic and amide band functional groups. The adsorption of metal ion onto defatted *C. papaya* seed biosorbent led to small shifts in the IR bands. The adsorption capacity of defatted *C. papaya* seed biosorbent was evaluated to be 1666.67 mg/g for Pb^{2+} and 1000.00 mg/g for Cd^{2+}. In binary metal ion solution, the defatted *C. papaya* seeds showed decreased adsorption capacity for either metal ion. The influence of different particle sizes was found to have negative impact on the adsorption capacity of *C. papaya* seed biosorbent in the removal of Pb^{2+} and Cd^{2+} from aqueous solution. The adsorption of both metal ions was observed to follow the Freudlich model better than the Langmuir model suggesting that the adsorption of both metal ions was on multisites on the

defatted *C. papaya* seed biosorbent. The adsorption was found to be highly feasible, spontaneous and exothermic in nature. Optimization results suggests 5 m^3 of 100 mg/L of Pb^{2+} and Cd^{2+} requires 43.3 and 49.2 kg of defatted *C. papaya* seeds to remove 95% of the metal ions from aqueous solution.

Keywords: Biosorbent; Exothermic; *Carica papaya* seeds; Particle size; Lead; Cadmium

S. Kalloum[a], H. Bouabdessalem[b], A. Touzi[a], A. Iddou[c, d], M.S. Ouali[d]. ([a]Unit of Research in Renewable Energies in Saharan Medium, Adrar, Algeria, [b]Normal Higher School of Technical Teaching, Oran, Algeria, [c]University of Sciences and Technology-Mohammed Boudiaf Oran, BO 1505 EL M'naouar, 31000, Oran, Algeria, [d]Laboratory of materials valorization and nuisances treatment, Ibn badis University of Mostaganem, BO 227, Mostaganem 27000, Algeria). Biogas production from the sludge of the municipal wastewater treatment plant of Adrar city (southwest of Algeria). Biomass and Bioenergy, Volume 35(7) (2011): 2554-2560

This study deals with the treatment and valorization of sludge issued from the municipal wastewater treatment plant of Adrar city (southwest of Algeria). The sludge considered was a complex mixture of substances, essentially organic matters with a rate of 54%. An acute biological activity of the crude substrate was noted (1.67 10^6 germs/1 ml). The diluted sludge with a content of 16 g/l of total solids (TS) was fermented in a digester of one litter capacity under anaerobic conditions during 33 days. The quantity of biogas produced was 280.31 Nml with a yield of 30 Nml of biogas/mg of COD removed. The COD, BOD and TS reduction yields were 88, 90 and 81% respectively, followed by a complete destruction of the pathogenic flora particularly *Escherichia coli*. This study presented an important energetic opportunity by producing 30,950 KWh.

Keywords: Sludge; Municipal wastewater treatment plant; Organic matter; Digestion; Biogas; Energy

Marco de Graaff[a, b], Martijn F.M. Bijmans[b], Ben Abbas[c], Gert-J.W. Euverink[b], Gerard Muyzer[c], Albert J.H. Janssen[a, d]. ([a]Sub-department of Environmental Technology, Wageningen University, Bomenweg 2, P.O. Box 8129, 6700 EV Wageningen, The Netherlands, [b]Wetsus, Centre of Excellence for Sustainable Water Technology, Agora 1, 8934 CJ, Leeuwarden, The Netherlands, [c]Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC, Delft, The Netherlands, [d]Shell, Kesslerpark 1, 2288 GS, Rijswijk, The Netherlands. Biological treatment of refinery spent caustics under halo-alkaline conditions. Bioresource Technology, Volume 102(15) (2011): 7257-7264

The present research demonstrates the biological treatment of refinery sulfidic spent caustics in a continuously fed system under halo-alkaline conditions (i.e. pH 9.5; Na^+ = 0.8 M). Experiments were performed in identical gas-lift bioreactors operated under aerobic conditions (80–90% saturation) at 35 °C. Sulfide loading rates up to 27 mmol L^{-1} day^{-1} were successfully applied at a HRT of 3.5 days. Sulfide was completely converted into sulfate by the haloalkaliphilic sulfide-oxidizing bacteria belonging to the genus *Thioalkalivibrio*. Influent benzene concentrations ranged from 100 to 600 μM. At steady state, benzene was removed by 93% due to high stripping efficiencies and biodegradation. Microbial community analysis revealed the presence of haloalkaliphilic heterotrophic bacteria belonging to the genera *Marinobacter*, *Halomonas* and *Idiomarina* which might have been involved in the observed benzene removal. The work shows

the potential of halo-alkaliphilic bacteria in mitigating environmental problems caused by alkaline waste.

Keywords: Spent caustics; Sulfide oxidation; Haloalkalipilic; *Thioalkalivibrio*; Benzene

Biotransformation

Heiko Niewerth, Klaus Bergander, Siri Ram Chhabra, Paul Williams and Susanne Fetzner. Synthesis and biotransformation of 2-alkyl-4(1*H*)-quinolones by recombinant *Pseudomonas putida* KT2440. Applied Genetics and Molecular Biotechnology, Applied Microbiology and Biotechnology, Volume 91(5) (2011): 1399-1408

2-Alkyl-4(1*H*)-quinolones (AQs) and related derivatives, which exhibit a variety of biological properties, are secondary metabolites produced by, e.g., *Pseudomonas* and *Burkholderia* spp. Due to their main role as signaling molecules in the quorum sensing system of *Pseudomonas aeruginosa*, 2-heptyl-4(1*H*)-quinolone (HHQ) and its 3-hydroxy derivative, termed the "*Pseudomonas* quinolone signal" (PQS), have received considerable attention. Since chemical synthesis of different AQs is complex, we assessed the applicability of recombinant *P. putida* KT2440 strains for the biosynthetic production of AQs. In mineral salts medium supplemented with octanoate and anthranilate, batch cultures of *P. putida* KT2440 [pBBR-*pqsABCD*] produced about 45 μM HHQ, 30% and 70% of which were localized in the culture supernatant and methanolic cell extract, respectively. 2,4-Dihydroxyquinoline and minor amounts of C_3- to C_{13}-saturated and $C_{7:1}$ to $C_{13:1}$ monounsaturated AQs were formed as by-products. Mass spectrometry and nuclear magnetic resonance analyses spectroscopy indicated that unsaturated AQs having the same molecular mass are *cis* and *trans* isomers rather than position isomers, with the double bond located between the α and β carbon of the alkyl chain. Supplementing the cultures with hexanoate instead of octanoate shifted the AQ profile towards increased formation of C_5-AQ. Individual AQs can be prepared from concentrated methanolic extracts by preparative high-performance liquid chromatography (HPLC). Regioselective hydroxylation of HHQ to PQS can be achieved in >90% yield by biotransformation with *P. putida* KT2440 [pBBR-*pqsH*]. PQS can be isolated from methanolic cell extracts by HPLC, or be precipitated as Fe(III)-PQS complex. Preparation of a library of AQs will facilitate studies on the biological functions of these compounds.

Keywords: 2-Alkyl-4(1*H*)-quinolone – 2-alkenyl-4(1*H*)-quinolone isomers – 2-alkyl-3-hydroxy-4(1*H*)-quinolone – 2-heptyl-4(1*H*)-quinolone – *Pseudomonas putida* – *Pseudomonas* quinolone signal

Jiyoung Seo, Su-Il Kang, Mihyang Kim, Jaehong Han and Hor-Gil Hur. Flavonoids biotransformation by bacterial non-heme dioxygenases, biphenyl and naphthalene dioxygenase. Applied Microbiology and Biotechnology, Volume 91(2) (2011): 219-228

This review details recent progresses in the flavonoid biotransformation by bacterial non-heme dioxygenases, biphenyl dioxygenase (BDO), and naphthalene dioxygenase (NDO), which can initially activate biphenyl and naphthalene with insertion of dioxygen in stereospecfic and

regiospecific manners. Flavone, isoflavone, flavanone, and isoflavanol were biotransformed by BDO from *Pseudomonas pseudoalcaligenes* KF707 and NDO from *Pseudomonas* sp. strain NCIB9816-4, respectively. In general, BDO showed wide range of substrate spectrum and produced the oxidized products, whereas NDO only metabolized flat two-dimensional substrates of flavone and isoflavone. Furthermore, biotransformation of B-ring skewed substrates, flavanone and isoflavanol, by BDO produced the epoxide products, instead of dihydrodiols. These results support the idea that substrate-driven reactivity alteration of the Fe-oxo active species may occur in the active site of non-heme dioxygenases. The study of flavonoid biotransformation by structurally-well defined BDO and NDO will provide the substrate structure and reactivity relationships and eventually establish the production of non-plant-originated flavonoids by means of microbial biotechnology.

Keywords: Biotransformation – Biphenyl dioxygenase – Naphthalene dioxygenase – Flavonoid – Polyphenol

Clarissa S. Capel, Ana C. D. de Souza, Tatiane C. de Carvalho, João P. B. de Sousa, Sérgio R. Ambrósio, Carlos H. G. Martins, Wilson R. Cunha, Rosario H. Galán and Niege A. J. C. Furtado. Biotransformation using *Mucor rouxii* for the production of oleanolic acid derivatives and their antimicrobial activity against oral pathogens. Journal of Industrial Microbiology & Biotechnology, Volume 38(9) (2011): 1493-1498

The goal of this study is to produce oleanolic acid derivatives by biotransformation process using *Mucor rouxii* and evaluate their antimicrobial activity against oral pathogens. The microbial transformation was carried out in shake flasks at 30°C for 216 h with shaking at 120 rpm. Three new derivatives, 7β-hydroxy-3-oxo-olean-12-en-28-oic acid, 7β,21β-dihydroxy-3-oxo-olean-12-en-28-oic acid, and 3β,7β,21β-trihydroxyolean-12-en-28-oic acid, and one know compound, 21β-hydroxy-3-oxo-olean-12-en-28-oic acid, were isolated, and the structures were elucidated on the basis of spectroscopic analyses. The antimicrobial activity of the substrate and its transformed products was evaluated against five oral pathogens. Among these compounds, the derivative 21β-hydroxy-3-oxo-olean-12-en-28-oic acid displayed the strongest activity against *Porphyromonas gingivalis*, which is a primary etiological agent of periodontal disease. In an attempt to improve the antimicrobial activity of the derivative 21β-hydroxy-3-oxo-olean-12-en-28-oic acid, its sodium salt was prepared, and the minimum inhibitory concentration against *P. gingivalis* was reduced by one-half. The biotransformation process using *M. rouxii* has potential to be applied to the production of oleanolic acid derivatives. Research and antimicrobial activity evaluation of new oleanolic acid derivatives may provide an important contribution to the discovery of new adjunct agents for treatment of dental diseases such as dental caries, gingivitis, and periodontitis.

Keywords: Biotransformation – *Mucor rouxii* – Oleanolic acid derivatives – Antimicrobial activity – Oral pathogens

Li Ma, Xiongmin Liu, Jingjuan Liang and Zuohui Zhang. Biotransformations of cinnamaldehyde, cinnamic acid and acetophenone with *Mucor*. World Journal of Microbiology and Biotechnology, Volume 27(9) (2011): 2133-2137

A strain JX23 was isolated from soil and identified as a species of *Mucor* according to the morphological characteristics and the nuclear ribosomal internal transcribed spacer sequence and designated as *Mucor* sp. JX23. Biotransformations of cinnamaldehyde (CMD), cinnamic acid

(CMA) and acetophenone (ACP) catalyzed by JX23 were investigated. After JX23 was cultured for 48 h, the substrates CMD, CMA and ACP were added to the growth medium respectively and the products were analyzed by GC–MS and HPLC. *Mucor* sp. JX23 exhibited considerable redox capability and different catalytic specificity to CMD, CMA and ACP. CMD was selectively hydrogenated to cinnamyl alcohol. CMA was biotransformed to ACP with α, β-oxidation like degradation, and ACP could not be reduced further by JX23. When ACP was added as substrate, it could be asymmetrically reduced to (*S*)-(−)-1-phenylethyl alcohol (*S*-PEA) with high stereoselectivity (90%). Further, the biotransformations of different binary mixture substrates with JX23 were also studied. The biocatalytic selectivity depended on the relationship between the binary mixtures in above-mentioned reaction.

Keywords: *Mucor* – Biotransformation – Cinnamaldehyde – Cinnamic acid – Acetophenone – Binary mixture substrates

Shaofeng Rong, Baomei Ding, Xiaoli Zhang, Xuesong Zheng and Yifei Wang. Enhanced Biotransformation of 2-Phenylethanol with Ethanol Oxidation in a Solid–Liquid Two-Phase System by Active Dry Yeast. Current Microbiology, Volume 63(5) (2011): 503-509

2-Phenylethanol (2-PE) can be produced from L-phenylalanine (L-Phe) with the oxidation degradation of ethanol by active dry yeast. In this study, the catalysis effect of ethanol on biotransforming L-Phe into 2-PE by yeast was evaluated and optimized. The results indicated that increasing ethanol concentration was beneficial for enhancing 2-PE concentration but lowered the 2-PE productivity. Initial ethanol concentration above 25 g/l could strongly inhibit the 2-PE production. To obtain 2-PE with desirable concentrations with an economical operation mode, three fed-batch biotransformation operation methods using ethanol or/and glucose were carried out in a solid–liquid two-phase system. When using ethanol alone with the initial concentration of 10 g/l, the total concentration and overall productivity of 2-PE were 7.6 g/l and 0.065 g l^{-1} h^{-1}, respectively. Furthermore, an experiment with controlled glucose solely (higher than 2 g/l) was finished. In this case, phenylacetaldehyde (PA) was detected along with ethanol accumulation, suggesting that reaction of PA → 2-PE in Ehrlich pathway was inhibited. To further enhance 2-PE production by using glucose only, a novel operation strategy to simultaneously control rates of glucose glycolysis and ethanol oxidative degradation with the aid of ISPR techniques was developed. With this strategy, 2-PE concentration and yield based on glucose consumption reached a higher level of 14.8 g/l and 0.12 g-PE/g-glucose, respectively, and these are the highest values reported up to date with the fed-batch biotransformation operation mode.

Jesper Svanfelt, Johan Eriksson and Leif Kronberg. Photochemical transformation of the thyroid hormone levothyroxine in aqueous solution. Environmental Science and Pollution Research, Volume 18(6) (2011): 871-876

The direct aqueous photolysis of the thyroid hormone levothyroxine (T_4) has been studied.

One of the major photoproducts, i.e., 4-[4-(2-amino-2-carboxy-ethyl)-2,6-diiodo-phenoxy]-penta-2,4-dienoic acid (P1), was isolated by liquid chromatography and structurally assigned by mass spectrometric (MS) and nuclear magnetic resonance spectroscopic methods. The identity of a second major product, i.e., 3,5-diiodo-L-thyrosine (P3), was confirmed through access to a

commercially available standard. Furthermore, the structures of three additional transformation products are proposed on the basis of data obtained by high-resolution MS analyses. UV absorption spectra were determined for T_4 and the two photoproducts P1 and P3. Disappearance quantum yields were calculated for T_4 (φ=0.014 at pH 12) and P3 (φ=0.024 at pH 12 and φ=0.010 at pH 8.5), whereas the compound P1 was found to be stable under the studied conditions ($T_{1/2}$=600 min).

The results indicate that solar UV light may have a significant impact on the fate of T_4 in the aquatic environment.

Keywords: Thyroid hormone – Photolysis – Photoproducts – Environmental fate

Maribel Cano[1]*, Myrna Solis[1], Joel Diaz[1], Aida Solis[2], Octavio Loera[3] and Maura M. Teutli[4]. ([1]Centro de Investigación en Biotecnología Aplicada del Instituto Politécnico Nacional, Carretera Estatal Santa Inés Tecuexcomac-Tepetitla Km. 1.5, Tepetitla de Lardizábal, Tlaxcala, C.P. 90700 México, [2]Universidad Autónoma Metropolitana-Unidad Xochimilco, México, [3]Universidad Autónoma Metropolitana-Unidad Iztapalapa, México, [4]Facultad de Ingeniería, Benemérita Universidad Autónoma de Puebla, México. *Corresponding author. E-mail: maribel_cano@hotmail.com. Tel: 0052-248-4842819). **Biotransformation of indigo carmine to isatin sulfonic acid by lyophilized mycelia from *Trametes versicolor.* African Journal of Biotechnology Vol. 10 (57) (2011): 12224-12231**

In this work, acrylonitrile (AN) and acrylic acid (AA) monomers were directly grafted onto chitosan using ammonium persulfate (APS) as an initiator and methylenebisacrylamide (MBA) as a crosslinking agent under an inert atmosphere. The hydrogels structure was characterized by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and thermogravimetric analysis (TGA). The effect of grafting variables, that is, AA/AN weight ratio and concentration of MBA and APS, was systematically optimized to achieve a hydrogel with swelling capacity as high as possible. The water absorbency increased with increasing the AA amount in the monomer feed due to formation of polyelectrolyte. The swelling of the hydrogel samples in saline solution (0.15 mol/l NaCl, $CaCl_2$ and $AlCl_3$) was examined. The results indicate that the swelling capacity decreased with an increase in the ionic strength of the swelling medium. This behavior can be attributed to charge screening effect for monovalent cations, as well as ionic crosslinking for multivalent cations. Furthermore, the swelling of superabsorbing hydrogels was examined in solutions with pH values ranging between 1 and 13.

Keywords: Hydrogel, chitosan, superabsorbent, acrylonitrile, acrylic acid.

Biomarker

Angela Serafim, Belisandra Lopes, Rui Company, Alexandra Cravo, Tânia Gomes, Vânia Sousa and Maria João Bebianno. A multi-biomarker approach in cross-transplanted mussels *Mytilus galloprovincialis.* Ecotoxicology, Volume 20(8) (2011): 1959-1974

The present work integrates the active biomonitoring (ABM) concept in mussels *Mytilus galloprovincialis* from the South coast of Portugal transplanted during 28 days between two sites with different sources of contamination, and vice versa, in order to assess biological effects in

these mussels. For that purpose a multibiomarker approach was used. The suit of biomarkers indicative of metal contamination were metallothioneins (MT) and the enzyme δ-aminolevulinic acid dehydratase (ALAD), for organic contamination mixed function oxidase system (MFO), glutathione-*S*-transferase (GST) and acetylcholinesterase (AChE), as oxidative stress biomarkers superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid peroxidation (LPO). These biomarkers were used to determine an index to evaluate the stress levels in these two sites. Site A is strongly influenced by metallic contamination, with higher Cu, Cr and Pb in *M. galloprovinciallis*, as well as higher MT levels, antioxidant enzymes activities and LPO concentrations, and lower ALAD activity. In site B organic compounds (PAHs) are prevalent and native mussels show higher activities of the MFO system components and GST. Transplanted mussels had significant alterations in some biomarkers that reflect the type of contaminants present in each site, which demonstrates the primary role of the environment in determining the physiological characteristics of resident mussels. Therefore the application of ABM using a battery of biomarkers turns out to be a useful approach in sites where usually complex mixtures of contaminants occurs. In this study the biomarkers that better differentiate the impact of different contaminants at each site were MT, CYP450, SOD and CAT.

Keywords: Active biomonitoring – Cross-transplant – Multi-biomarker – *Mytilus galloprovincialis*

Gwenola Sabatte, Harma Feitsma, Toon H. Evers, Menno W.J. Prins. (Philips Research, High Tech Campus 11, 5656 AE Eindhoven, The Netherlands). Protein biomarker enrichment by biomarker antibody complex elution for immunoassay biosensing. Biosensors and Bioelectronics, Volume 29(1) (2011): 18-22

It is very challenging to perform sample enrichment for protein biomarkers because proteins can easily change conformation and denature. In this paper we demonstrate protein enrichment suited for high-sensitivity integrated immuno-biosensing. The method enhances the concentration of the biomarkers and simultaneously removes matrix components that could interfere with the immunoassay. Biomarkers are captured using antibody coated magnetic particles and the biomarker antibody complexes are released by enzymatic elution. The eluted complexes are subsequently detected in a sandwich immunoassay biosensor. A scaling study of the enrichment process demonstrates an enrichment factor of 15 in buffer and plasma. We analyze the enrichment factor in terms of the three basic steps of the assay (capture, concentration, elution) and we quantify their respective efficiencies. The process is suited for integration into bio-analytical tools.

Keywords: Enrichment; Protein; Biomarker; Sample preparation; Immunoassay; Magnetic particles; Biosensor

Biofertilizer

Yueyue Ding, Shuang Li, Chang Dou, Yang Yu and He Huang. Production of Fumaric Acid by *Rhizopus oryzae*: Role of Carbon–Nitrogen Ratio. Applied Biochemistry and Biotechnology, Volume 164(8) (2011): 1461-1467

Cytosolic fumarase, a key enzyme for the accumulation of fumaric acid in *Rhizopus oryzae*, catalyzes the dehydration of L-malic acid to fumaric acid. The effects of carbon–nitrogen ratio on the acid production and activity of cytosolic fumarase were investigated. Under nitrogen limitation stress, the cytosolic fumarase could keep high activity. With the urea concentration decreased from 2.0 to 0.1 g l^{-1}, the cytosolic fumarase activity increased by 300% and the production of fumaric acid increased from 14.4 to 40.3 g l^{-1} and L-malic acid decreased from 2.1 to 0.3 g l^{-1}. Cytosolic fumarase could be inhibited by substrate analog 3-hydroxybutyric acid. With the addition of 3-hydroxybutyric acid (50 mM) in the fermentation culture, fumaric acid production decreased from 40.3 to 14.1 g l^{-1} and L-malic acid increased from 0.3 to 5.4 g l^{-1}.

Keywords: Fumaric acid – Cytosolic fumarase – Substrate analog – *Rhizopus oryzae*

Biocomposting

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In order to asses the implication of endosulfan in the soil amended with vermicompost, *Zinnia elegans* (Family Asteraceae) was grown, under strict laboratory conditions. Seed germination, size of internode, total length of the plant, leaf area of the plant, tufts of rootlets, which emerged, were measured and recorded. Vermicompost at a concentration of 12.5% was used as source of amendment; treatment was made with endosulfan the concentration of which ranged from 2.5, 5 and 7.5% respectively. From the data obtained after 4 days of treatment, it appeared that the addition of endosulfan in plain soil (7.5%), affects germination to negative value. Reduced germination and plant growth even during prolonged treatment (up to 9 days) was noticed during treatment with 2.5 and 5% of endosulfan. In a soil amended with vermicompost, however, germination to total length of the plant was found to increase quite considerably. This trend has continued, even during extended period of treatment. The entire texture of the plant was found to change to a healthier look in the presence of vermicompost. Better growth of the plant, larger number of rootlets, and bigger leaf area, can be suggested to be additive role of vermicompost on growth and development of *Zinnia elegans*. This also indicated possible involvement of the plant

in remediation of pesticide endosulfan. On this account, *Z. elegans* like related members of this family can be considered as a candidate involved in remediation of pesticides from polluted soil

Keyword: Vermicompost, endosulfan, *Zinnia elegans*.

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The bioaccumulation of heavy metals (Cd, Zn, Ni, Pb and Cr) and the relationship between them was investigated on earthworm (*Eisenia fetida)* physiology during the process of vermicomposting. The soil samples were obtained from Roudehen city in the eastern area of Tehran. *E. fetida* specimens were exposed to a mixture of metal compounds in various concentrations to estimate the rate of heavy metal bioaccumulation. After 14-days of (the results of the experiments showed that earthworms accumulated this elements in 14 days) exposure, the metal accumulation was measured using atomic absorption spectroscopy. Cluster analysis was used to interprete the data. Analysis of the 14-day experimental results revealed a strong relationship between the bioaccumulation of Pb and Cr and between Ni and Cd (correlations 0.8 and 0.63).

Keywords: Earthworm, *Eisenia fetida*, vermicomposting, heavy metal, bioaccumulation.

Adarsh Pal Vig[a], Jaswinder Singh[b], Shahid Hussain Wani[a], Salwinder Singh Dhaliwal[c]. ([a]Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, India, [b]Department of Zoology, Khalsa College, Amritsar, Punjab, India, [c]Department of Soil Science, Punjab Agricultural University, Ludhiana, Punjab, India). Vermicomposting of tannery sludge mixed with cattle dung into valuable manure using earthworm *Eisenia fetida* (Savigny). Bioresource Technology, Volume 102(17) (2011): 7941-7945

The present study revealed the role of earthworm in converting tannery sludge into a valuable product. Tannery sludge was toxic to earthworm, therefore it was mixed with cattle dung in different proportions viz. 0:100 (T_0), 10:90 (T_{10}), 25:75 (T_{25}), 50:50 (T_{50}) and 75:25 (T_{75}) on dry weight basis. The minimum mortality and highest population buildup of worms was in T_0 mixture. Nitrogen, sodium, phosphorus and pH increased from initial in the range of 7.3–66.6%, 16.90–70.58%, 8.57–44.8% and 2.8–13.65%, respectively. On the other hand potassium, organic carbon and electrical conductivity decreased in the range of 4.34–28.5%, 7.54–22.35% and 32.35–53.12%, respectively. C:N ratio decreased from 20.53% to 47.36% in the final products. Transition metals increased significantly from the initial value and within the permissible limit. The result indicated that vermicomposting with *Eisenia fetida* is better for changing this sludge into nutrient rich manure in a short period of time.

Keywords: *Eisenia fetida*; Tannery sludge; Vermicomposting; Leather industry; Solid waste

K. Liu, G.W. Price . (**Department of Engineering, Nova Scotia Agricultural College, Truro, Nova Scotia, P.O. Box 550, Canada B2N 5E3). Evaluation of three composting systems for the management of spent coffee grounds. Bioresource Technology, Volume 102(17) (2011): 7966-7974**

This study was conducted to evaluate the optimum composting approach for the management of spent coffee grounds from the restaurant and ready-to-serve coffee industry. Three composting systems were assessed, including in-vessel composting, vermicomposting bins, and aerated static pile bin composting, over study periods ranging from 47 to 98 days. Total carbon content was reduced by 5–7% in the spent coffee ground treatments across the three composting systems. Nitrogen and other mineral nutrient contents were conserved or enhanced from the initial to the final composts in all the composting systems assessed. Earthworm growth and survival (15–80%) was reduced in all the treatments but mortality rates were lower in coffee treatments with cardboard additions. A decline in earthworm mortality with cardboard additions was the result of reduced exposure to organic compounds and chemicals released through the decomposition of spent coffee grounds.

Keywords: Spent coffee grounds; Total carbon; Total nitrogen; *Eisenia fetida*; Composting systems

Aija Rainisalo, Martin Romantschuk, Merja H. Kontro . (**Helsinki University, Department of Environmental Sciences, Niemenkatu 73, 15140 Lahti, Finland**). **Evolution of clostridia and streptomycetes in full-scale composting facilities and pilot drums equipped with on-line temperature monitoring and aeration. Bioresource Technology, Volume 102(17) (2011): 7975-7983**

The evolution of sporulating bacteria in full-scale composting facilities with online temperature monitoring has been poorly studied, although organic matter recycling increases. We analysed *Clostridium perfringens* and sulphite-reducing clostridia (SRC) by cultivation, and streptomycetes by real-time PCR in five full-scale, temperature-monitored and aerated composting processes, and two pilot-scale drum composters. Facilities composted woodchips, sawdust, peat, or bark amended sludge or source-separated biowaste. Streptomycetes genes of $0.21–110 \times 10^7$ copies/g feed increased fast to $0.019–33 \times 10^9$ copies/g, and then were equal or decreased. SRC of $0.06–2.2 \times 10^7$ cfu/g feed decreased to 0–600 cfu/g, with re-growth in two facilities. End products were clean of *C. perfringens*, detected in sludge composts. Although processes contained large quantities of spore-forming bacteria, in the best facilities end products had the high quality. Temperature (>55 °C, >2d) was not related to the end compost quality, but relations between waste and bulking agent qualities, aeration, and processing time should be better controlled.

Keywords: Clostridia; Streptomycetes; Composting; Full-scale facilities; Pilot drums

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Biofiltration of composting gases using different municipal solid waste-pruning residue composts: Monitoring by using an electronic nose. Bioresource Technology, Volume 102(17) (2011): 7984-7993

The concentration of volatile organic compounds (VOCs) during the composting of kitchen waste and pruning residues, and the abatement of VOCs by different compost biofilters was studied. VOCs removal efficiencies greater than 90% were obtained using composts of municipal solid waste (MSW) or MSW-pruning residue as biofilter material. An electronic nose identified qualitative differences among the biofilter output gases at very low concentrations of VOCs. These differences were related to compost constituents, compost particle size (2–7 or 7–20 mm), and a combination of both factors. The total concentration of VOCs determined by a photoionization analyser and inferred from electronic nose data sets were correlated over an ample range of concentrations of VOCs, showing that these techniques could be specially adapted for the monitoring of these processes.

Keywords: Biofiltration; Volatile organic compounds; Municipal solid waste; Composting; Electronic nose

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Influence of humic substances (HS) on bioavailability of Cu and Zn was characterized during 120 days co-composting of sewage sludge and maize straw. At the initial stage of composting, Cu and Zn in sewage sludge were released as organic matter was degraded, and water soluble Cu and Zn increased markedly. Water soluble Cu and FA content decreased after 21 days whereas water soluble Zn increased during the whole process. Both HA–Cu and HA–Zn were significantly and positively correlated with HA and H/F, respectively. At the end of composting, the distribution coefficients of HA–Cu and HA–Zn reached 27.50% and 3.33% respectively with HA–Cu/HA–Zn ratio increased from 1.29 to 2.73. The results suggest that Cu combined with HA more strongly than Zn, and composting treatment could decrease bioavailability of Cu markedly.

Keywords: Sewage sludge; Composting; Heavy metals; Humic substances; Bioavailability

Karuna Shrestha, Pramod Shrestha, Kerry B. Walsh, Keith M. Harrower, David J. Midmore. (Centre for Plant and Water Science (CPWS), Faculty of Science, Engineering & Health, CQUniversity, Rockhampton, 4702 QLD, Australia). Microbial enhancement of compost extracts based on cattle rumen content compost – Characterisation of a system. Bioresource Technology, Volume 102(17) (2011): 8027-8034

Microbially enhanced compost extracts ('compost tea') are being used in commercial agriculture as a source of nutrients and for their perceived benefit to soil microbiology, including plant disease suppression. Rumen content material is a waste of cattle abattoirs, which can be value-

added by conversion to compost and 'compost tea'. A system for compost extraction and microbial enhancement was characterised. Molasses amendment increased bacterial count 10-fold, while amendment based on molasses and 'fish and kelp hydrolysate' increased fungal count 10-fold. Compost extract incubated at 1:10 (w/v) dilution showed the highest microbial load, activity and humic/fulvic acid content compared to other dilutions. Aeration increased the extraction efficiency of soluble metabolites, and microbial growth rate, as did extraction of compost without the use of a constraining bag. A protocol of 1:10 dilution and aerated incubation with kelp and molasses amendments is recommended to optimise microbial load and fungal-to-bacterial ratio for this inoculum source.

Keywords: Compost tea; Plate counts; FDA hydrolysis; Additives; Organic acids

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The present work proposes the use of *Pleurotus ostreatus* immobilized on Amberlite XAD-4 as new biosorbent in trace metal determination. The effects of experimental parameters, such as "pH and flow rate of sample solution, amount of solid phase, eluent type, and concentration" on the recovery of the metal ions were investigated. Maximum adsorption of Cr(III), Cd(II) and Cu(II) ions took place in the pH range 4–5. These metal ions can be desorbed with 1 M HCl (recovery 95–100%). 0.2 g adsorbent amount and 2.5 mL min^{-1} flow rate was found to be optimum of all preconcentration experiments. The sorption capacity after 10 cycles of sorption and desorption does not vary more than 2.0%. The influences of the contaminant ions on the retentions of the analytes were also examined. The results showed that *P. ostreatus* immobilized on Amberlite XAD-4 can be considered as very promising material in trace metal determination.

Keywords: Chromium(III); Cadmium(II); Copper(II); Preconcentration; *Pleurotus ostreatus*

H. Deka[a], S. Deka[a], C.K. Baruah[c], J. Das[d], S. Hoque[b], N.S. Sarma[b]. ([a]Resource Management and Environment Division, Institute of Advanced Study in Science and Technology, Guwahati-35, Assam, India, [b]Material Science Division, Institute of Advanced Study in Science and Technology, Guwahati-35, Assam, India, [c]Department of Botany, Gauhati University, Guwahati-14, Assam, India, [d]Department of Zoology, Gauhati University, Guwahati-14, Assam, India). Vermicomposting of distillation waste of citronella plant (*Cymbopogon winterianus* Jowitt.) employing *Eudrilus eugeniae.* Bioresource Technology, Volume 102(13) (2011): 6944-6950

Laboratory experiment on vermicomposting of distillation waste of java citronella (*Cymbopogon winterianus* Jowitt.) was carried out employing *Eudrilus eugeniae*, in two seasonal trials, covering summer and winter periods. Two vermicomposting treatments were conducted in earthen pots, one with citronella plant waste only (CW) and the other, a mixture of citronella waste and cowdung in the proportion 5:1 (CW + CD). Vermicomposting of citronella waste resulted reduction in C/N ratio (83.5–87.7%), enhancement of ash content and a number of macro and micronutrients. The FT-IR spectroscopy of the vermicompost revealed the reduction in aliphatic and aromatic compound as well as increase in amide group after the 105 days

stabilization process. The vermicompost output was significantly enhanced in CW + CD treatment than CW treatment. Even, nutrient content of the vermicompost was also higher in CW + CD treatment than CW alone indicating the positive role of cowdung in improvement of quantity and quality.

Keywords: Vermicomposting; Citronella waste; *Eudrilus eugeniae*; FT-IR spectroscopy; Seasonal impact

Guang'en Luo[a], P. James Strong[b], Hailong Wang[b], Wuzhong Ni[a], Weiyong Shi[a]. ([a]Key Laboratory of Polluted Environment Remediation and Ecological Health, Ministry of Education, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou Zhejiang 310029, China, [b]Scion, Private Bag 3020, Rotorua 3046, New Zealand). Kinetics of the pyrolytic and hydrothermal decomposition of water hyacinth. Bioresource Technology, Volume 102(13) (2011): 6990-6994

The kinetics of water hyacinth decomposition using pyrolysis and hydrothermal treatment was compared. With pyrolysis, initial vaporization occurred at 453 K as determined by thermogravimetric analysis, while initial solubilisation occurred at 433 K with subcritical hydrothermal treatment. The "kinetic triplet" was determined for the ranges of 423–483 K (range I) and 473–553 K (range II) using the Coats–Redfern method for both treatments. The calculated activation energies for ranges I and II were 110 and 116 kJ/mol for conventional pyrolysis and 145 and 90 kJ/mol for hydrothermal treatment. The similar activation energies for the two temperature ranges observed for pyrolysis implied that only hemicellulose decomposition occurred. For hydrothermal treatment, both hemicellulose and cellulose decomposition occurred in temperature range II, in which a notable lower activation energy was observed. This implied hydrothermal treatment was more suitable for conversion lignocellulosic biomass under these conditions.

Keywords: Coats–Redfern method; Hydrothermal; Kinetic; Water hyacinth

Biopesticides

S.J. Taylor, D.A. Downie, I.D. Paterson. (Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa). Genetic diversity of introduced populations of the water hyacinth biological control agent *Eccritotarsus catarinensis* (Hemiptera: Miridae). Biological Control, Volume 58(3) (2011): 330-336

Genetic bottlenecks can be deleterious to populations. In biological control, agent populations may be subject to severe bottlenecks during selection, importation and while in culture. The genetic variability of two collections of the water hyacinth biological control agent *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae) was measured using Inter-simple Sequence Repeats (ISSR) and mtDNA cytochrome oxidase I (COI) sequences. The first collection (Brazilian) went through a bottleneck of a single gravid female, while the second collection (Peru) originated from 1000 individuals and has been maintained at a large size in culture. Two naturalised South African populations from the Brazilian collection were also sampled (Nseleni and Mbozambo).

Polymorphism for ISSR was high in the Peruvian and two naturalised samples, but much less so in the Brazilian sample. The Peruvian population was shown to be highly differentiated from the Brazilian and its naturalised populations by high values of F_{ST} and Nei's genetic distance, as well as in a Multidimensional Scaling (MDS) plot and an unrooted neighbour joining tree derived from Jaccard's coefficient of similarity. In addition, sequencing of the COI region of the mitochondrial DNA revealed only two haplotypes, one Brazilian and one Peruvian, with a 5.2% sequence divergence, suggesting that recombination and not mutation is the cause of most variation in the ISSR regions. The results suggest that substantial genetic variation may be retained or recovered after a bottleneck. This may mitigate deleterious effects that are a concern for the fate of biological control agents after release.

Keywords: COI; *Eichhornia crassipes*; Genetic bottleneck; ISSR; Population genetics

M.A. Rafter[a], W.A. Palmer[b], G.H. Walter[a]. ([a]School of Biological Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia, [b]Biosecurity Queensland, Department of Employment, Economic Development & Innovation, Ecosciences Precinct, GPO Box 46, Brisbane, Qld 4001, Australia). Assessing the biological control potential of an adventitiously-established "pest", *Scirtothrips aurantii* (Faure), on a weed, *Bryophyllum delagoense* (Eckl. & Zeyh.), in Queensland. Biological Control, Volume 58(3) (2011): 346-353

South African citrus thrips (*Scirtothrips aurantii*) established adventitiously in Australia. Although it is a major horticultural pest in Africa, it is now advocated as a possible biological control agent against *Bryophyllum delagoense* Eckl. & Zeyh. (Crassulaceae). To evaluate the biocontrol potential of *S. aurantii* a two year field study was conducted on the western Darling Downs of southern Queensland. Imidacloprid insecticide was applied to two quadrats at each of 18 field sites to assess, in the absence of *S. aurantii*, the persistence of individual plants and to quantify propagule production and recruitment by this declared weed. A third quadrat was left, as a control, to be infested naturally by *S. aurantii.* When released from herbivory by thrips in the field, plants grew significantly more, flowered more, and were significantly more fecund than plants in the quadrats with *S. aurantii*. Increases in growth and fecundity translated into significantly increased plant numbers but not increased recruitment. Recruitment even declined in experimental quadrats, through the indirect effects of releasing plants from herbivory. Field sampling also revealed that *S. aurantii* may be sensitive to seasonal climatic fluctuations. These and other local climatic influences may limit the biological control potential of the insect.

Keywords: Insecticide exclusion study; Mother-of-millions; South African citrus thrips; Biocontrol potential

A. Raio[a], G. Puopolo[b], A. Cimmino[c], R. Danti[a], G. Della Rocca[a], A. Evidente[c]. ([a]Istituto per la Protezione delle Piante, CNR-Area di Ricerca di Firenze, 50019 Sesto fiorentino (FI), Italy, [b]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", 80055 Portici (NA), Italy, [c]Dipartimento di Scienze del Suolo, della Pianta, dell′Ambiente e delle Produzioni Animali, Università degli Studi di Napoli "Federico II", 80055 Portici (NA), Italy). Biocontrol of cypress canker by the phenazine producer *Pseudomonas chlororaphis* subsp. *aureofaciens* strain M71. Biological Control, Volume 58(2) (2011): 133-138

Pseudomonas chlororaphis subsp. *aureofaciens* strain M71 was tested for its efficacy in controlling *L. Seiridium cardinale*, the fungus responsible for bark canker of common cypress (*Cupressus sempervirens*). The bacterium was able to completely inhibit the mycelial growth and conidium germination of the fungus *in vitro* and prevented canker induction in field trials. Strain M71 produced two phenazine compounds, phenazine-1-carboxyilic acid and 2-hydroxyphenazine. They were extracted from the bacterial culture, purified, identified and tested for their activity against *S. cardinale* and three other fungi responsible for cypress canker, viz. *Diplodiacupressi*, *Seiridium cupressi* and *Seiridium unicorne*. Phenazine-1-carboxylic acid was the sole compound active against the four fungi. The application *in vivo* of this phenazine molecule against *S. cardinale* reduced canker size indicating that the compound is directly involved in the control of the fungal pathogen by *P. chlororaphis* subsp. *aureofaciens* strain M71. Furthermore, the antagonist showed an interesting capacity for epiphytic fitness since it was able to establish itself on the crown of cypress plants and survive on it for more than three months.

Keywords: Phenazine-1-carboxyilic acid; Phyllosphere; *Seiridium cardinale*

Sandrine Cheyppe-Buchmann, Marie-Claude Bon, Sylvie Warot, Walker Jones, Thibaut Malausa, Xavier Fauvergue and Nicolas Ris. Molecular characterization of *Psyttalia lounsburyi*, a candidate biocontrol agent of the olive fruit fly, and its *Wolbachia* symbionts as a pre-requisite for future intraspecific hybridization. BioControl, Volume 56(5) (2011): 713-724

Numerous arthropod species are genetically differentiated across their distribution area. Diversifying the geographical origins of a biocontrol agent species can be used to favour their perennial establishment by the sampling of pre-adapted genotypes and/or the production of new genotypes through hybridization. Hybridization can be nevertheless challenged by reproductive isolations induced by some common microbial endosymbionts. In this study, we aimed at characterizing (i) the genetic diversity of six populations of *Psyttalia lounsburyi* (Hymenoptera: Braconidae), a candidate biocontrol agent of the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) and (ii) the diversity of their *Wolbachia* endosymbionts. Both mitochondrial and microsatellite markers evidence clustering between the South African population and several Kenyan/Namibian populations. The survey of the *Wolbachia* also distinguished two main variants with a spatial heterogeneity in the infection status. All these results are discussed in the context of the use of these *P. lounsburyi* populations for hybridization and further field releases.

Keywords: *Bactrocera oleae* – Classical biological control – Hybridization – *Psyttalia lounsburyi* – *Wolbachia*

L. Ebrahimi, G. Niknam and E. E. Lewis. Lethal and sublethal effects of Iranian isolates of *Steinernema feltiae* and *Heterorhabditis bacteriophora* on the Colorado potato beetle, *Leptinotarsa decemlineata*. BioControl, Volume 56(5) (2011): 781-788

To determine the LC_{50} values of two entomopathogenic nematodes against *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) prepupae, different concentrations of the nematodes were tested in soil. Because of the different temperature requirements of the two nematode species, bioassay experiments were conducted at $20 \pm 1°C$ and $27 \pm 2°C$ for

Steinernema feltiae Filipjev (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), respectively. Both the isolates were effective against *L. decemlineata*. LC_{50} values of *H. bacteriophora* against progeny of field-collected adults and laboratory-reared adults were estimated as 8.5 and 7.6 IJ per prepupa, respectively. For *S. feltiae* the value was calculated as 51.2 IJ per prepupa against offspring of laboratory-reared adults of *L. decemlineata* only. Cellular encapsulation of both nematode species was observed. Sublethal nematode concentrations caused wing deformation and delayed metamorphosis which may affect Colorado potato beetle adult fitness.

Keywords: Chrysomelidae – Insect fitness – Heterorhabditidae – LC_{50} – Steinernematidae – Prepupa

Azza Misk and Christopher Franco. Biocontrol of chickpea root rot using endophytic actinobacteria. BioControl, Volume 56(5) (2011): 811-822

Eleven actinobacterial strains were isolated from different plants, lentil (*Lens esculentus*), chickpea (*Cicer arietinum* L.), pea (*Pisum sativum*), faba bean (*Vicia faba*) and wheat (*Triticum vulgare*) from Paskerville, South Australia. Isolates were characterized and identified morphologically as well as using 16S ribosomal RNA gene sequencing. Of the actinobacteria tested, 72% produced siderophores, 33% were positive for cyanogens production, and 11% showed phosphate solubility. All isolates had antimicrobial activity against *Phytophthora medicaginis, Pythium irregulare* and *Botrytis cinerea.* In a greenhouse experiment, actinobacteria with the highest biocontrol capabilities were tested for their ability to control *Phytophthora* root rot on chickpea. Both *Streptomyces* sp. BSA25 and WRA1 successfully suppressed *Phytophthora* root rot when coinoculated with either *Mesorhizobium ciceri* WSM1666 or Kaiuroo 3. *Streptomyces* sp. BSA25 with either rhizobial strain enhanced vegetative growth of root (7–11 fold) and shoot dry weights (2–3 fold) compared to infected control, whereas *Streptomyces* sp. WRA1 increased root and shoot dry weights by 8- and 4-fold, respectively when inoculated with *M. ciceri* WSM1666. We suggest that careful selection of actinobacteria should be considered when coinoculated with beneficial microorganisms as plant symbionts.

Keywords: Actinobacteria – Rhizobia – Coinoculation – Biocontrol

Patrick De Clercq, Peter G. Mason and Dirk Babendreier. Benefits and risks of exotic biological control agents. BioControl, Volume 56(4) (2011): 681-698

The use of exotic (=alien) arthropods in classical and augmentative biological control programs has yielded huge economic and ecological benefits. Exotic species of arthropods have contributed to the suppression of key pests in agriculture and forestry or have aided in restoring natural systems affected by adventive species. However, adverse non-target effects of exotic biological control agents have been observed in a number of projects. Non-target effects range from very small effects, e.g. 2% parasitization on a non-target insect on a local level, to massive effects on a large scale. Until now, no consensus on how to judge the magnitude of non-target effects and whether these effects can be tolerated or are unacceptable has emerged. In this paper, we briefly review both the benefits of biological control as well as the associated risks including to human and animal health, plant health and particularly the environment. We also make an attempt at identifying the major challenges for assessing risks and for balancing benefits and risks. There is general agreement that sound risk assessment procedures should precede the

release of exotic invertebrate biological control agents and a recent shift—especially for arthropod biological control—from introductions done without meaningful risk assessment studies to projects conducting thorough host range testing can be observed. However, overly stringent regulations that would preclude promising agents from being developed must be avoided.

Keywords: Augmentative biological control – Augmentation – Classical biological control – Exotic species – Non-target effects – Risk assessment – Risk-benefit analysis

Irom Manoj Singha, Yelena Kakoty, Bala Gopalan Unni, Mohan Chandra Kalita, Jayshree Das, Ashok Naglot, Sawlang Borsingh Wann and Lokendra Singh. Control of *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* using leaf extract of *Piper betle* L.: a preliminary study. World Journal of Microbiology and Biotechnology, Volume 27(11) (2011): 2583-2589

The main objective of this study was to evaluate the effectiveness of crude chloroform extract of *Piper betle* L. (PbC) in controlling *Fusarium* wilt of tomato (*Lycopersicon esculentum*) caused by *Fusarium oxysporum* f. sp. *lycopersici*. It was observed that 1% (w/w) amendment of the PbC in soil was more efficient in reducing the *Fusarium* population in soil than carbendazim and the combined amendment of carbendazim and PbC. *Fusarium* wilt control studies were carried out in a greenhouse. Variation in different parameters like shoot growth, root growth and mean fresh weights of tomato seedlings in all the treatments were recorded. Accumulation of total phenolics was also studied from the root tissues of tomato. Higher accumulation of total phenolics was observed in the *Fusarium*-infested plants as compared to that of healthy control and PbC-treated plants. Moreover, it was observed that the extract could reduce the symptoms and disease development. Electron microscopy studies were also done to observe the *Fusarium* infestation in the vascular bundles and to show the accumulation of total phenolics in the vacuoles of root tissue.

Keywords: *Fusarium* wilt – *Piper betle* L. – Tomato

Ali Suliman Al-Akel* and Elamin Mohamed Suliman. (Department of Zoology, College of Science, King Saud University, P. O. Box 2455, Riyadh-11451, Saudi Arabia. *Corresponding author. E-mail: alaklasr@hotmail.com). Biological control agent for mosquito larvae: Review on the killifish, *Aphanius dispar dispar* (Rüppel, 1829). African Journal of Biotechnology, Vol. 10 (44)(2011): 8683-8688

This review attempts to give an account on the recent advances on the killifish *Aphanius dispar dispar* as a biological control agent for mosquito larvae. Thirty six (36) articles of literature (scientific papers, technical and workshop reports) on this subject covering the period between 1980 and 2009 were reviewed. The larviciding process by using chemicals to control mosquitoes in the past resulted in a very harmful effects on the environment (bioaccumulation of DDT), resistance of mosquito vectors, destruction of non-target organisms and human health hazards. Biological control of mosquito larvae by using fish has shown many advantages over chemicals, but exogenous fishes such as *Gambusia affinis* may have negative effects on fishes and destroy the local habitat. Eco-friendly indigenous larvivorous fish with less harm to the environment and the local fish fauna is suitable for biological control of mosquito larvae. Furthermore, *A. dispar*

is capable of natural and artificial reproduction to maintain a fish stock in order to eliminate mosquito larvae and protect people from many mosquito borne diseases such as malaria, dengue, rift valley fever (RVF), encephalitis and many others (Suliman, 2010). Hence, the indigenous killifish, *A. dispar* is found to play this role effectively and efficiently. Problems associated with its artificial breeding and fungal infection of its eggs can be further investigated. In addition to this, integrated methods of biological control should be carried out in order to reach the best targets of mosquito control.

Key words: Biological control, mosquito larvae, indigenous fish, *Aphanius dispar.*

Xiao Huang, Yumei Wang, Wei Liu, Jie Bao. (State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China). Biological removal of inhibitors leads to the improved lipid production in the lipid fermentation of corn stover hydrolysate by *Trichosporon cutaneum*. Bioresource Technology, Volume 102(20) (2011): 9705-9709

Corn stover (CS) hydrolysate was used as the fermentation feedstock of *Trichosporon cutaneum* CX1 for production of microbial lipid as the potential raw material of biodiesel. Two major technical barriers of the lipid fermentation were investigated: one was the strong inhibition of lignocellulose degradation compounds generated in the CS pretreatment; the other was the low carbon-to-nitrogen molar ratio (C/N ratio) of the CS hydrolysate. The newly established biodetoxification method was applied to remove the inhibitors in the pretreated CS. The enhancement of the pretreatment severity and the biodetoxification intensity on the lipid fermentation was investigated. The results show that the biodetoxification not only efficiently removed the inhibitor substances, but also led to the reduction of nitrogen content and the increase of C/N ratio. The cell lipid content of *T. cutaneum* CX1 using the biodetoxified CS hydrolysate reached 23.5%, which was doubled than that using the non-detoxified value.

Keywords: Microbial lipid; Corn stover hydrolysate; *Trichosporon cutaneum* CX1; Biodetoxification; Carbon-to-nitrogen molar ratio (C/N ratio)

Biodegradation

Maíra Nicolau de Almeida, Valéria Monteze Guimarães, Kenneth M. Bischoff, Daniel Luciano Falkoski, Olinto Liparini Pereira, Dayelle S. P. O. Gonçalves and Sebastião Tavares de Rezende. Cellulases and Hemicellulases from Endophytic *Acremonium* Species and Its Application on Sugarcane Bagasse Hydrolysis. Applied Biochemistry and Biotechnology, Volume 165(2) (2011): 594-610

The aim of this work was to have cellulase activity and hemicellulase activity screenings of endophyte *Acremonium* species (*Acremonium zeae* EA0802 and *Acremonium* sp. EA0810). Both fungi were cultivated in submerged culture (SC) containing L-arabinose, D-xylose, oat spelt xylan, sugarcane bagasse, or corn straw as carbon source. In solid-state fermentation, it was tested as carbon source sugarcane bagasse or corn straw. The highest FPase, endoglucanase, and xylanase activities were produced by *Acremonium* sp. EA0810 cultivated in SC containing sugarcane bagasse as a carbon source. The highest β-glucosidase activity was produced by *Acremonium* sp. EA0810 cultivated in SC using D-xylose as carbon source. *A. zeae* EA0802 has highest α-arabinofuranosidase and α-galactosidase activities in SC using xylan as a carbon

source. FPase, endoglucanase, β-glucosidase, and xylanase from *Acremonium* sp. EA0810 has optimum pH and temperatures of 6.0, 55 °C; 5.0, 70 °C; 4.5, 60 °C; and 6.5, 50 °C, respectively. α-Arabinofuranosidase and α-galactosidase from *A. zeae* EA0802 has optimum pH and temperatures of 5.0, 60 °C and 4.5, 45 °C, respectively. It was analyzed the application of *Acremonium* sp. EA0810 to hydrolyze sugarcane bagasse, and it was achieved 63% of conversion into reducing sugar and 42% of conversion into glucose.

Keywords: Cellulase – Hemicellulase – *Acremonium* – Endophyte – Ethanol – Agroindustrial residue

Chunqiao Xiao, Ruan Chi, Xiaohong Li, Min Xia and Zhongwu Xia. Biosolubilization of Rock Phosphate by Three Stress-Tolerant Fungal Strains. Applied Biochemistry and Biotechnology, Volume 165(2) (2011): 719-727

Three stress-tolerant phosphate-solubilizing fungal strains identified as *Aspergillus niger*, *Aspergillus japonicus*, and *Penicillium simplicissimum* were isolated from wheat rhizospheric soil. The strains demonstrated different capabilities of phosphate solubilization in National Botanical Research Institute's phosphate medium containing rock phosphate (RP) as sole phosphorus (P) source, and the solubilization of RP by *P. simplicissimum* was the most effective among these strains, followed by *A. niger* and *A. japonicus*. All the strains exhibited high levels of stress tolerance like 1045°C temperature, 411 pH, 03.5% NaCl, and 035% PEG 10000. The strains also differed in their abilities to survive and release soluble P from RP under different stresses. *A. niger* showed significantly higher tolerance to temperature and pH over the other two strains. Higher amount of spores and content of soluble P in the medium were observed in the presence of 3.5% NaCl with *P. simplicissimum*, followed by *A. niger* and *A. japonicus*. *P. simplicissimum* could not solubilize RP in the presence of 35% PEG 10000, which exhibited the lowest tolerance to desiccation stress among the three strains.

Keywords: Biosolubilization – Rock phosphate (RP) – Phosphate-solubilizing fungi – Stress – Phosphorus (P)

Saad Aldin, Fuzhou Tu, George Nakhla and Madhumita B. Ray. Simulating the Degradation of Odor Precursors in Primary and Waste-Activated Sludge During Anaerobic Digestion. Applied Biochemistry and Biotechnology, Volume 164(8) (2011): 1292-1304

Degradation of known odor precursors in sludge during anaerobic digestion was systematically studied and simulated using the Anaerobic Digestion Model Number 1 (ADM1). The degradation of various protein fractions (particulate, soluble, and bound), volatile fatty acids (VFAs), lipids, and amino acids of primary sludge (PS) and waste-activated sludge (WAS) were monitored during anaerobic digestion. The degradation kinetic constants of the odor precursors namely, protein, lipid, and VFAs were determined. Relationships between degradations of protein fractions and volatile suspended solid were established; a strong relationship between bound protein, a major odor precursor, and volatile suspended solid degradation was found. No statistically significant difference in bound protein reduction was observed between PS and WAS. ADM1 was successfully used to simulate the lab scale continuous anaerobic digestion; model results with optimized parameters showed good agreement with the experimental data for

methane production and several other sludge parameters including odor precursors such as lipids, VFAs, and proteins.

Keywords: Anaerobic digestion – Odors precursors – Cell protein – Bound protein – ADM1 model

Joost van den Brink and Ronald P. de Vries. Fungal enzyme sets for plant polysaccharide degradation. Applied Microbiology and Biotechnology, Volume 91(6) (2011): 1477-1492

Enzymatic degradation of plant polysaccharides has many industrial applications, such as within the paper, food, and feed industry and for sustainable production of fuels and chemicals. Cellulose, hemicelluloses, and pectins are the main components of plant cell wall polysaccharides. These polysaccharides are often tightly packed, contain many different sugar residues, and are branched with a diversity of structures. To enable efficient degradation of these polysaccharides, fungi produce an extensive set of carbohydrate-active enzymes. The variety of the enzyme set differs between fungi and often corresponds to the requirements of its habitat. Carbohydrate-active enzymes can be organized in different families based on the amino acid sequence of the structurally related catalytic modules. Fungal enzymes involved in plant polysaccharide degradation are assigned to at least 35 glycoside hydrolase families, three carbohydrate esterase families and six polysaccharide lyase families. This mini-review will discuss the enzymes needed for complete degradation of plant polysaccharides and will give an overview of the latest developments concerning fungal carbohydrate-active enzymes and their corresponding families.

Keywords: Polysaccharides – Plant biomass – Fungal enzymes – *Aspergillus* – *Trichoderma*

Sathyanarayana N. Gummadi and B. Bhavya. Enhanced degradation of caffeine and caffeine demethylase production by *Pseudomonas* sp. in bioreactors under fed-batch mode. Biotechnological Products and Process Engineering, Applied Microbiology and Biotechnology, Volume 91(4) (2011): 1007-1017

The growth of *Pseudomonas* sp. was studied in fed-batch process with an aim to improve the caffeine degradation rate and caffeine demethylase activity. The effects of varying initial caffeine concentrations in the batch mode, increase in the number of feeds, varying feed flow rates, and added nutrients to the feed on the fed-batch process were investigated. A maximum caffeine degradation rate of 0.82 g/L h and maximum caffeine demethylase activity of 2.6 U/mg were achieved using manual intermittent pulse feeds of caffeine with substrate concentration as feedback parameter for the fed batch started with an initial caffeine concentration of 3 g/L. A slight increase in the caffeine degradation rate (0.85 g/L h) and caffeine demethylase activity (3.4 U/mg) was observed when the additional nutrients were added along with caffeine in the feed. This is the first report showing complete degradation of large magnitudes of caffeine amounting to 237 g in 75 h. These results show that the fed-batch conditions achieved in this study using *Pseudomonas* sp. facilitate the development of a sustainable bioprocess to degrade the high concentrations of caffeine in industrial effluents.

Keywords: *Pseudomonas* sp. – Caffeine degradation – Caffeine demethylase – Bioreactor – Fed-batch

Li Li Zhang, Shou Qin Leng, Run Ye Zhu and Jian Meng Chen. Degradation of chlorobenzene by strain *Ralstonia pickettii* L2 isolated from a biotrickling filter treating a chlorobenzene-contaminated gas stream. Environmental Biotechnology, Applied Microbiology and Biotechnology, Volume 91(2) (2011): 407-415

A *Ralstonia pickettii* species able to degrade chlorobenzene (CB) as the sole source of carbon and energy was isolated from a biotrickling filter used for the removal of CB from waste gases. This organism, strain L2, could degrade CB as high as 220 mg/L completely. Following CB consumption, stoichiometric amounts of chloride were released, and CO_2 production rate up to 80.2% proved that the loss of CB was mainly via mineralization and incorporation into cell material. The Haldane modification of the Monod equation adequately described the relationship between the specific growth rate and substrate concentration. The maximum specific growth rate and yield coefficient were 0.26 h^{-1} and 0.26 mg of biomass produced/mg of CB consumed, respectively. The pathways for CB degradation were proposed by the identification of metabolites and assay of ring cleavage enzymes in cell extracts. CB was degraded predominantly via 2-chlorophenol to 3-chlorocatechol and also partially via phenol to catechol with subsequent *ortho* ring cleavage, suggesting partially new pathways for CB-utilizing bacteria.

Keywords: *Ralstonia pickettii* – Chlorobenzene – Biodegradation – Metabolite – Pathway

Guangfei Liu, Jiti Zhou, Jing Wang, Xiujuan Wang, Ruofei Jin and Hong Lv. Decolorization of azo dyes by *Shewanella oneidensis* MR-1 in the presence of humic acids. Environmental Biotechnology, Applied Microbiology and Biotechnology, Volume 91(2) (2011): 417-424

The effects of humic acid (HA) on azo dye decolorization by *Shewanella oneidensis* MR-1 were studied. It was found that HA species isolated from different sources could all accelerate the decolorization of Acid Red 27 (AR27). Anoxic and anaerobic conditions were required for the enhancement of azo dye decolorization by HA. In the presence of 50 mg DOC L^{-1} Aldrich HA, 15–29% increases in decolorization efficiencies of azo dyes with different structures were achieved in 11 h. The enhancing effects increased with the increase of HA concentrations ranging from 25 to 150 mg DOC L^{-1}, and the decolorization rates were directly proportional to the HA concentrations when they were below 100 mg DOC L^{-1}. Lactate and formate were good electron donors for AR27 decolorization in the presence of HA. Both nitrate (0.1–3.0 mM) and nitrite (0.3–1.2 mM) inhibited AR27 decolorization in the presence of HA, and negligible decolorization was observed before their removal. Soluble $FeCl_3$ could accelerate the decolorization process in the presence of HA, whereas insoluble hematite could not. These findings may affect the understanding of bioremediation of azo dye-polluted environments and help improve the treatment of azo dye wastewaters.

Keywords: Azo dye – Decolorization – Humic acid – *Shewanella oneidensis* MR-1

Simone Larcher and Viviane Yargeau. Biodegradation of sulfamethoxazole by individual and mixed bacteria. Applied Microbiology and Biotechnology, Volume 91(1) (2011): 211-218

Antibiotic compounds, like sulfamethoxazole (SMX), have become a concern in the aquatic environment due to the potential development of antibacterial resistances. Due to excretion and disposal, SMX has been frequently detected in wastewaters and surface waters. SMX removal in conventional wastewater treatment plants (WWTPs) ranges from 0% to 90%, and there are opposing results regarding its biodegradability at lab scale. The objective of this research was to determine the ability of pure cultures of individual and mixed consortia of bacteria (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Rhodococcus equi*, *Rhodococcus erythropolis*, *Rhodococcus rhodocrous*, and *Rhodococcus zopfii*) known to exist in WWTP activated sludge to remove SMX. Results showed that *R. equi* alone had the greatest ability to remove SMX leading to 29% removal (with glucose) and the formation of a metabolite. Degradation pathways and metabolite structures have been proposed based on the potential enzymes produced by *R. equi*. When *R. equi* was mixed with other microorganisms, a positive synergistic effect was not observed and the maximum SMX removal achieved was 5%. This indicates that pure culture results cannot be extrapolated to mixed culture conditions, and the methodology developed here to study the biodegradability of compounds under controlled mixed culture conditions offers an alternative to conventional studies using pure bacterial cultures or inocula from activated sludge sources consisting of unknown and variable microbial populations.

Keywords: Sulfamethoxazole (SMX) – Antibiotics – Biodegradation – *R. equi* – Mixed cultures

Marcie G. Towell[a], Graeme I. Paton[b], Kirk T. Semple[a]. ([a]Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK, [b]Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 3UU, UK). The biodegradation of cable oil components: Impact of oil concentration, nutrient addition and bioaugmentation. Environmental Pollution, Volume 159(12) (2011) :3777-3783

The effect of cable oil concentration, nutrient amendment and bioaugmentation on cable oil component biodegradation in a pristine agricultural soil was investigated. Biodegradation potential was evaluated over 21 d by measuring cumulative CO_2 respiration on a Micro-Oxymax respirometer and ^{14}C-phenyldodecane mineralisation using a ^{14}C-respirometric assay. Cable oil concentration had a significant effect upon oil biodegradation. Microbial respiratory activity increased with increasing cable oil concentration, whereas ^{14}C-phenydodecane mineralisation decreased. Bioaugmentation achieved the best cable oil biodegradation performance, resulting in increases in cumulative CO_2 respiration, and maximum rates and extents of ^{14}C-phenyldodecane mineralisation. Generally, nutrient amendment also enhanced cable oil biodegradation, but not to the extent that degrader amendment did. Cable oil biodegradation was a function of (i) cable oil concentration and (ii) catabolic ability of microbial populations. Bioaugmentation may enhance cable oil biodegradation, and is dependent upon composition, cell number and application of catabolic inocula to soil.

Keywords: Cable oil; Biodegradation; Phenyldodecane; Nutrients; Bioaugmentation and mineralisation

Hiroto Suhara, Ai Adachi, Ichiro Kamei and Nitaro Maekawa. Degradation of chlorinated pesticide DDT by litter-decomposing basidiomycetes. Biodegradation, Volume 22(6) (2011): 1075-1086

One hundred and two basidiomycete strains (93 species in 41 genera) that prefer a soil environment were examined for screening of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) biodegradation. Three strains within two litter-decomposing genera, *Agrocybe* and *Marasmiellus*, were selected for their DDT biotransformation capacity. Eight metabolites; 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD), two monohydroxy-DDTs, monohydroxy-DDD, 2,2-dichloro-1,1-bis(4-chlorophenyl)ethanol, putative 2,2-bis(4-chlorophenyl)ethanol and two unidentified compounds were detected from the culture with *Marasmiellus* sp. TUFC10101. A P450 inhibitor, 1-ABT, inhibited the formation of monohydroxy-DDTs and monohydroxy-DDD from DDT and DDD, respectively. These results indicated that oxidative pathway which was catalyzed by P450 monooxygenase exist beside reductive dechlorination of DDT. Monohydroxylation of the aromatic rings of DDT (and DDD) by fungal P450 is reported here for the first time.

Keywords: Bioremediation – Cytochrome P450 monooxygenase – Insecticide – DDT – Persistent organic pollutants (POPs) – *Marasmiellus*

Pardeep Kumar, Mehdi Nemati and Gordon A. Hill. Biodegradation kinetics of 1,4-benzoquinone in batch and continuous systems. Biodegradation, Volume 22(6) (2011): 1087-1093

Combining chemical and biological treatments is a potentially economic approach to remove high concentration of recalcitrant compounds from wastewaters. In the present study, the biodegradation of 1,4-benzoquinone, an intermediate compound formed during phenol oxidation by chlorine dioxide, was investigated using *Pseudomonas putida* (ATCC 17484) in batch and continuous bioreactors. Batch experiments were conducted to determine the effects of 1,4-benzoquinone concentration and temperature on the microbial activity and biodegradation kinetics. Using the generated data, the maximum specific growth rate and biodegradation rate were determined as 0.94 h^{-1} and 6.71 mg of 1,4-benzoquinone l^{-1} h^{-1}. Biodegradation in a continuous bioreactor indicated a linear relationship between substrate loading and biodegradation rates prior to wash out of the cells, with a maximum biodegradation rate of 246 mg l^{-1} h^{-1} observed at a loading rate of 275 mg l^{-1} h^{-1} (residence time: 1.82 h). Biokinetic parameters were also determined using the steady state substrate and biomass concentrations at various dilution rates and compared to those obtained in batch cultures.

Keywords: Phenol oxidation – 1,4-Benzoquinone – *Pseudomonas putida* – Biodegradation – Kinetics

Nga B. Le and Nicholas V. Coleman. Biodegradation of vinyl chloride, *cis*-dichloroethene and 1,2-dichloroethane in the alkene/alkane-oxidising *Mycobacterium* strain NBB4. Biodegradation, Volume 22(6) (2011): 1095-1108

Mycobacterium chubuense strain NBB4 can grow on both alkanes and alkenes as carbon sources, and was hypothesised to be an effective bioremediation agent for chlorinated aliphatic pollutants. In this study, the ability of NBB4 to biodegrade vinyl chloride (VC), cis-dichloroethene (cDCE) and 1,2-dichloroethane (DCA) was investigated under pure-culture conditions and in microcosms. Ethene-grown NBB4 cells were capable of biodegrading VC and cDCE, while ethane-grown cells could biodegrade cDCE and DCA. The stoichiometry of inorganic chloride release (1 mol/mol in each case) indicated that VC was completely dechlorinated, while cDCE and DCA were only partially dechlorinated, yielding chloroacetate in the case of DCA, and unknown metabolites in the case of cDCE. The apparent maximum specific activities (k) of whole cells against ethene, cDCE, ethane and DCA were 93 ± 4.6, 89 ± 18, 39 ± 5.5, and 4.8 ± 0.9 nmol/min/mg protein, respectively, while the substrate affinities (K_S) of whole cells with the same substrates were 2.0 ± 0.15, 46 ± 11, 11 ± 0.33 and 4.0 ± 3.2 μM, respectively. In microcosms containing contaminated aquifer sediments and groundwater, NBB4 cells removed 85-95% of the pollutants (cDCE or DCA at 2 mM) within 24 h, and the cells remained viable for >1 month. Due to its favourable kinetic parameters, and robust survival and biodegradation activities, strain NBB4 is a promising candidate for bioremediation of chlorinated aliphatic pollutants.

Keywords: *Mycobacterium* – Biodegradation – Bioremediation – Microcosm – Vinyl chloride – *Cis*-dichloroethene – 1,2-dichloroethane – Alkane – Alkene – Monooxygenase

S. S. Singh and A. K. Dikshit. Decolourization of anaerobically digested and polyaluminium chloride treated distillery spentwash in a fungal stirred tank aerobic reactor. Biodegradation, Volume 22(6) (2011): 1109-1117

Decolourization of anaerobically digested and polyaluminium chloride treated distillery spentwash was studied in a fungal stirred tank aerobic reactor without dilution of wastewater. *Aspergillus niger* isolate IITB-V8 was used as the fungal inoculum. The main objectives of the study were to optimize the stirrer speed for achieving maximum decolourization and to determine the kinetic parameters. A mathematical model was developed to describe the batch culture kinetics. Volumetric oxygen transfer coefficient ($k_L a$) was obtained using dynamic method. The maximum specific growth rate and growth yield of fungus were determined using Logistic equation and using Luedeking–Piret equation. 150 rpm was found to be optimum stirrer speed for overall decolourization of 87%. At the optimum stirrer speed, volumetric oxygen transfer coefficient ($k_L a$) was 0.4957 min^{-1} and the maximum specific growth rate of fungus was 0.224 h^{-1}. The values of yield coefficient ($Y_{x/s}$) and maintenance coefficient (m_s) were found to be 0.48 g cells (g substrate)$^{-1}$ and 0.015 g substrate (g cells)$^{-1}$ h^{-1}.

Keywords: Molasses spentwash – Decolourization – *Aspergillus niger* – Stirrer speed – Kinetic model

Charumathi Deivasigamani and Nilanjana Das. Biodegradation of Basic Violet 3 by *Candida krusei* isolated from textile wastewater. Biodegradation, Volume 22(6) (2011): 1169-1180

Basic Violet 3 (BV) belongs to the most important group of synthetic colorants and is used extensively in textile industries. It is considered as xenobiotic compound which is recalcitrant to biodegradation. As *Candida krusei* could not use BV as sole carbon source, experiments were conducted to study the effect of cosubstrates on decolorization of BV in semi synthetic medium

using glucose, sucrose, lactose, maltose, yeast extract, peptone, urea and ammonium sulphate. Maximum decolorization (74%) was observed in media supplemented with sucrose. Use of sugarcane bagasse extract as sole nutrient source showed 100% decolorization of BV within 24 h under optimized condition. UV–visible, FTIR spectral analysis and HPLC analysis confirmed the biodegradation of BV. Six degradation products were isolated and identified. We propose the biodegradation pathway for BV which occurs via stepwise reduction and demethylation process to yield mono-, di-, tri-, tetra-, penta- and hexa-demethylated BV species which was degraded completely. The study of the enzymes responsible for decolorization showed the activities of lignin peroxidase, lacasse, tyrosinase, NADH-DCIP reductase, MG reductase and azoreductase in cells before and after decolorization. A significant increase in activities of NADH-DCIP reductase and laccase was observed in the cells after decolorization. The yeast *C. krusei* could show the ability to decolorize the textile dye BV using inexpensive source like sugarcane bagasse extract for decolorization.

Keywords: Biodegradation – Basic Violet 3 (BV) – *Candida krusei* – Biodegradation pathway – NADH-DCIP reductase – Laccase

Preethy Chandran and Nilanjana Das. Degradation of diesel oil by immobilized *Candida tropicalis* and biofilm formed on gravels. Biodegradation, Volume 22(6) (2011): 1181-1189

The performance of diesel oil degradation by *Candida tropicalis* immobilized on various conventional matrices (sodium alginate, carboxyl methyl cellulose, chitosan) and biowaste materials (wheat bran, sawdust, peanut hull powder) was investigated using the method of entrapment and physical adsorption. The yeast species immobilized in wheat bran showed enhanced efficiency in degrading diesel oil (98%) compared to free cells culture (80%) over a period of 7 days. Copious amount of exopolysaccharides were also produced in the presence of diesel oil. The biofilm forming ability of *C. tropicalis* on PVC strips was evaluated using XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) reduction assay and monitored by scanning electron microscopy and atomic force microscopy. Yeast biofilm formed on gravels showed 97% degradation of diesel oil over a period of 10 days. The potential use of the biofilms for preparing trickling filters (gravel particles), for attenuating hydrocarbons in oily liquid wastes before their disposal in the open environment is suggested and discussed. This is the first successful attempt for 'artificially' establishing hydrocarbon degrading yeast biofilm on solid substrates.

Keywords: AFM – Biofilm – *Candida tropicalis* – Diesel oil – PVC – SEM

Gustavo Yáñez-Ocampo, Enrique Sánchez-Salinas and M. Laura Ortiz-Hernández. Removal of methyl parathion and tetrachlorvinphos by a bacterial consortium immobilized on tezontle-packed up-flow reactor. Biodegradation, Volume 22(6) (2011): 1203-1213

A tezontle-packed up-flow reactor (TPUFR) with an immobilized bacterial consortium for biological treatment of methyl-parathion and tetrachlorvinphos was evaluated. These organophosphate pesticides are widely used in Mexico for insect and mite control, respectively. With the aim of developing a tool for pesticide biodegradation, four flow rates (0.936, 1.41, 2.19, and 3.51 l/h) and four hydraulic residence times (0.313, 0.206, 0.133, and 0.083 h) were

evaluated in a TPUFR. In the bioreactor, with an operating time of 8 h and a flow of 0.936 l/h, we obtained 75% efficiency in the removal of methyl-parathion and tetrachlorvinphos. Their adsorptions in the volcanic rock were 9% and 6%, respectively. It was demonstrated that the removal of pesticides was due to the biological activity of the immobilized bacterial consortium. We confirmed the decrease in toxicity in the treated effluent from the bioreactor through the application of acute toxicity tests on *Eisenia foetida*. Immobilization of a bacterial consortium using tezontle as a support is innovative and an economical tool for the treatment of mixtures of organophosphorus pesticide residues.

Keywords: Methyl-parathion – Tetrachlorvinphos – Bacterial consortium – Tezontle – Removal

Nichina Gomi, Shuji Yoshida, Kazutsugu Matsumoto, Masayuki Okudomi, Hiroki Konno, Toru Hisabori and Yasushi Sugano. Degradation of the synthetic dye amaranth by the fungus *Bjerkandera adusta* Dec 1: inference of the degradation pathway from an analysis of decolorized products. Biodegradation, Volume 22(6) (2011): 1239-1245

We examined the degradation of amaranth, a representative azo dye, by *Bjerkandera adusta* Dec 1. The degradation products were analyzed by high performance liquid chromatography (HPLC), visible absorbance, and electrospray ionization time-of-flight mass spectroscopy (ESI-TOF-MS). At the primary culture stage (3 days), the probable reaction intermediates were 1-aminonaphthalene-2,3,6-triol, 4-(hydroxyamino) naphthalene-1-ol, and 2-hydroxy-3-[2-(4-sulfophenyl) hydrazinyl] benzenesulfonic acid. After 10 days, the reaction products detected were 4-nitrophenol, phenol, 2-hydroxy-3-nitrobenzenesulfonic acid, 4-nitrobenzene sulfonic acid, and 3,4′-disulfonyl azo benzene, suggesting that no aromatic amines were created. Manganese-dependent peroxidase activity increased sharply after 3 days culture. Based on these results, we herein propose, for the first time, a degradation pathway for amaranth. Our results suggest that Dec 1 degrades amaranth via the combined activities of peroxidase and hydrolase and reductase action.

Keywords: *Bjerkandera adusta* Dec 1 – Amaranth – Azo – Degradation – Peroxidase

Jan Frouz, Tomáš Cajthaml and Ondřej Mudrák. The effect of lignin photodegradation on decomposability of *Calamagrostis epigeios* grass litter. Biodegradation, Volume 22(6) (2011): 1247-1254

The common grass *Calamagrostis epigeions* produces a large amount of dead biomass, which remain above the soil surface for many months. In this study, we determined how exposure of dead biomass above the soil affects its subsequent decomposition in soil. Collected dead standing biomass was divided in two parts, the first one (initial litter) was stored in a dark, dry place. The other part was placed in litterbags in the field. The litterbags were located in soil, on the soil surface, or hanging in the air without contact with soil but exposed to the sun and rain. After 1 year of field exposure, litter mass loss and C and N content were measured, and changes in litter chemistry were explored using NMR and thermochemolysis-GC–MS. The potential decomposability of the litter was quantified by burying the litter from the litterbags and the initial litter in soil microcosms and measuring soil respiration. Soil respiration was greater with litter that had been hanging in air than with all other kinds of litter. These finding could not be explained by changes in litter mass or C:N ratio. NMR indicated a decrease in polysaccharides relative to lignin in litter that was buried in soil but not in litter that was placed on soil surface or

that was hanging in the air. Thermochemolysis indicated that the syringyl units of the litter lignin were decomposed when the litter was exposed to light. We postulate that photochemical decay of lignin increase decomposability of dead standing biomass.

Keywords: Thermochemolysis-GC–MS – ^{13}C NMR – Decomposition – Plant litter – Post mining sites – Light

Ngangbam Sarat Singh and Dileep K. Singh. Biodegradation of endosulfan and endosulfan sulfate by *Achromobacter xylosoxidans* strain C8B in broth medium. Biodegradation, Volume 22(5) (2011): 845-857

Endosulfan is one of the most widely used wide spectrum cyclodiene organochlorine insecticide. In environment, endosulfan can undergo either oxidation or hydrolysis reaction to form endosulfan sulfate and endosulfan diol respectively. Endosulfan sulfate is as toxic and as persistent as its parent isomers. In the present study, endosulfan degrading bacteria were isolated from soil through selective enrichment technique using sulfur free medium with endosulfan as sole sulfur source. Out of the 8 isolated bacterial strains, strain C8B was found to be the most efficient endosulfan degrader, degrading 94.12% α-endosulfan and 84.52% β-endosulfan. The bacterial strain was identified as *Achromobacter xylosoxidans* strain C8B on the basis of 16S rDNA sequence similarity. *Achromobacter xylosoxidans* strain C8B was also found to degrade 80.10% endosulfan sulfate using it as sulfur source. No known metabolites were found to be formed in the culture media during the entire course of degradation. Besides, the bacterial strain was found to degrade all the known endosulfan metabolites. There was marked increase in the quantity of released CO_2 from the culture media with endosulfan as sulfur source as compared to $MgSO_4$ suggesting that the bacterial strain, *Achromobacter xylosoxidans* strain C8B probably degraded endosulfan completely through the formation of endosulfan ether.

Keywords: Endosulfan – Endosulfan sulfate – *Achromobacter xylosoxidans* strain C8B – Mineralization

Pengfei Xiao, Toshio Mori, Ichiro Kamei and Ryuichiro Kondo. A novel metabolic pathway for biodegradation of DDT by the white rot fungi, *Phlebia lindtneri* and *Phlebia brevispora.* Biodegradation, Volume 22(5) (2011): 859-867

1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) was used as the substrate for a degradation experiment with the white rot fungi *Phlebia lindtneri* GB-1027 and *Phlebia brevispora* TMIC34596, which are capable of degrading polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated biphenyls (PCBs). Pure culture of *P. lindtneri* and *P. brevispora* with DDT (25 μmol l^{-1}) showed that 70 and 30% of DDT, respectively, disappeared in a low-nitrogen medium after a 21-day incubation period. The metabolites were analyzed using gas chromatography/mass spectrometry (GC/MS). Both fungi metabolized DDT to 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD), 2,2-bis(4-chlorophenyl)acetic acid (DDA) and 4,4-dichlorobenzophenone (DBP). Additionally, DDD was converted to DDA and DBP. DDA was converted to DBP and 4,4-dichlorobenzhydrol (DBH). While DBP was treated as substrate, DBH and three hydroxylated metabolites, including one dihydroxylated DBP and two different isomers of monohydroxylated DBH, were produced from fungal cultures, and these hydroxylated metabolites were efficiently inhibited by the addition of a cytochrome P-450

inhibitor, piperonyl butoxide. These results indicate that the white rot fungi *P. lindtneri* and *P. brevispora* can degrade DBP/DBH through hydroxylation of the aromatic ring. Moreover, the single-ring aromatic metabolites, such as 4-chlorobenzaldehyde, 4-chlorobenzyl alcohol and 4-chlorobenzoic acid, were found as metabolic products of all substrate, demonstrating that the cleavage reaction of the aliphatic-aryl carbon bond occurs in the biodegradation process of DDT by white rot fungi.

Keywords: Biodegradation – DDT – White rot fungi – Cytochrome P-450 – Hydroxylation – Metabolite

Jun Wu, Liguan Li, Hongwei Du, Lijuan Jiang, Qiong Zhang, Zhongbo Wei, Xiaolin Wang, Lin Xiao and Liuyan Yang. Biodegradation of leuco derivatives of triphenylmethane dyes by *Sphingomonas* sp. CM9. Biodegradation, Volume 22(5) (2011): 897-904

A leuco derivatives of triphenylmethane dyes degrading bacterium, strain CM9, was isolated from an aquafarm field. Based on morphology, physiologic tests, 16S rDNA sequence, and phylogenetic characteristics, it was identified as *Sphingomonas* sp. This strain was capable of degrading leucomalachite green (LMG), leucocrystal violet and leucobasic fuchsin completely. The relationship between bacterium growth and LMG degradation suggested that strain CM9 could use LMG as the sole source of carbon. The most LMG degradation activity of CM9 crude extract was observed at pH 7.0 and at 30°C. Many metal ions had little inhibition effect on the degradation activity of the crude extract. CM9 also showed strong decolorization of triphenylmethane dyes to their leuco derivatives. GC/MS analysis detected two novel metabolic products, methylbenzene and 4-aminophenol, during the LMG degradation by CM9.

Keywords: Leuco derivatives of triphenylmethane dyes – Degradation – Decolorization – Metabolic products

Osman Nuri Ağdağ. Biodegradation of olive-mill pomace mixed with organic fraction of municipal solid waste. Biodegradation, Volume 22(5)(2011): 931-938

This study investigated the effects of organic fraction of municipal solid waste (OFMSW) addition on the anaerobic treatment of the olive-mill pomace. Biodegradability of olive-mill pomace mixed with OFMSW was examined in anaerobic bioreactors. Only OFMSW was loaded in the first (control) bioreactor, while run 1 and run 2 bioreactors included different ratio of OFMSW and olive-mill pomace. COD, BOD_5, NH_4–N, pH, VFA, CH_4 quantity and percentage in anaerobic bioreactors were regularly monitored. In addition, inert COD and anaerobic toxicity assay (ATA) were measured in leachate samples. The results of the study showed that 70% of OFMSW addition to olive-mill pomace has an advantage in terms of pollution parameters and methane generation. Since olive-mill pomace is not easy biodegradable, addition of high proportion of OFMSW promotes biodegradability of olive-mill pomace. Decreasing in BOD_5/COD ratios in the run 1 and run 2 reactors carried out as 62 and 52%, respectively.

Keywords: Olive-mill pomace – Biodegradation – Anaerobic digestion – Municipal solid wastes

San-Lang Wang, Wan-Nine Tseng and Tzu-Wen Liang. Biodegradation of shellfish wastes and production of chitosanases by a squid pen-assimilating bacterium, *Acinetobactercalcoaceticus* TKU024. Biodegradation, Volume 22(5) (2011): 939-948

Two chitosanases (CHSA1 and CHSA2) were purified from the culture supernatant of *Acinetobacter calcoaceticus* TKU024 with squid pen as the sole carbon/nitrogen source. The molecular masses of CHSA1 and CHSA2 determined by SDS-PAGE were approximately 27 and 66 kDa, respectively. The optimum pH, optimum temperature, pH stability, and thermal stability of CHSA1 and CHSA2 were (pH 6, 50°C, pH 4–10, <90°C) and (pH 7, 60°C, pH 6–11, <70°C), respectively. CHSA1 and CHSA2 had broad pH and thermal stability. CHSA1 and CHSA2 were both inhibited by EDTA and were inhibited completely by 5 mM Mn^{2+}. CHSA1 and CHSA2 degraded chitosan with DD ranging from 60 to 98%, and also degraded some chitin. The most susceptible substrate was 60% deacetylated chitosan. Furthermore, TKU024 culture supernatant (1.5% SPP) incubated for 5 days has the most reducing sugars (0.63 mg/ml). With this method, we have shown that shellfish wastes may have a great potential for the production of bioactive materials.

Keywords: Chitosanase – Squid pen wastes – *Acinetobacter calcoaceticus* – Reducing sugar

Shanwei Xu, G. Douglas Inglis, Tim Reuter, O. Grant Clark, Miodrag Belosevic, Jerry J. Leonard and Tim A. McAllister. Biodegradation of specified risk material and characterization of actinobacterial communities in laboratory-scale composters. Biodegradation, Volume 22(5) (2011): 1029-1043

As a result of bovine spongiform encephalopathy in Canada, specific tissues at risk of harbouring prions are not allowed to enter the food chain. Composting may be a viable alternative to rendering and land filling for the disposal of specified risk material (SRM). Two types of laboratory-scale composters, actively-heated and ambient systems were constructed to assess the biodegradation of SRM over 30 days. A second heating cycle was generated by mixing the compost after 15 days. Compared to ambient composters, temperature profiles in actively-heated composters were above 50°C for 5 and 4 days longer in the first and second composting cycles, respectively. Degradation of SRM was similar between two composter types during two composting cycles, averaging 52.2% in the first cycle and 43.9% in second cycle. Denaturing gradient gel electrophoresis (DGGE) revealed that changes in the actinobacteria populations in the first composting cycle were of a temporal nature, whereas alterations in populations in the second composting cycle were more related to active heating of compost. Sequencing of the dominant DGGE bands showed the predominance of *Corynebacterium*, *Promicromonospora*, *Pseudonocardia*, and *Thermobifida* in the first composting cycle and *Corynebacterium*, *Mycobacterium, Nocardia, Saccharomonospora*, and *Streptomyces* in the second composting cycle. Active heating can alter the nature of actinobacteria populations in compost, but does not appear to have a major impact on the extent of degradation of SRM.

Keywords: Specified risk material (SRM) – Laboratory-scale composter – Biodegradation – Actinobacteria – Denaturing gradient gel electrophoresis (DGGE)

Hugo Ribeiro, Ana P. Mucha, C. Marisa R. Almeida and Adriano A. Bordalo. Hydrocarbon degradation potential of salt marsh plant–microorganisms associations. Biodegradation, Volume 22(4) (2011): 729-739

Estuaries are often considered sinks for contaminants and the cleanup of salt marshes, sensitive ecosystems with a major ecological role, should be carried out by means of least intrusive approaches, such as bioremediation. This study was designed to evaluate the influence of plant–microorganisms associations on petroleum hydrocarbons fate in salt marshes of a temperate estuary (Lima River, NW Portugal). Sediments un-colonized and colonized (rhizosediments) by different plants *(Juncus maritimus, Phragmites australis, Triglochin striata* and *Spartina patens)* were sampled in four sites of the lower and middle estuary for hydrocarbon degrading microorganisms (HD), total cell counts (TCC) and total petroleum hydrocarbons (TPHs) assessment. In general, TPHs, HD and TCC were significantly higher ($P < 0.05$) in rhizosediments than in un-colonized sediments. Also recorded were differences on the abundance of hydrocarbon degraders among the rhizosediment of the different plants collected at the same site (*J. maritimus* < *P. australis* < *T. striata*), with statistically significant differences ($P < 0.05$) between *J. maritimus* and *T. striata*. Moreover, strong positive correlations—0.81 and 0.84 ($P < 0.05$), between biotic (HD) and abiotic (organic matter content) parameters and TPHs concentrations were also found. Our data clearly suggest that salt marsh plants can influence the microbial community, by fostering the development of hydrocarbon-degrading microbial populations in its rhizosphere, an effect observed for all plants. This effect, combined with the plant capability to retain hydrocarbons around the roots, points out that salt marsh plant–microorganisms associations may actively contribute to hydrocarbon removal and degradation in estuarine environments.

Keywords: Bioremediation – Hydrocarbons – Estuary – Salt marsh – Rhizosphere – Lima River estuary

David Hughes, Benjamin R. Clark and Cormac D. Murphy. Biodegradation of polyfluorinated biphenyl in bacteria. Biodegradation, Volume 22(4) (2011): 741-749

Fluorinated aromatic compounds are significant environmental pollutants, and microorganisms play important roles in their biodegradation. The effect of fluorine substitution on the transformation of fluorobiphenyl in two bacteria was investigated. *Pseudomonas pseudoalcaligenes* KF707 and *Burkholderia xenovorans* LB400 used 2,3,4,5,6-pentafluorobiphenyl and 4,4′-difluorobiphenyl as sole sources of carbon and energy. The catabolism of the fluorinated compounds was examined by gas chromatography–mass spectrometry and fluorine-19 nuclear magnetic resonance spectroscopy (^{19}F NMR), and revealed that the bacteria employed the upper pathway of biphenyl catabolism to degrade these xenobiotics. The novel fluorometabolites 3-pentafluorophenyl-cyclohexa-3,5-diene-1,2-diol and 3-pentafluorophenyl-benzene-1,2-diol were detected in the supernatants of biphenyl-grown resting cells incubated with 2,3,4,5,6-pentafluorobiphenyl, most likely as a consequence of the actions of BphA and BphB. 4-Fluorobenzoate was detected in cultures incubated with 4,4′-difluorobiphenyl and ^{19}F NMR analysis of the supernatant from *P. pseudoalcaligenes* KF707 revealed the presence of additional water-soluble fluorometabolites.

Keywords: PCB – Fluorometabolites – NMR – Mass spectrometry

J. Alonso-Gutiérrez[1,2], M. Teramoto[2], A. Yamazoe[2], S. Harayama[2], A. Figueras[1], B. Novoa[1]. Alkane-degrading properties of *Dietzia* sp. H0B, a key player in the *Prestige* oil spill biodegradation (NW Spain). Journal of Applied Microbiology, Volume 111(4) (2011): 800–810

Investigation of the alkane-degrading properties of *Dietzia* sp. H0B, one of the isolated Corynebacterineae strains that became dominant after the *Prestige* oil spill.

Using molecular and chemical analyses, the alkane-degrading properties of strain *Dietzia* sp. H0B were analysed. This Grampositive isolate was able to grow on *n*-alkanes ranging from C_{12} to C_{38} and branched alkanes (pristane and phytane). 8-Hexadecene was detected as an intermediate of hexadecane degradation by *Dietzia* H0B, suggesting a novel alkane-degrading pathway in this strain. Three putative alkane hydroxylase genes (one *alkB* homologue and two CYP153 gene homologues of cytochrome P450 family) were PCR-amplified from *Dietzia* H0B and differed from previously known hydroxylase genes, which might be related to the novel degrading activity observed on *Dietzia* H0B. The alkane degradation activity and the *alkB* and CYP153 gene expression were observed constitutively regardless of the presence of the substrate, suggesting additional, novel pathways for alkane degradation.

The results from this study suggest novel alkane-degrading pathways in Dietzia H0B and a genetic background coding for two different putative oil-degrading enzymes, which is mostly unexplored and worth to be subject of further functional analysis.

This study increases the scarce information available about the genetic background of alkane degradation in genus *Dietzia* and suggests new pathways and novel expression mechanisms of alkane degradation.

Keywords: alkane degradation; AlkB; constitutive expression; CYP153; *Dietzia*; oil spill; Prestige

T.J. Bootten[1,†], K.N. Joblin[2,‡], B.H. McArdle[3], P.J. Harris[1]. Degradation of lignified secondary cell walls of lucerne (*Medicago sativa* L.) by rumen fungi growing in methanogenic co-culture. Journal of Applied Microbiology, Volume 111(5) (2011): 1086–1096

To compare the abilities of the monocentric rumen fungi *Neocallimastix frontalis*, *Piromyces communis* and *Caecomyces communis*, growing in coculture with *Methanobrevibacter smithii,* to colonize and degrade lignified secondary cell walls of lucerne (alfalfa) hay.

The cell walls of xylem cylinders isolated from stems of lucerne contained mostly xylans, cellulose and lignin together with a small proportion of pectic polysaccharides. All of these major components were removed during incubation with the three fungi, and differing cell wall polysaccharides were degraded to different extents. The greatest dry weight loss was found with *N. frontalis* and least with *C. communis*, and scanning electron microscopy revealed that these extensively colonized different cell types. *C. communis* specifically colonized secondary xylem fibres and showed much less degradation than *N. frontalis* and *P. communis*.

Neocallimastix frontalis and *P. communis* were efficient degraders of the cell walls of lucerne xylem cylinders. Degradation occurred of pectic polysaccharides, xylan and cellulose. Loss of lignin from the xylem cylinders probably resulted from the cleavage of xylan releasing xylan–lignin complexes.

Unlike rumen bacteria, the rumen fungi *N. frontalis*, *P. communis* and *C. communis* are able to degrade lignified secondary walls in lucerne stems. These fungi could improve forage utilization by ruminants and may have potential in the degradation of lignocellulosic biomass in the production of biofuels.

Keywords: *Caecomyces*; degradation; lignified cell wall; *Medicago sativa*; *Neocallimastix*; *Piromyces*; rumen fungi

María Soledad Fuentes, Juliana María Sáez, Claudia Susana Benimeli and María Julia Amoroso. Lindane Biodegradation by Defined Consortia of Indigenous *Streptomyces* Strains. Water, Air, & Soil Pollution, Volume 222(1-4) (2011): 217-231

The current study aimed to compare lindane degradation by pure and mixed cultures of *Streptomyces* sp. Cell-free extracts were assayed for potentiating dechlorinase activity and, based on these results, consortia of two to six microorganisms were assayed for their growth on and degradation of lindane. Furthermore, the role of bacterial consortia of lindane-degrading strains was examined in lindane decontamination soil assays. Four actinobacteria, previously isolated from a pesticide-contaminated area, were selected because of their tolerance to lindane and their ability to use the pesticide as sole carbon source. These strains as well as *Streptomyces* sp. M7 and *Streptomyces coelicolor* A3 were used to study specific dechlorinase activity (SDA) and lindane removal in mixed cultures. Pure cultures presented SDA in the presence of 1.66 mg L^{-1} lindane as carbon source. SDA was improved by certain mixed cultures until 12 times compared with pure cultures. Mixed cultures with two, three, and four strains showed maximum lindane removal of 46% to 68%, whereas combinations of five and six strains did not efficiently remove the pesticide from the culture medium. The *Streptomyces* sp. A2, A5, M7, and A11 consortium presented the lowest ratio between residual lindane concentration and SDA and could be a promising tool for lindane biodegradation.

Keywords: Lindane – Bioremediation – Actinobacteria – Microbial consortium

Brijesh Kumar Yadav and S. Majid Hassanizadeh. An Overview of Biodegradation of LNAPLs in Coastal (Semi)-arid Environment. Water, Air, & Soil Pollution, Volume 220(1-4) (2011):225-239

Contamination of soil and water due to the release of light non-aqueous phase liquids (LNAPLs) is a ubiquitous problem. The problem is more severe in arid and semi-arid coastal regions where most of the petroleum production and related refinery industries are located. Biological treatment of these organic contaminated resources is receiving increasing interests and where applicable, can serve as a cost-effective remediation alternative. The success of bioremediation greatly depends on the prevailing environmental variables, and their remediation favoring customization requires a sound understanding of their integrated behavior on fate and transport of LNAPLs under site-specific conditions. The arid and semi-arid coastal sites are characterized by specific environmental extremes; primarily, varying low and high temperatures, high salinity, water table dynamics, and fluctuating soil moisture content. An understanding of the behavior of these

environmental variables on biological interactions with LNAPLs would be helpful in customizing the bioremediation for restoring problematic sites in these regions. Therefore, this paper reviews the microbial degradation of LNAPLs in soil–water, considering the influences of prevailing environmental parameters of arid and semi-arid coastal regions. First, the mechanism of biodegradation of LNAPLs is discussed briefly, followed by a summary of popular kinetic models used by researchers for describing the degradation rate of these hydrocarbons. Next, the impact of soil moisture content, water table dynamics, and soil–water temperature on the fate and transport of LNAPLs are discussed, including an overview of the studies conducted so far. Finally, based on the reviewed information, a general conclusion is presented with recommendations for future research subjects on optimizing the bioremediation technique in the field under the aforesaid environmental conditions. The present review will be useful to better understand the feasibility of bioremediation technology, in general, and its applicability for remediating LNAPLs polluted lands under aforesaid environments, in particular.

Keywords: Bioremediation – Arid and semi-arid environments – LNAPL – Coastal region – Biodegradation

Magdalena Błaszak, Robert Pełech and Paulina Graczyk. Screening of Microorganisms for Biodegradation of Simazine Pollution (Obsolete Pesticide Azotop 50 WP). Water, Air, & Soil Pollution, Volume 220(1-4)(2011): 373-385

The capability of environmental microorganisms to biodegrade simazine—an active substance of 2-chloro-*s*-triazine herbicides (pesticide waste since 2007)—was assessed. An enormous metabolic potential of microorganisms impels to explore the possibilities of using them as an alternative way for thermal and chemical methods of utilization. First, the biotope rich in microorganisms resistant to simazine was examined. Only the higher dose of simazine (100 mg/l) had an actual influence on quantity of bacteria and environmental fungi incubated on substrate with simazine. Most simazine-resistant bacteria populated activated sludge and biohumus (vermicompost); the biggest strain of resistant fungi was found in floral soil and risosphere soil of maize. Compost and biohumus were the sources of microorganisms which biodegraded simazine, though either of them was the dominant considering the quantity of simazine-resistant microorganisms. In both cases of periodic culture (microorganisms from biohumus and compost), nearly 100% of simazine (50 mg/l) was degraded (within 8 days). After the repeated enrichment culture with simazine, the rate of its degradation highly accelerated, and just after 24 h, the significant decrease of simazine (20% in compost and 80% in biohumus) was noted. Although a dozen attempts of isolating various strains responsible for biodegradation of simazine from compost and biohumus were performed, only the strain identified as *Arthrobacter urefaciens* (NC) was obtained, and it biodegraded simazine with almost 100% efficiency (within 4 days).

Keywords: Biodegradation – *Arthrobacter urefaciens* – Simazine – Obsolete pesticide

Yuefen Yin, Junhui Guo, Li Zheng, Li Tian and Xiaoru Wang. Capability of polychlorinated biophenyl (PCBs) degrading fungi segregated from sediments. World Journal of Microbiology and Biotechnology, Volume 27(11)(2011): 2567-2574

Four strains of fungi isolated from the sediments of badly contaminated Li-Cun River were studied to investigate their ability to remove 6 target PCBs with and without sodium dodecyl sulfate as the surfactant. Having different viability at different conditions, these four fungi all showed relatively better PCB degradation capability and higher efficiency for total PCB removal in the absence of the ligninolytic enzymes. The findings that chloride concentration changes and some acids are generated as the metabolic products contribute to our understanding of PCBs degradation pathways. Combinations of different PCB concentrations and fresh fungi weight inoculated were attempted to achieve maximum efficiency. After the bio-treatment, the concentrations of PCBs are significantly reduced with the chloride ion concentration increasing slightly. Not only in the PCB degradation process but also in the total PCB removed did these four fungi show high capability, but no apparent effect of SDS on the performance of the fungi is found.

Keywords: Fungal biodegradation – Polychlorinated biophenyl – Chloride ion

Bao-zhan Wang, Yun Ma, Wei-you Zhou, Jin-wei Zheng, Jian-chun Zhu, Jian He and Shun-peng Li. Biodegradation of synthetic pyrethroids by *Ochrobactrum tritici* strain pyd-1. World Journal of Microbiology and Biotechnology, Volume 27(10)(2011): 2315-2324

A synthetic pyrethroid (SP)-degrading bacterium, designated pyd-1, was isolated from SPcontaminated soil. Based on its phenotypic and genotypic properties, the strain was identified as *Ochrobactrum tritici*. Strain pyd-1 was able to degrade a wide range of SPs, and its degradation efficiencies were dependent on the molecular structure of the SP. Interestingly, the strain degraded cis- and trans-permethrin (cypermethrin) at nearly the same rate and possessed approximately equal hydrolysis activities toward the two enantiomers of fenpropathrin. These results suggest that different isomers of SPs are degraded with equal efficiency by strain pyd-1. We studied the metabolic pathway of fenpropathrin degradation in strain pyd-1 by metabolite identification and enzymatic analysis. Fenpropathrin is degraded by hydrolysis of the carboxylester linkage to yield 2,2,3,3-tetramethylcyclopropanecarboxylic acid and 3-phenoxybenzaldehyde, which is converted to 3-phenoxybenzoic acid (PBA). PBA is further metabolized to 4-hydroxy-3-phenoxybenzoic acid (4-hydroxy-PBA). 4-Hydroxy-PBA is oxidized to protocatechuate and p-hydroquinone. Protocatechuate is further oxidized through an ortho-cleavage pathway, and p-hydroquinone is degraded via 1,2,4-benzenetriol.

Keywords: Biodegradation – Synthetic pyrethroids – *Ochrobactrum tritici* pyd-1 – Isomer selectivity – Degradation pathway

Ze-long Wang, Dan Wang, Qiang Li, Wang-liang Li, Huang Tang and Jian-min Xing. Enhanced biodesulfurization by expression of dibenzothiophene uptake genes in *Rhodococcus erythropolis*. World Journal of Microbiology and Biotechnology, Volume 27(9) (2011): 1965-1970

The transfer of dibenzothiophene (DBT) and its derivatives into cells is a critical step for biodesulfurization. The desulfurization reactions of resting cells and cell lysate were studied, which showed that the desulfurization rate of DBT, especially 4, 6-dimethyldibenzothiophene (4, 6-DMDBT) in *Rhodococcus erythropolis* LSSE8-1 was seriously affected by the transfer into cells. The inhibited effect of NaN_3 on desulfurization reactions was studied, which confirmed that the transfer of DBT into cells was an active transport in *R. erythropolis* LSSE8-1. The uptake-genes of DBT and its derivatives (*Hcu*ABC) of *Pseudomonas delafieldii* R-8 were

introduced into the specific desulfurization bacterium, *R. erythropolis* LSSE8-1. Compared with the wild type, the strains bearing *Hcu*ABC genes showed a higher desulfurization activity. The desulfurization ratio of DBT showed a 19% increase, and 13% increase of 4, 6-DMDBT.

Keywords: Biodesulfurization – Dibenzothiophene uptake – Cell lysate – *Hcu*ABC

Veena Sagar and D. P. Singh. Biodegradation of lindane pesticide by non white- rots soil fungus *Fusarium* sp. World Journal of Microbiology and Biotechnology, Volume 27(8) (2011): 1747-1754

Lindane or γ- hexachlorocyclohexane (γ-HCH) is a chlorinated pesticide and its toxic effects on biota necessitate its removal. Microbial degradation is an important process for pesticide bioremediation and the role of soil fungi in recycling of organic matter prompted us to study the biodegradation of lindane using fungi. This study aims at enrichment, isolation and screening of soil fungi capable of metabolizing lindane. Two *Fusarium* species (*F. poae* and *F. solani*) isolated from the pesticide contaminated soil showed better growth on the plates supplemented with lindane as a sole carbon source, when compared with the growth performance of other fungal isolates from the same contaminated soil. However, ANOVA revealed a significant difference in fungal biomass production in both *F.* poae ($F = 22.02$; $N = 15$; $P < 0.001$) and *F.* solani ($F = 268.75$; $N = 15$; $P < 0.001$) across different lindane concentrations (0–600 μg ml^{-1}). Growth of both *Fusarium* sp. was maximum at a lindane concentration of 100 μg ml^{-1}, while minimum at 600 μg ml^{-1} concentrations. Results on the time dependent release of chlorine by the *Fusarium* strains in the presence of various concentration of lindane showed the highest mineralization of the pesticide on 10th day of incubation. Time dependent variations in the release of chlorine from 1st to 10th day by both the selected fungal strains were found to be statistically significant. A significant positive relationship exists between fungal biomass increase and chlorine release existed for both *F. solani* ($R^2 = 0.960$) and *F. poae* ($R^2 = 0.628$). The results of gas chromatograph analysis of γ- HCH confirmed the biodegradation and utilization of γ- HCH by *F. poae* and *F. solani*. The data on lindane degradation by the two fungal strains demonstrated that the biodegradation of lindane by *F. solani* (59.4%) was slightly higher than that by the *F. poae* (56.7%).

Keywords: Bioremediation – Free chlorine – Fungi – Lindane – Soil

Anupama Shrivastav, Sanjiv K. Mishra, Imran Pancha, Deepti Jain, Sourish Bhattacharya, Sheetal Patel and Sandhya Mishra. Biodegradability studies of polyhydroxyalkanoate (PHA) film produced by a marine bacteria using Jatropha biodiesel byproduct as a substrate. World Journal of Microbiology and Biotechnology, Volume 27(7) (2011): 1531-1541

Polyhydroxyalkanoates are water-insoluble, hydrophobic polymers and can be degraded by microorganisms that produce extracellular PHA depolymerase. The present work was aimed to evaluate the degradability of Polyhydroxyalkanoate film produced by *Halomonas hydrothermalis* using Jatropha biodiesel byproduct as a substrate. PHB films were subjected to degradation in soil and compared with the synthetic polymer (acrylate) and blend prepared using the synthetic polymer (acrylate) and PHB. After 50 days, 60% of weight loss in PHB film and after 180 days 10% of blended film was degraded while no degradation was found in the

synthetic film. Scanning electron microscopy and confocal microscopy revealed that after 50 days the PHB film and the blended film became more porous after degradation while synthetic film was not porous. The degradative process was biologically mediated which was evident by the control in which the PHB films were kept in sterile soil and the films showed inherent integrity over time. The TGA and DSC analysis shows that the melting temperatures were changed after degradation indicating physical changes in the polymer during degradation.

Keywords: Biodegradation – Polyhydroxyalkanoate (PHA) – Jatropha – Biodiesel byproduct – Polyhydroxybutyrate (PHB) – Thermogravimetric analysis (TGA) – Differential scanning calorimetry (DSC)

Sikandar I. Mulla, T. P. Manjunatha, Robertcyril S. Hoskeri, Preeti N. Tallur and Harichandra Z. Ninnekar. Biodegradation of 3-Nitrobenzoate by *Bacillus flexus* strain XJU-4. World Journal of Microbiology and Biotechnology, Volume 27(7) (2011): 1587-1592

Bacillus flexus strain XJU-4 utilized 3-nitrobenzoate at 12 mM as a sole source of carbon and energy. This strain also utilized 4-nitrobenzoate, 2-nitrotoluene and nitrobenzene as growth substrates. The optimum conditions for degradation of 3-nitrobenzoate by the organism were found to be at pH 7.0 and temperature 30°C. Metabolite analysis, growth and enzymatic studies have revealed that the organism degraded 3-nitrobenzoate by oxidative mechanism through protocatechuate with the release of nitrite. The cells grown on 3-nitrobenzoate utilized protocatechuate but not 3-hydroxybenzoate, 3-aminobenzoate, 4-hydroxy-3-nitrobenzoate and 4-nitrocatechol. The cell-free extract of *Bacillus flexus* strain XJU-4 grown on 3-nitrobenzoate contained the activity of protocatechuate 2,3-dioxygenase, which suggest that protocatechuate was further degraded by a novel 2,3-dioxygenative *meta*-cleavage pathway.

Keywords: Degradation – *Bacillus flexus* strain XJU-4 – 3-Nitrobenzoate – Protocatechuate

Pankaj Kumar Arora and Rakesh Kumar Jain. Pathway for Degradation of 2-Chloro-4-Nitrophenol in *Arthrobacter* sp. SJCon. Current Microbiology, Volume 63(6) (2011): 568-573

Degradation of 2-Chloro-4-nitrophenol (2C4NP) was studied by *Arthrobacter* sp. SJCon, isolated from the soil of a pesticide contaminated site. This strain utilized 2C4NP as sole source of carbon and energy and degraded 2C4NP with stoichiometric release of nitrite and chloride ions. A metabolite was detected during the study of 2C4NP degradation and identified as chlorohydroquinone (CHQ) by thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC–MS). Inhibition study using 2,2′-dipyridyl showed that CHQ is a terminal aromatic compound in degradation pathway of 2C4NP. CHQ dioxygenase activity was observed in the crude extract of 2C4NP induced cells of the strain SJCon that suggested the cleavage of the CHQ to maleylacetate (MA). Our study clearly showed that *Arthrobacter* sp. SJCon degraded 2C4NP via formation of CHQ that further cleaved to MA by CHQ dioxygenase. This mechanism of degradation of 2C4NP differs from previously reported degradation pathways of 2C4NP

Tianming Cai, Liwei Chen, Jing Xu and Shu Cai. Degradation of Bromoxynil Octanoate by Strain *Acinetobacter* sp. XB2 Isolated from Contaminated Soil. Current Microbiology, Volume 63(2) (2011): 218-225

Bromoxynil octanoate (BOO), the most widespread herbicide applied to maize, is potentially toxic to both animals and humans. In this article, a highly effective BOO-degrading bacterial strain, XB2, was isolated from the soil of a herbicide factory. The strain was identified as an *Acinetobacter* sp. based on its 16S rRNA gene sequence analysis, morphological, physiological, and biochemical properties. This strain could use BOO as its sole carbon source and could degrade 100 mg l^{-1} BOO to non-detectable levels in 72 h (h). The optimal pH and temperature for strain XB2's growth and degradation of BOO in MSM are 7.0 and 30°C, respectively. We propose the following pathway of BOO degradation by strain XB2: the first step is the scission of the ester bond to form bromoxynil, bromoxynil then transformed to 3,5-dibromo-4-hydroxybenzoic acid due to the hydrolysis of nitriles, and debromination finally results in the formation of 3-bromo-4-hydroxybenzoic acid. Inoculating BOO-treated soil samples with strain XB2 resulted in a higher rate of BOO degradation than in non-inoculated soil, regardless of whether the soil had previously been sterilized.

Keywords: *Acinetobacter* sp. XB2 – Biodegradation – Bromoxynil octanoate – Metabolites – Soil remediation

Hedi Ben Mansour, Kamel Ghedira, Daniel Barillier, Leila Chekir Ghedira and Ridha Mosrati. Degradation and detoxification of acid orange 52 by *Pseudomonas putida* mt-2: a laboratory study. Environmental Science and Pollution Research, Volume 18(9) (2011): 1527-1535

Acid orange 52 (AO52), extensively used in textile industries, was decolorized by *Pseudomonas putida* mt-2. AO52 azoreduction products such as N,N′-dimethyl-*p*-phenylenediamine (DMPD) and 4-aminobenzenesulfonic acid (4-ABS), were identified in the static degradation mixture. These amines were identified only in media of static incubation, which is consistent with their biotransformation under shaken incubation (aerobic conditions).

Tests with azo products were carried out, and whole cells were found able to easily degrade DMPD contrary to 4-ABS. However, this last could be attacked by cell extract, and an oxygen uptake was observed during the reaction.

Degradation of DMPD by entire cells led to the formation of catechol. These results show that *P. putida* was able to decolorize AO52 and metabolize its derivative amines. In addition, the ability of tested compounds was evaluated in vitro to reduce human plasma butyrylcholinesterase (BuChE) activity.

Azoreduction products seem to be responsible for BuChE inhibition activity observed in static biodegradation extract. However, toxicity of AO52 completely disappears after shaken incubation with *P. putida,* suggesting that bacterium has a catabolism which enables it to completely degrade AO52 and especially, to detoxify the dye mixture.

Keywords: *Pseudomonas putida* mt-2 – Biodegradation – Textile dyes – Detoxification – Acid orange 52

Yuan Zhang, Yong-Guan Zhu, Sabine Houot, Min Qiao, Naoise Nunan and Patricia Garnier. Remediation of polycyclic aromatic hydrocarbon (PAH) contaminated soil through composting with fresh organic wastes. Environmental Science and Pollution Research, Volume 18(9) (2011): 1574-1584

Composting may enhance bioremediation of PAH-contaminated soils by providing organic substrates that stimulate the growth of potential microbial degraders. However, the influence of added organic matter (OM) together with the microbial activities on the dissipation of PAHs has not yet been fully assessed.

An in-vessel composting-bioremediation experiment of a contaminated soil amended with fresh wastes was carried out. Four different experimental conditions were tested in triplicate during 60 days using laboratory-scale reactors: treatment S (100% soil), W (100% wastes), SW (soil/waste mixture), and SWB (soil/waste mixture with inoculation of degrading microorganisms).

A dry mass loss of 35±5% was observed in treatments with organic wastes during composting in all the treatments except treatment S. The dissipation of the 16 USEPA-listed PAHs was largely enhanced from no significant change to 50.5±14.8% (for SW)/63.7±10.0% (for SWB). More obvious dissipation was observed when fresh wastes were added at the beginning of composting to the contaminated soil, without significant difference between the inoculated and non-inoculated treatments. Phospholipid fatty acid (PLFA) profiling showed that fungi and G- bacteria dominated at the beginning of experiment and were probably involved in PAH dissipation. Subsequently, greater relative abundances of G + bacteria were observed as PAH dissipation slowed down.

The results suggest that improving the composting process with optimal organic compositions may be a feasible remediation strategy in PAH-contaminated soils through stimulation of active microbial populations.

Keywords: Composting – Contaminated soil – PAHs – Bioremediation – Microbial communities – PLFAs

Maria Arena, Cristina Abbate, Kikku Fukushima and Mara Gennari. Degradation of poly (lactic acid) and nanocomposites by *Bacillus licheniformis.* Environmental Science and Pollution Research, Volume 18(6) (2011): 865-870

The disposal problem due to non-degradable petroleum-based plastics has raised the demand for biodegradable polymers. The degradation of poly (lactic acid) (PLA) has been studied for several years, but the understanding of involved mechanisms is still incomplete. Based on our previous studies, and it is hypothesized an enzymatic involvement, the aim of this study was to continue investigations on the degradation of PLA and its nanocomposites by *Bacillus licheniformis.*

Biodegradation of PLA and its nanocomposites (CLOISITE 30B and SOMASIF MEE) was performed on compression-molded, 25×25×0.6-mm films. Firstly, two plastic films were dipped into sterile nutrient broth inoculated with *B. licheniformis* and incubated at 32°C. Then, to verify

if biodegradation was due to extracellular esterase, the culture broth was filtered to remove *B. licheniformis* cells, and the plastic materials were put into this broth.

PLA degradation by *B. licheniformis* was accelerated by the presence of organoclays. After 5 months in liquid culture, nanocomposites showed only the 10% of residual mass, compared with the 60% of pure PLA. Extracellular esterase activity was detected in the filtered culture broth confirming that PLA biodegradation was probably due to this enzyme action.

Keywords: *Bacillus licheniformis* – Scanning electron microscopy – Nanocomposites – Organoclay – Polylactic acid

Georgia Gatidou and Evaggelia Iatrou. Investigation of photodegradation and hydrolysis of selected substituted urea and organophosphate pesticides in water. Environmental Science and Pollution Research, Volume 18(6) (2011): 949-957

Photodegradation and hydrolysis of two substituted urea herbicides, monolinuron [3-(4-chlorophenyl)-1-methoxy-1-methylurea] and linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], and one organophosphorous insecticide, phoxim [2-(diethoxyphosphinothioyloxyimino)-2-phenylacetonitrile], were studied using buffered sterilized distilled water (pH 4, 7 and 9).

Experiments were performed in the absence and presence of light (320–740 nm), while the effect of nitrates and humic acids on photodegradation was investigated for all pH values. An analytical method was developed and validated for the determination of target compounds in water samples using liquid chromatography positive ion electrospray–mass spectrometry.

According to the results, substituted ureas neither hydrolyzed, at all tested pH values, nor photodegraded at pH 7 and 9. Slow photodegradation of the compounds was observed at pH 4. During 70 days of light exposure, initial concentrations of linuron and monolinuron were decreased by 54% and 31%, respectively, while the presence of nitrates slightly enhanced photodegradation of these compounds. On the other hand, phoxim was found to be very unstable for all the tested conditions and an increase of pH resulted to higher degradation. During hydrolysis experiments, the degradation of the compound ranged from 41% (pH 4) to 85% (pH 9) and the half-lives varied from 10 h (pH 9) to 204 h (pH 4). The presence of light enhanced phoxim degradation and as a result half-lives of 37, 22 and 9h were calculated for pH 4, 7 and 9, respectively. The addition of nitrates and humic acids did not significantly affect the photodegradation of phoxim.

The results indicated that among the three tested pesticides, phoxim found to be the most sensitive in both photodegradation and hydrolysis.

Keywords: Fate – Removal – Phenylureas – Organophosphates – Photodegradation – Hydrolysis

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Branch, Islamic Azad University, Tehran, Iran. *Corresponding author. E-mail: Jamalfhosseini@srbiau.ac.ir). Challenges in commercialization of nano and biotechnologies in agricultural sector of Iran. African Journal of Biotechnology Vol. 10 (34)(2011): 6516-6521

The major purpose of this study was to determine challenges in commercialization of nano and biotechnologies in agicultural sector of Iran. The total population for this study was 50 participants who attended a workshop on commercialization of nano and biotechnologies in agriculture at biotech 2010 exhibition in Tehran. The results showed that the social and cultural challenges caused 39% of variance on the perception of the respondents about challenges influencing the commercialization of nano and biotechnologies in agricultural sector of Iran. The commercialization of nano and biotechnologies in Iran faces challenges and obstacles and require location-specific approaches.

Keywords: Commercialization, challenges, agriculture sector, Iran, nanotechnologies, biotechnologies.

Magda M. Aly[1,2]*. ([1]Faculty of Science, Biology Department, King Abdulaziz University, Saudi Arabia, [2]Faculty of Science, Botany Department, Kafr El Sheikh University, Egypt. E-mail: magdaaali@hotmail.com. Tel: 00966546407565). Degradation of morpholine by *Mycobacterium* sp. isolated from contaminated wastewater collected from Egypt. African Journal of Biotechnology Vol. 10 (42)(2011): 8351-8358

The biodegradation of morpholine has attracted much interest because morpholine causes environmental pollution. Ten species belonging to nine genera were tested for their abilities to degrade morpholine in mineral salts medium containing morpholine (1 g/l). *Mycobacterium* sp. isolated from polluted water sample collected from Abu Za"baal lakes, effectively utilized morpholine as carbon, nitrogen and energy source. The tested *Mycobacterium* was able to grow in high concentrations of morpholine but the rapidly increase in pH of the growth medium and accumulation of ammonia inhibited bacterial growth and complete mineralization of morpholine. The molar conversion ratio of morpholine to ammonia was 1:0.89. Growing of the selected bacterium in liquid medium with 1 g/l morpholine at 37°C and pH 6.5, enhanced morpholine degradation. Addition of metyrapone to the growth medium inhibited morpholine degradation. Immobilization of *Mycobacterium* cells in sodium alginate increased morpholine degradation compared with free cells. At high concentrations of morpholine (4 to 6 g/l), there was a decrease in both cell viability and respiration of *Mycobacterium* but no genotoxicity was found.

Keywords: Morpholine, *Mycobacterium*, biodegradation, pollution, ammonia, cytochrome P450, metyrapone, immobilization.

YouShuang Zhu[1], HaiBo Zhang[1,2], YingLong Zhang[1,3] and Feng Huang[1]*. ([1]State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, China, [2]Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266071, China, [3]Biotechnology Department, Shandong Institute of Commerce and Technology, Jinan 250103, China. *Corresponding author. E-mail: lignin302304@yahoo.cn. Tel: 86-531-88565234. Fax: 86-531-88565234). Lignocellulose degradation, enzyme production and protein enrichment by *Trametes versicolor* during solid-state fermentation of corn stover. African Journal of Biotechnology, Vol. 10 (45) (2011): 9182-9192

Microbial conversion of corn stover by white rot fungi has the potential to increase its ligninolysis and nutritional value, thereby transforming it into protein-enriched animal feed. Response surface methodology was applied to optimize conditions for the production of lignocellulolytic enzymes by *Trametes versicolor* during solid-state fermentation of corn stover, as well as enhance ligninolysis and increase the crude protein content. The effects of an additional carbon source (glucose), copper sulfate ($CuSO_4$) and initial moisture content on lignocellulolytic enzymes, changes in chemical constituents and the crude protein content of corn stover were investigated. *T. versicolor* produced high laccase, moderate xylanase, and low CMCase activity, whereas neither LiP nor MnP activity was detected. An overall 20-fold increase in laccase activity (45.1 U/g corn stover) was achieved under the optimized conditions. The maximum degradation of lignin and hemicellulose was up to 34.8 and 21.9%, respectively. However, the maximum cellulose loss was less than 10.5%. The crude protein content of the fermented corn stover was doubled under the optimized conditions. Therefore, *T. versicolor* is a potential organism for laccase production using solid-state fermentation, as well as the simultaneous enhancement of delignification and improvement of the crude protein content in corn stover.

Keywords: Corn stover, central composite design, laccase, ligninolysis, *Trametes versicolor*, crude protein.

Shazia Nouren*, Haq Nawaz Bhatti and Sadia Ilyas. (Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan. *Corresponding author. E-mail: shazianouren@yahoo.com. Fax: +92-41-9200764). **Bioleaching of copper, aluminum, magnesium and manganese from brown shale by *Ganoderma lucidum*.** **African Journal of Biotechnology, Vol. 10 (52) (2011): 10664-10673**

The present study was done to check the bioleaching feasibility of brown shale for the recovery of copper (Cu), aluminum (Al), magnesium (Mg) and manganese (Mn) ions using *Ganoderma lucidum*. Different experimental parameters were optimized for the enhanced recovery of metals ions. Effect of different substrates like glucose, molasses, saw dust and cotton seed cake on the recovery of metals ions was investigated under shaking as well as non-shaking conditions. Significant difference in leaching of metal ions by *G. lucidum* was observed under shaking and non shaking conditions. Maximum leaching of Al (90.7%), Mg (96.46%), Mn (66.3%) and Cu (73.45%) was observed using glucose under shaking conditions with 5, 3, 4 and 3% pulp densities respectively. The results show that maximum solubilization up to 68.89, 77.03 and 38.37% was achieved for Cu, Al and Mg ions respectively using molasses as substrate, whereas, 57.74% recovery of Mn was achieved with saw dust. The recovery of metal ions indicated that this low grade discarded ore may be a potential source of metal ions in future.

Keywords: Recovery, brown shale, *Ganoderma lucidum*, organic acids, pulp density.

Bazgha Ahmad*, Haq Nawaz Bhatti and Sadia Ilyas. (Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan. *Corresponding author. Email: bazgha_ahmad@hotmail.com. Fax#: 92-41-9200764). **Bio-extraction of metal ions from laterite ore by *Penicillium chrysogenum*.** **African Journal of Biotechnology, Vol. 10 (54) (2011): 11196-11205**

The main objective of this study was to find a more feasible and economical method to extract metal ions from laterite ore by *Penicillium chrysogenum*. The effect of different substrates on microbial recovery of metal ions from laterite ore using indigenous strain of *P. chrysogenum* was observed. Maximum recovery of aluminum (86.78%), iron (97.78%), manganese (77.61%), nickel (57.31%) and chromium (34.32%) was recorded in case of shaking flasks experiments up to 24 days of incubation. Metal ions solubilization was also compared with the samples, which were not shaken and maximum recovery of Al (83.54 %), Fe (96.12 %), Mn (88.56 %), Ni (46.53 %) and Cr (37.82 %), were attained up to 24 days of incubation period. Enhanced recovery of Fe and Al may be due to the result of the acidic effect of the environment and the chelating capacity of organic acids.

Keywords: Bioleaching, *Penicillium chrysogenum*, agriculture wastes, laterite ore.

A. Govender and B. Pillay*. (Discipline of Microbiology, Faculty of Science and Agriculture, University of KwaZulu-Natal (Westville Campus), Private Bag X54001, Durban 4000, Republic of South Africa. *Corresponding author. E-mail: pillayb1@ukzn.ac.za. Tel: +27 312607404. Fax: +27 312607809). **Biochemical activities of 1,2-dichloroethane (DCA) degrading bacteria. African Journal of Biotechnology, Vol. 10 (55) (2011): 11574-11581**

One of the most widely produced industrial solvents is 1,2-dichloroethane (DCA), a known carcinogen, toxic to both terrestrial and aquatic ecosystems. Annual production in the United States, Japan and Europe alone are in excess of thirteen thousand metric tons. Entry into the environment is mainly due to poor handling, accidental spillages and illegal dumping. Five indigenous DCA degrading bacterial isolates capable of completely degrading DCA under aerobic conditions recently isolated from South African waste water treatment facilities, were found to belong to the genus *Ancylobacter*. The specific activities of the enzymes in DCA catabolism were compared with previously characterized DCA degrading bacterial isolates using crude cell lysates and different intermediates within the catabolic route as substrates. The catabolic route by which DCA is degraded was found to be similar to previously characterized isolates of *Ancylobacter* as well as *Xanthobacter autotrophicus* GJ10. The specific activities of all the enzymes within the catabolic route of the South African isolates were found to be lower when compared with previously characterized isolates. Polyacrylamide gel electrophoresis of crude cell lysates did not indicate over-expression of the hydrolytic dehalogenase as observed in a previously characterized isolate of *Ancylobacter*. Although, the overall specific activities of each of the enzymes in the DCA catabolic route were found to be lower in this study, these isolates may still have potential use in the bioremediation of DCA contaminated sites in South Africa.

Keywords: 1,2- Dichloroethane, halogenated hydrocarbon, dehalogenase.

Noura El-Ahmady El-Naggar[1]*, A. A. Sherief [2] and Sarah Shawky Hamza[2]. ([1]Department of Bioprocess Development, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technology Applications, Alexandria, Egypt, [2]Department of Botany, Faculty of Science, Mansoura University, Egypt. *Corresponding author. E-mail: nouraelahmady@yahoo.com. Tel: (002)0103738444. Fax: (002)03 4593423). **Bioconversion process of rice straw by thermotolerant cellulolytic *Streptomyces viridiochromogenes* under solid-state fermentation conditions for bioethanol production. African Journal of Biotechnology, Vol. 10 (56) (2011): 11998-12011**

Enzymatic hydrolysis of the cellulose fraction of rice straw to glucose using solid-state fermentation for bioethanol production is a focus of current attention. A total of 10 actinomycetes isolates were isolated from soils and decayed rice straw. All these isolates were purified and screened for their cellulolytic activity; one strain was selected for further study and identified as *Streptomyces viridiochromogenes***.** Optimization of fermentation conditions showed highest cellulolytic enzymes production on the 5th day at pH 6.5 and at 40°C. The production of enzymes reached its maximal value at 4.0 g of rice straw/250 ml flask. Avicelase and total cellulase productivity were highly increased by the addition of NH_4Cl as N-source, while *maximum activity* of CMCase was *recorded by the addition of* peptone as N-source to the fermentation medium. The influence of various physico-chemical factors on enzyme activity was also investigated. The half life time of avicelase, total cellulase and CMCase at 60°C was 39.4, 50.0 and 78.58 min, respectively. A maximum of ethanol production 1.428±0.074% (v/v) by *Saccharomyces cereviseae* using dilute acid pretreated rice straw hydrolysate with initial soluble sugar 2.340±0.072% was recorded after 2 days of fermentation.

Keywords: Bioethanol, cellulolytic enzymes, rice straw, solid-state fermentation, *Streptomyces viridiochromogenes*.

Abbreviations: CMC, Carboxymethyl cellulose; **CMCase,** carboxymethyl-cellulase; ***s*,** *Streptomyces.*

Shanooba Palamthodi[1]*, Dhiraj Patil[2] and Yatin Patil[2]. ([1]Department of Biotechnology Engineering, Tatyasaheb Kore Institute of Engineering and Technology, Warananagar, India, [2]Department of Biotechnology Engineering, Kolhapur Institute of Engineering and Technology, Kolhapur, India. *Corresponding author. E-mail: Shanooba_pm@tkietwarana.org or shanooba.pm@gmail.com. Tel: 09960495337 or 09960495436). Microbial degradation of textile industrial effluents. African Journal of Biotechnology, Vol. 10 (59) (2011): 12657-12661

Textile waste water is a highly variable mixture of many polluting substance ranging from inorganic compounds and elements to polymers and organic products. To ensure the safety of effluents, proper technologies need to be used for the complete degradation of dyes. Traditionally, treatments of textile waste water involve physical or chemical methods. But both physical and chemical methods have many short comings. Biodegradation is an eco friendly activity it can produce little or no secondary hazard. In this work, the *in situ* degradation of textile industrial effluent was carried out. The degradation of two different dyes, blue and green colour has been studied. The isolated organism which showed the ability to degrade dye was characterized and identified as *Paenibacillus azoreducers* using various biochemical techniques. The degradation of dye was confirmed via the decolourisation assay and by the measurement of COD and BOD values. A trickling bed reactor was designed and the treatment of effluent from a textile industry was effectively carried out.

Keywords: Biodegradation, textile wastewater, secondary hazard, *Paenibacillus azoreducens*, decolourisation, trickling bed reactor.

Juan Men[1,2] and Fa Cheng[1]*. ([1]Department of chemistry, School of Science, Tianjin University, Tianjin 300072, China, [2]Environmental Protection Monitoring Station of Tanggu, Tianjin 300450, China. *Corresponding author. E-mail: menanlan@126.com. Tel: +86-22-25866616. Fax: +86-22-25866615). Biodegradation and growth characteristics of a toluene-degrading strain. African Journal of Biotechnology, Vol. 10 (61) (2011): 13299-13306

A toluene-degrading strain was isolated from active sludge in this study. Both growth characteristic and the performance to degrade toluene by the strain in batch culture mode were evaluated. Results showed that the isolated strain presented a good ability to remove toluene with the maximum removal efficiency of 93.8%. Growth and toluene degradation occurred at 20 to 50°C but the optimum was found to be 30°C for both. The optimal pH for growth and toluene degradation was 6.5. Lower toluene concentrations (1.19 to 2.45 mg/l) promoted faster growth rates than higher concentrations (3.28 to 6.17 mg/l) during the first 20 h; this could be probably due to the substrate inhibition effects. The removal efficiencies of toluene (90 to 95%) were almost the same within the concentrations ranges (1.19 to 6.17 mg/l). Kinetic analysis results indicated that the biodegradation of toluene followed first-order kinetics, and the removal rate constant (*k*) was 0.0385. Finally, the isolated strain was identified as *Pseudomonas* sp. using 16S rDNA sequencing.

Keywords: Biodegradation, growth characteristic, toluene, *Pseudomonas.*

Bronislava Uhnáková[a], Roland Ludwig[b], Jana Pěknicová[c], Ladislav Homolka[d], Ludmila Lisá[d], Miroslav Šulc[e], Alena Petříčková[a], Fatima Elzeinová[c], Helena Pelantová[e], Daniela Monti[f], Vladimír Křen[a], Dietmar Haltrich[b], Ludmila Martínková[a]. ([a]nstitute of Microbiology, Laboratory of Biotransformation, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic, [b]niversity of Natural Resources and Life Sciences, Department of Food Science and Technology, Muthgasse 18, 1190 Vienna, Austria, [c]nstitute of Biotechnology, Laboratory of Diagnostics for Reproductive Medicine, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic, [d]Institute of Microbiology, Laboratory of Environmental Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic, [e]nstitute of Microbiology, Laboratory of Molecular Structure Characterization, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic, [f]stituto di Chimica del Riconoscimento Molecolare, C.N.R., Via Mario Bianco 9, 20131 Milano, Italy). Biodegradation of tetrabromobisphenol A by oxidases in basidiomycetous fungi and estrogenic activity of the biotransformation products.Bioresource Technology, Volume 102(20) (2011): 9409-9415

Tetrabromobisphenol A (TBBPA) degradation was investigated using white rot fungi and their oxidative enzymes. Strains of the *Trametes, Pleurotus, Bjerkandera and Dichomitus* genera eliminated almost 1 mM TBBPA within 4 days. Laccase, whose role in TBBPA degradation was demonstrated in fungal cultures, was applied to TBBPA degradation alone and in combination with cellobiose dehydrogenase from *Sclerotium rolfsii*. Purified laccase from *Trametes versicolor* degraded approximately 2 mM TBBPA within 5 h, while the addition of cellobiose dehydrogenase increased the degradation rate to almost 2.5 mM within 3 h. Laccase was used to prepare TBBPA metabolites 2,6-dibromo-4-(2-hydroxypropane-2-yl) phenol (**1**), 2,6-dibromo-4-(2-methoxypropane-2-yl) phenol (**2**) and 1-(3,5-dibromo-4-hydroxyphen-1-yl)-2,2′,6,6′-

tetrabromo-4,4′-isopropylidene diphenol (**3**). As compounds **1** and **3** were identical to the TBBPA metabolites prepared by using rat and human liver fractions (Zalko et al., 2006), laccase can provide a simple means of preparing these metabolites for toxicity studies. Products **1** and **2** exhibited estrogenic effects, unlike TBBPA, but lower cell toxicity.

Keywords: White rot fungi; Laccase; Cellobiose dehydrogenase; Tetrabromobisphenol A metabolites; Estrogenic activity

Wen-Da Oh, Poh-Eng Lim, Chye-Eng Seng, Amat Ngilmi Ahmad Sujari. (School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia). Bioregeneration of granular activated carbon in simultaneous adsorption and biodegradation of chlorophenols. Bioresource Technology,Volume 102(20) (2011) : 9497-9502

The objectives of this study are to obtain the time courses of the amount of chlorophenol adsorbed onto granular activated carbon (GAC) in the simultaneous adsorption and biodegradation processes involving 4-chlorophenol (4-CP) and 2,4-dichlorophenol (2,4-DCP), respectively, and to quantify the bioregeneration efficiency of GAC loaded with 4-CP and 2,4-DCP by direct measurement of the amount of chlorophenol adsorbed onto GAC. Under abiotic and biotic conditions, the time courses of the amount of chlorophenol adsorbed onto GAC at various GAC dosages for the initial 4-CP and 2,4-DCP concentrations below and above the biomass acclimated concentrations of 300 and 150 mg/L, respectively, were determined. The results show that the highest bioregeneration efficiency was achieved provided that the initial adsorbate concentration was lower than the acclimated concentration. When the initial adsorbate concentration was higher than the acclimated concentration, the highest bioregeneration efficiency was achieved if excess adsorbent was used.

Keywords: Bioregeneration; Granular activated carbon; Adsorption; Biodegradation; Chlorophenols

Liping Huang[a], Linlin Gan[a], Qingliang Zhao[b], Bruce E. Logan[c], Hong Lu[a], Guohua Chen[a, d]. ([a]Key Laboratory of Industrial Ecology and Environmental Engineering, Ministry of Education (MOE), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China, [b]State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China, [c]Department of Civil and Environmental Engineering, The Pennsylvania State University, University Park, PA 16802, USA, [d]Department of Chemical and Biomolecular Engineering, Kowloon, Hong Kong University of Science and Technology, Hong Kong, China). Degradation of pentachlorophenol with the presence of fermentable and non-fermentable co-substrates in a microbial fuel cell. Bioresource Technology, Volume 102(19) (2011): 8762-8768

Pentachlorophenol (PCP) was more rapidly degraded in acetate and glucose-fed microbial fuel cells (MFCs) than in open circuit controls, with removal rates of 0.12 ± 0.01 mg/L h (14.8 ± 1.0 mg/g-VSS-h) in acetate-fed, and 0.08 ± 0.01 mg/L h (6.9 ± 0.8 mg/g-VSS-h) in glucose-fed MFCs, at an initial PCP concentration of 15 mg/L. A PCP of 15 mg/L had no effect on power generation from acetate but power production was decreased with glucose. Coulombic balances indicate the predominant product was electricity (16.1 ± 0.3%) in PCP-acetate MFCs, and lactate

(19.8 ± 3.3%) in PCP-glucose MFCs. Current generation accelerated the removal of PCP and co-substrates, as well as the degradation products in both PCP-acetate and PCP-glucose reactors. While 2,3,4,5-tetrachlorophenol was present in both reactors, tetrachlorohydroquinone was only found in PCP-acetate MFCs. These results demonstrate PCP degradation and power production were affected by current generation and the type of electron donor provided.

Keywords: Microbial fuel cell; Pentachlorophenol; Co-metabolism; Acetate; Glucose

Chenggang Zheng[a], Jianglin He[b], Yongli Wang[c], Manman Wang[a], Zhiyong Huang[a]. ([a]Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China, [b]Chengdu Institute of Geology and Mineral Resources, Chengdu, Sichuan 610081, China, [c]Lanzhou Institute of Geology, Chinese Academy of Sciences, Lanzhou 730000, China). **Hydrocarbon degradation and bioemulsifier production by thermophilic *Geobacillus pallidus* strains. Bioresource Technology, Volume 102(19) (2011): 9155-9161**

Geobacillus pallidus XS2 and XS3 were isolated from oil contaminated soil samples in Yumen oilfield, China, and were able to produce bioemulsifiers on different hydrocarbons. Biodegradation assays exhibited that approximately 70% of PAH (250 mg/L) or 85% of crude oil (500 mg/L) was removed by the thermophilic bacteria after 20 days. The bioemulsifiers of the two strains were isolated and obtained a productive yield of 4.24 ± 0.08 and 3.82 ± 0.11 g/L, respectively. GPC analysis revealed that the number-average molecular weights (M_n) of the two bioemulsifiers were 271,785 Da and 526,369 Da, with *PDI* values of 1.104 and 1.027, respectively. Chemical composition studies exhibited that the bioemulsifier XS2 consisted of carbohydrates (68.6%), lipids (22.7%) and proteins (8.7%) while the bioemulsifier XS3 was composed by carbohydrates (41.1%), lipids (47.6%) and proteins (11.3%). Emulsification assays approved the effectiveness of bioemulsifiers over a wide range of temperature, pH and salinity.

Keywords: *Geobacillus pallidus*; Hydrocarbon; Biodegradation; Bioemulsifier; Emulsifying activity

Lailatul Qadariyah[a], Mahfud[a], Sumarno[a], Siti Machmudah[a, b], Wahyudiono[c], Mitsuru Sasaki[c], Motonobu Goto[b]. ([a]Chemical Engineering Department, Faculty of Industrial Technology, Sepuluh Nopember Institute of Technology, Indonesia, [b]Bioelectrics Research Center, Kumamoto University, Japan, [c]Graduate School of Science and Technology, Kumamoto University, Japan). **Degradation of glycerol using hydrothermal process. Bioresource Technology, Volume 102(19) (2011): 9267-9271**

Sub-critical or supercritical water was utilized for the degradation of glycerol in an environmentally benign reaction. The reaction was carried out in a batch reactor in the temperature range of 473–673 K, pressure of 30 MPa, and reaction time of 20–60 min. The effects of temperature and reaction time were observed. The degradation of glycerol produced acetaldehyde, acrolein, allyl alcohol and un-identified products. The highest yield of acrolein, acetaldehyde and allyl alcohol were 0.20, 7.17, 96.69 mol%, respectively. Glycerol conversion was 99.92 mol%. While acetaldehyde was formed only in sub-critical water and allyl alcohol only in supercritical water, acrolein was formed in both. The kinetics of the global reaction displayed a pseudo-first-order. The activation energy at subcritical water was 39.6 kJ/mol. Based on the results, this method could be an efficient method for glycerol degradation because the high conversion of glycerol was obtained.

Keywords: Degradation; Glycerol; Sub-critical water; Supercritical water

Anouk F. Duque, Vânia S. Bessa, Maria F. Carvalho, Paula M.L. Castro. (CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal). Bioaugmentation of a rotating biological contactor for degradation of 2-fluorophenol. Bioresource Technology, Volume 102(19) (2011): 9300-9303

The performance of a laboratory scale rotating biological contactor (RBC) towards shock loadings of 2-fluorophenol (2-FP) was investigated. During a period of ca. 2 months organic shock loadings of 25 mg L^{-1} of 2-FP were applied to the RBC. As no biodegradation of 2-FP was observed, bioaugmentation of the RBC with a 2-FP degrading strain was carried out and, along ca. 6 months, organic shock loadings within a range of 25–200 mg L^{-1} of 2-FP were applied. Complete biodegradation of 50 mg L^{-1} of 2-FP was observed during operation of the reactor. The RBC showed to be robust towards starvation periods, as after ca. 1 month of non-supply of the target compound, the reactor resumed 2-FP degradation. The inoculated strain was retained within the biofilm in the disks, as the 2-FP degrading strain was recovered from the biofilm by the end of the experiment, thus bioaugmentation was successfully achieved.

Keywords: Rotating biological contactor (RBC); 2-Fluorophenol (2-FP); Bioaugmentation; Biodegradation

Xing-Biao Wang[a, b, d, 1], Chang-Qiao Chi[a], Yong Nie[a], Yue-Qin Tang[a, 1], Yan Tan[c], Gang Wu[b], Xiao-Lei Wu[a]. ([a]Department of Energy and Resources Engineering, College of Engineering, Peking University, Beijing 100871, PR China, [b]State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Bejing 100085, PR China, [c]Department of Environmental Science and Engineering, Tsinghua University, Beijing 100084, PR China, [d]Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China). Degradation of petroleum hydrocarbons (C6–C40) and crude oil by a novel *Dietzia* strain. Bioresource Technology, Volume 102 (17) (2011): 7755-7761

A novel bacterial strain, DQ12-45-1b, was isolated from the production water of a deep subterranean oil-reservoir. Morphological, physiological and phylogenetic analyses indicated that the strain belonged to the genus *Dietzia* with both *alkB* (coding for alkane monooxygenase) and CYP153 (coding for P450 alkane hydroxylase of the cytochrome CYP153 family) genes and their induction detected. It was capable of utilizing a wide range of *n*-alkanes (C6–C40), aromatic compounds and crude oil as the sole carbon sources for growth. In addition, it preferentially degraded short-chain hydrocarbons (□C25) in the early cultivation phase and accumulated hydrocarbons with chain-lengths from C23 to C27 during later cultivation stage with crude oil as the sole carbon source. This is the first study to report the different behaviors of a bacterial species toward crude oil degradation as well as a species of *Dietzia* degrading a wide range of hydrocarbons.

Keywords: *Dietzia*; Biodegradation; Alkane; Hydrocarbon; Crude oil

Shaohua Chen, Qiongbo Hu, Meiying Hu, Jianjun Luo, Qunfang Weng, Kaiping Lai. (Key Laboratory of Pesticide and Chemical Biology, Ministry of Education, Laboratory of Insect Toxicology, South China Agricultural University, Guangzhou 510642, PR China). Isolation and characterization of a fungus able to degrade pyrethroids and 3-phenoxybenzaldehyde. Bioresource Technology, Volume 102(17) (2011): 8110-8116

Fungal strain HU, isolated from activated sludge and identified as a member of the genus *Cladosporium* based on morphology and sequencing of 28S rRNA, was shown to degrade 90% of fenvalerate, fenpropathrin, β-cypermethrin, deltamethrin, bifenthrin, and permethrin (100 mg L^{-1}) within 5 days. Fenvalerate was utilized as sole carbon and energy source and co-metabolized in the presence of sucrose. Degradation of fenvalerate occurred at pH 5–10 at 18–38 °C. The fungus first hydrolyzed the carboxylester linkage to produce α-hydroxy-3-phenoxy-benzeneacetonitrile and 3-phenoxybenzaldehyde, and subsequently degraded these two compounds with a q_{max}, K_s and K_i of 1.73 d^{-1}, 99.20 mg L^{-1} and 449.75 mg L^{-1}, respectively. Degradation followed first-order kinetics. These results show that the fungal strain may possess potential to be used in bioremediation of pyrethroid-contaminated environments.

Keywords: Pyrethroids; 3-Phenoxybenzaldehyde; Biodegradation; *Cladosporium* sp.; Kinetics

Mohammad Zain khan[a], Pijush Kanti Mondal[b], Suhail Sabir[a], Vinod Tare[c]. ([a]Environmental Research Laboratory, Department of Chemistry, Aligarh Muslim University, Faculty of Science, Aligarh 202 002, UP, India, [b]Environmental Research Laboratory, Department of Applied Chemistry, Faculty of Engineering and Technology, Aligarh Muslim University, Aligarh 202 002, UP, India, [c]Environmental Engineering and Management, Department of Civil Engineering, Indian Institute of Technology, Kanpur 208 016, UP, India). Degradation pathway, toxicity and kinetics of 2,4,6-trichlorophenol with different co-substrate by aerobic granules in SBR. Bioresource Technology, Volume 102(13) (2011): 7016-7021

The present study deals with cultivation of 2,4,6-trichlorophenol (TCP) degrading aerobic granules in two SBR systems based on glucose and acetate as co-substrate. Biodegradation of TCP containing wastewater starting from 10 to 360 mg L^{-1} with more than 90% efficiency was achieved. Sludge volume index decreases as the operation proceeds to stabilize at 35 and 30 mL g^{-1} while MLVSS increases from 4 to 6.5 and 6.2 g L^{-1} for R1 (with glucose as co-substrate) and R2 (with sodium acetate as co-substrate), respectively. FTIR, GC and GC/MS spectral studies shows that the biodegradation occurred via chlorocatechol pathway and the cleavage may be at *ortho*-position. Haldane model for inhibitory substrate was applied to the system and it was observed that glucose fed granules have a high specific degradation rate and efficiency than acetate fed granules. Genotoxicity studies shows that effluent coming from SBRs was non-toxic.

Keywords: Bioremediation; TCP; Glucose; Acetate; Genotoxicity

Biosensor

Ashok Mulchandani and Rajesh. Microbial Biosensors for Organophosphate Pesticides. Applied Biochemistry and Biotechnology, Volume 165(2) (2011): 687-699

Organophosphates, amongst the most toxic substance known, are used widely in agriculture around the world. Their extensive use, however, has resulted in their occurrence in the water and food supply threatening humans and animals. Therefore, there is a need for determination of these neurotoxic compounds sensitively, selectively, and rapidly in the field. The present work is a brief review on the recent advancements in amperometric, potentiometric, and optical biosensors using genetically engineered microorganisms expressing organophosphate hydrolyzing enzyme intracellularly or anchored on the cell surface for the detection of organophosphate pesticides. The benefits and limitations associated with such microbial biosensors are delineated.

Keywords: Biosensor – Microbial – Organophosphate – Nerve agents – Pesticides

T. Sawidis[a], J. Breuste[b], M. Mitrovic[c], P. Pavlovic[c], K. Tsigaridas[a]. ([a]Department of Botany, University of Thessaloniki, 54124 Thessaloniki, Greece, [b]Department of Geography and Geology, University of Salzburg, 5010 Salzburg, Austria, [c]Department of Ecology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia). **Trees as bioindicator of heavy metal pollution in three European cities. Environmental Pollution, Volume 159(12) (2011): 3560-3570**

Concentrations of four heavy metals were determined in tree leaves and bark collected from polluted and non-polluted areas of three European cities (Salzburg, Belgrade and Thessaloniki) for a comparative study. *Platanus orientalis L.* and *Pinus nigra Arn.*, widespread in urban northern and southern Europe, were tested for their suitability for air quality biomonitoring. Leaves and barks were collected uniformly of an initial quantity of about 30 g of each sample. Analysis was accomplished by electrothermal atomic absorption spectrometry after total digestion. Site-dependent variations were found with the highest concentration level measured in Belgrade, followed by Thessaloniki and Salzburg. A higher accumulation of heavy metals was found in bark compared to leaves. Pine tree bark, accumulating higher concentrations of trace metals compared to plane tree bark, shows a higher efficiency as bioindicator for urban pollution. Both indicator species are suitable for comparative studies on bioindication of urban air pollution.

Keywords: Heavy metals; Urban air pollution; Bioindicators; *Platanus orientalis*; *Pinus nigra*

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atmospheric trace metals – With special references to megacities. Environmental Pollution, Volume 159(8-9) (2011) : 1991-1995

After considering t the particular problems of atmospheric pollution in megacities, i.e. agglomerations larger than 5 mio. inhabitants, with urbanization of World's population going on steadily, possibilities of active biomonitoring by means of green plants are discussed. Based on specific definitions of active and passive bioindication the chances of monitoring heavy metals in Sao Paulo megacity were demonstrated (first results published before). This is to show that there is need for increased use of bioindication to tackle the particular problems of megacities concerning environmental "health", the data to be processed according to the Multi-Markered-Bioindication-Concept (MMBC). Comparison to other work shows this approach to be reasonable.

Keywords: Chemical pollution; Health care; Multi-Markered-Bioindication-Concept (MMBC); Atmospheric deposition; São Paulo

Ashok Mulchandani, Nosang V Myung. (Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521, United States). Conducting polymer nanowires-based label-free biosensors. Current Opinion in Biotechnology, Volume 22(4) (2011): 502-508

Label-free sensing technologies have recently attracted a great deal of interest for sensitive, rapid and facile analysis for applications in health care, environmental monitoring, food safety and homeland security. One-dimensional (1-D) nanostructures such as nanowires, configured as field-effect transistors (FETs)/chemiresistors that change conductance upon binding of charged macromolecules to receptors linked to the device surfaces are extremely attractive for label-free biosensors. Herein, we review recent advances in label-free biosensors based on conducting polymer nanowires based FET/chemiresistor. Specifically, we address the fabrication, functionalization, assembly/alignment and sensing applications of FET/chemiresistor based on these nanomaterials. The advantages and disadvantages of various fabrication, functionalization, and assembling procedures of these nanosensors are reviewed and discussed.

Hatice Ceylan Koydemir[a, c], Haluk Külah[a, b], Canan Özgen[c], Alpaslan Alp[d], Gülşen Hasçelik[d]. ([a]METU-MEMS Center, Middle East Technical University, 06800, Çankaya, Ankara, Turkey, [b]Department of Electrical and Electronics Engineering, Middle East Technical University, 06800, Çankaya, Ankara, Turkey, [c]Department of Chemical Engineering, Middle East Technical University, 06800, Çankaya, Ankara, Turkey, [d]Medical Microbiology, Hacettepe University, 06100, Sıhhiye, Ankara, Turkey). MEMS biosensors for detection of methicillin resistant *Staphylococcus aureus*. Biosensors and Bioelectronics, Volume 29(1) (2011): 1-12

This review presents the current state of the conventional methods, microfluidic based biosensors, and the commercial products used in the detection of methicillin resistant *Staphylococcus aureus* (MRSA), which is one of the most important threats of nosocomial infections in many parts of the world. The early detection of MRSA in the specimens of the patients is important to enable the appropriate treatment, to decrease morbidity and mortality rates, and to manage control actions in the healthcare units. Thus, rapid and inexpensive diagnostic systems with high sensitivity and specificity are essential to prevent MRSA to be an emerging public health threat. The design and fabrication of new diagnostic systems necessitates

working in collaboration between different disciplines to make new challenges in the field of clinical diagnosis and to meet the demands of clinicians. It is certain that in the near future, MEMS and nanotechnology based detection methods will take the place of current methods in clinical diagnosis. The evaluation of new trends for specificity, sensitivity, cost effectiveness, disposability, low weight, ease of use, and facile access should be taken into consideration.

Keywords: MEMS; MRSA; *Staphylococcus aureus*; Detection; Biosensors

Zhenping Chen, Dongmei Sun, Yiming Zhou, Jiayue Zhao, Tianhong Lu, Xiaohua Huang, Chenxin Cai, Jian Shen. (Jiangsu Key Laboratory of New Power Batteries, Jiangsu Key Laboratory of Biofunctional Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210046, PR China). Nano polyurethane-assisted ultrasensitive biodetection of H_2O_2 over immobilized Microperoxidase-11. Biosensors and Bioelectronics, Volume 29(1) (2011): 53-59

Nanostructured polyurethane (PU) synthesized by an emulsion polymerization with narrow size distribution was employed for the first time directly as a novel matrix for enzyme immobilization to develop sensitively amperometric biosensors. When Microperoxidase-11 (MP-11) was selected as a model protein, the resulting hydrogen peroxide (H_2O_2) biosensor exhibited improved sensitivity of 29.6 μA mM^{-1} cm^{-2} with quite good response time of (1.3 ± 0.4) s and remarkable limit of detection as low as 10 pM (S/N 3) over existing protocols. A linear calibration curve for hydrogen peroxide was obtained up to 1.3 μM under the optimized conditions with a relative low calculated Michaelis–Menten constant (K_M^{app})(1.87 ± 0.05) μM, which indicated the enhanced enzymatic affinity of MP-11 to H_2O_2 via PU. The possible interferents had negligible effect on the response current and time of the prepared biosensor. Results suggest that the PU nanoparticles (PU-NPs) with good biocompatibility and sufficient interfacial adhesion hold promise as an attractive support material for construction of ultrasensitive amperometric biosensor.

Keywords: Biosensor; Nano-polyurethane; Microperoxidase-11; Hydrogen peroxide

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Chrono-impedance technique (CIT) was implemented as a new transduction method for real time measurement of glucose in a biosensor system based in carbon paste (CP)/Ferrocene (FC)/glucose oxidase (GOx). The system presents high selectivity because the optimal stimulation signal composed by a 165 mV DC potential and 50 mV_{RMS} AC signal at 0.4 Hz was used. The low DC potential used decreased the interfering species effect and the biosensor showed a linear impedance response toward glucose detection at concentrations from 0 mM to 20 mM,with 0.9853 and 0.9945 correlation coefficient for impedance module (|Z|) and phase

(Φ), respectively. The results of quadruplicate sets reveal the high repeatability and reproducibility of the measurements with a relative standard deviation (RSD) less than 10%. CIT presented good accuracy (within 10% of the actual value) and precision did not exceed 15% of RSD for high concentration values and 20% for the low concentration ones. In addition, a high correlation coefficient (R^2 = 0.9954) between chrono-impedance and colorimetric methods was obtained. On the other hand, when two samples prepared at the same conditions were measured in parallel with both methods (the measurement was repeated four times), it should be noticed that student's *t*-test produced no difference between the two mentioned methods (p = 1). The biosensor system hereby presented is highly specific to glucose detection and shows a better linear range than the one reported on the previous article.

Keywords: Second generation biosensors; Electrochemical Impedance Spectroscopy; Chrono-impedance technique

Chan Woo Park, Jong-Heon Yang, Chil Seong Ah, Chang-Geun Ahn, Yo Han Choi, Kwang Hyo Chung, Wan-Joong Kim, Gun Yong Sung. (Biosensor Research Team, Electronics and Telecommunications Research Institute (ETRI), Daejeon 305-700, Republic of Korea). Toxin detection by Si photosensitive biosensors with a new measurement scheme. Biosensors and Bioelectronics, Volume 29(1) (2011): 219-223

We propose a new type of photosensitive biosensor with a CMOS compatible Si photodiode integrated circuit, for the high-sensitive detection of small mycotoxin molecules requiring competitive assay approach. In this work, a photodiode is connected to the gate of a field effect transistor (FET) so that the open circuit voltage (V_{OC}) of the illuminated photodiode is transferred into the drain/source current (I_{DS}) of the FET. The sensing scheme employs competitive binding of toxin molecules (within the sample solution) and toxin–BSA conjugates (immobilized on the photodiode surface) with Au-nanoparticle-labeled antibodies, followed by silver enhancement to generate opaque structures on the photodiode surface. By utilizing the non-linear dependence of the V_{OC} on the light intensity, we can maintain a sufficiently high signal resolution at low toxin concentrations (with most of the incident light blocked) for the competitive assay. By monitoring the I_{DS} of the FET whose gate is driven by the V_{OC}, quantitative detection of Aflatoxin B1 has been achieved in the range of 0–15 ppb.

Keywords: Photosensitive biosensor; CMOS compatible; Photodiode; Competitive assay; Mycotoxin

Amardeep Singh[1], Srikanta Patra[1], Jeong-Ah Lee, Kang Hyun Park, Haesik Yang. (Department of Chemistry and Chemistry Institute of Functional Materials, Pusan National University, Busan 609-735, Republic of Korea). An artificial enzyme-based assay: DNA detection using a peroxidase-like copper–creatinine complex. Biosensors and Bioelectronics, Volume 26(12) (2011): 4798-4803

We report an artificial enzyme-based DNA assay using a peroxidase-like copper (Cu)–creatinine complex as a catalyst for 3,3′,5,5′-tetramethylbenzidine (TMB) oxidation. The assay employs double signal amplification and a homogeneous catalytic reaction: (i) fast catalytic growth of Cu on a gold (Au) nanoparticle (NP) label forms a thick Cu layer (first amplification); (ii) dissolution of the Cu layer generates many Cu–creatinine complexes per NP (generation of homogeneous catalysts); (iii) peroxidase-like Cu–creatinine complexes rapidly convert TMB into a colored product (second amplification). To investigate the effect of ligand on the catalytic

activities of Cu complexes, the kinetics of catalytic TMB oxidation is tested with and without using imidazole ring-containing ligands (creatinine, imidazole, and poly(L-histidine)). Both fast oxidation of TMB and slow further oxidation of the colored product are required for high signal-to-background ratios. Cu–creatinine complex allows relatively fast oxidation and slow further oxidation. Fast seed-mediated Cu growth on Au NP and slow Cu autonucleation (i.e., slow formation of Cu NP in the absence of Au NP) are also required for high signal-to-background ratios. In tris–EDTA (tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetic acid) buffer (pH 7.7) containing high concentrations of Cu^{2+} (90 mM), ascorbic acid (50 mM), and Mg^{2+} (200 mM), Cu growth on Au NP is very fast and autonucleation is significantly suppressed. Fast catalytic oxidation by Cu–creatinine complex along with fast Cu growth on Au NP allows a detection limit of 0.1 pM for DNA in a simple microplate format.

Keywords: Artificial enzyme; DNA sensor; Copper complex; Gold nanoparticle; Peroxidase

Andrea Ventrella[a], Lucia Catucci[a, b], Tiziana Placido[a, b], Francesco Longobardi[a], Angela Agostiano[a, b, c]. ([a]Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", Via Orabona 4, 70126 Bari, Italy, [b]IPCF-CNR, sez. Bari, Via Orabona 4, 70126 Bari, Italy, [c]INSTM, Via G. Giusti 9, 50121 Firenze, Italy). Biomaterials based on photosynthetic membranes as potential sensors for herbicides. Biosensors and Bioelectronics, Volume 26(12) (2011): 4747-4752

In this study, ultrathin film multilayers of Photosystem II-enriched photosynthetic membranes (BBY) were prepared and immobilized on quartz substrates by means of a Layer by Layer procedure exploiting electrostatic interactions with poly(ethylenimine) as polyelectrolyte. The biomaterials thus obtained were characterized by means of optical techniques and Atomic Force Microscopy, highlighting the fact that the Layer by Layer approach allowed the BBYs to be immobilized with satisfactory results. The activity of these hybrid materials was evaluated by means of optical assays based on the Hill Reaction, indicating that the biosamples, which preserved about 65% of their original activity even ten weeks after preparation, were both stable and active. Furthermore, an investigation of the biochips' sensitivity to the herbicide terbutryn, as a model analyte, gave interesting results: inhibition of photosynthetic activity was observed at terbutryn concentrations higher than 10^{-7} M, thus evidencing the potential of such biomaterials in the environmental biosensor field.

Keywords: Photosynthetic material; Layer by Layer; Biosensor; Herbicide; Optical assay

Yo Morita[1], Wataru Yoshida[1], Nasa Savory, Sung Woong Han, Masayuki Tera, Kazuo Nagasawa, Chikashi Nakamura, Koji Sode, Kazunori Ikebukuro. (Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan). Development of a novel biosensing system based on the structural change of a polymerized guanine-quadruplex DNA nanostructure. Biosensors and Bioelectronics, Volume 26(12) (2011): 4837-4841

By inserting an adenosine aptamer into an aptamer that forms a G-quadruplex, we developed an adaptor molecule, named the Gq-switch, which links an electrode with flavin adenine dinucleotide-dependent glucose dehydrogenase (FADGDH) that is capable of transferring electron to a electrode directly. First, we selected an FADGDH-binding aptamer and identified

that its sequence is composed of two blocks of consecutive six guanine bases and it forms a polymerized G-quadruplex structure. Then, we inserted a sequence of an adenosine aptamer between the two blocks of consecutive guanine bases, and we found it also bound to adenosine. Then we named it as Gq-switch. In the absence of adenosine, the Gq-switch–FADGDH complex forms a 30-nm high bulb-shaped structure that changes in the presence of adenosine to give an 8-nm high wire-shaped structure. This structural change brings the FADGDH sufficiently close to the electrode for electron transfer to occur, and the adenosine can be detected from the current produced by the FADGDH. Adenosine was successfully detected with a concentration dependency using the Gq-switch–FADGDH complex immobilized Au electrode by measuring response current to the addition of glucose.

Keywords: Aptamers; FADGDH; G-quadruplexes; Nanostructures; Biosensors; Enzymes

F.J. Sanza[a], M. Holgado[a], F.J. Ortega[b], R. Casquel[a], D. López-Romero[c], M.J. Bañuls[b], M.F. Laguna[a], C.A. Barrios[c], R. Puchades[b], A. Maquieira[b]. ([a]Centro Láser, Universidad Politécnica de Madrid, Campus Sur, 28031 Madrid, Spain, [b]Instituto de Reconocimiento Molecular y Desarrollo Tecnológico, Departamento de Química, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain, [c]Instituto de Sistemas Optoelectrónicos y Microtecnología, Universidad Politécnica de Madrid, ETSI de Telecomunicación, Ciudad Universitaria s/n, 28040 Madrid, Spain). Bio-Photonic Sensing Cells over transparent substrates for anti-gestrinone antibodies biosensing. Biosensors and Bioelectronics, Volume 26(12) (2011): 4842-4847

In a previous work we introduced the term Bio-Photonic Sensing Cells (BICELLs), referred to periodic networks of nano-pillar suitable for biosensing when are vertically interrogated. In this article, we demonstrate the biosensing capabilities of a type of micrometric size BICELLs made of SU-8 nano-pillars fabricated over transparent substrates. We verify the biochips functionality comparing the theoretical simulations with the experimental results when are optically interrogated in transmission. We also demonstrate a sensitivity enhancement by reducing the pitch among nano-pillars from 800 to 700 nm. Thus, the Limit of Detection achievable in these types of BICELLs is in the order of 64 pg/mL for 700 nm in pitch among nano-pillars in comparison with 292 pg/mL for 800 nm in pitch when are interrogated by Fourier Transform Visible and Infrared Spectrometry. The experiments exhibited a good reproducibility with a relative standard deviation of 0.29% measured within 8 days for a specific concentration. Finally, BICELLs functionality was tested in real conditions with unpurified rabbit serum for detecting anti-gestrinone antibodies, demonstrating the high performance of this type of BICELLs to detect specific antibodies having immobilized the suitable bioreceptors onto the sensing surface.

Keywords: Optical biosensing; SU-8 nano-pillar; Anti-gestrinone detection

Won-young Chung[a], Ye Lim Jung[a], Ki Soo Park[a], Cheulhee Jung[a], Sung Chul Shin[a], Sang-Joon Hwang[b], Dae-Yeon Cho[b], Sun Ho Cha[c], Si Kyu Lim[c], Hyun Gyu Park[a]. ([a]Department of Chemical & Biomolecular Engineering (BK 21 program), KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea, [b]LabGenomics Clinical Research Institute, LabGenomics Co., Ltd., 1575-11 Seocho 3-dong, Seocho-gu, Seoul 137-874, Republic of Korea, [c]GenoTech Co., Ltd., 59-5 Jang-dong, Yuseong-gu, Daejeon 305-343, Republic of Korea). A Sexually Transmitted Disease (STD) DNA chip for the diagnosis of genitourinary infections. Biosensors and Bioelectronics, Volume 26(11) (2011): 4314-4319

The results of an investigation aimed at the development of a DNA chip for the detection of genitourinary infections are described. Through analysis of over 35,000 clinical cases, 14 pathogens which are most abundantly found among Koreans were selected and candidate sequences for capture probes were accordingly chosen by considering their sequences and β-globin house-keeping gene. Among this group, the most suitable capture probe sequences were selected by employing repeated chip tests in which they are immobilized on a glass chip by using a recently developed novel gold nanoparticles-based method. A multiplex PCR method was established to generate fluorescence-labeled sequences for all 14 pathogens along with the β-globin gene. By using optimized hybridization conditions, the final chip was constructed and employed to diagnose reliably both single and multiple infections in clinical human samples for 14 target pathogens. The results show that the novel chip methodology serves as a highly reliable and convenient tool for the diagnosis of Sexually Transmitted Diseases (STDs). Furthermore, this study has its great significance in that it demonstrates the entire process from statistical analysis of a large number of clinical cases to the final development of STD DNA chip just ready to be applied or commercialized in the clinical diagnostic field.

Keywords: Genitourinary infection; STD pathogen; DNA chip; Multiplex PCR

Xin Ting Zheng[b, c], Weihua Hu[a, b, c], Houxiao Wang[d], Hongbin Yang[b, c], Wei Zhou[d], Chang Ming Li[a, b, c]. ([a]Institute for Clean Energy & Advanced Materials, Southwest University, Chongqing 400715, PR China, [b]School of Chemical and Biomedical Engineering, Nanyang Technological University, 70 Nanyang Drive, Singapore 637457, Singapore, [c]Center for Advanced Bionanosystems, Nanyang Technological University, 70 Nanyang Drive, Singapore 637457, Singapore, [d]School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore). Bifunctional electro-optical nanoprobe to real-time detect local biochemical processes in single cells). Biosensors and Bioelectronics, Volume 26(11) (2011): 4484-4490

A bifunctional electro-optical nanoprobe with integrated nanoring electrode and optical nanotip was fabricated and investigated to simultaneously detect both electrical and optical signals in real-time with high spatial resolution. Concurrent measurements of the oxidant generation and the intracellular antioxidant levels in single cells correlate the stronger oxidant generation with an altered initial antioxidant response in the breast cancer cells in comparison to the normal ones suggesting that the cell malignancy is associated with the strength of oxidative stress, and the higher antioxidant level may be the cause of the drug resistance. While the optical detection indicates the fluctuation of the intracellular redox homeostasis, the chronoamperometric signals allow quantitative real-time detection of the H_2O_2 release and decay. Furthermore, the nanoscale probe enables localized simultaneous detections thus discovering that activated enzymes responsible for the oxidative stress target at specific membrane regions. This method promises applications in study of the dynamics of important physiological processes, and provides the opportunity to unravel the interplay of various signaling pathways.

Keywords: Nanoprobe; Simultaneous detection; Electrochemistry; Fluorescence; Reactive oxygen species

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University, West Lafayette, IN 47907, United States, [c]School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN 47907, United States). Au nanospheres and nanorods for enzyme-free electrochemical biosensor applications. Biosensors and Bioelectronics, Volume 26(11) (2011): 4514-4519

Au nanocrystals with different morphologies were prepared and used for enzyme-free electrochemical biosensor applications. To investigate the electrocatalytic properties of Au nanocrystals as a function on their morphologies, Au nanocrystals, Au nanospheres (NSs) on silica, Au NSs, and Au nanorods (NRs) with aspect ratios of 1:3 and 1:5, were coated on the screen printed electrodes and further measure the amperometic responses to hydrogen peroxide via three-electrode system. The electrodes modified with Au nanocrystals showed biosensing properties without any enzyme being attached or immobilized at their surface. The hydrogen peroxide detection limits of the biosensors with Au NSs, Au NRs (1:3), and Au NRs (1:5) were 6.48, 8.65, and 9.38 μM (S/N = 3), respectively. The biosensors with Au NSs, Au NRs (1:3), and Au NRs (1:5) showed the sensitivities of 11.13, 54.53, and 58.51 μA/mM, respectively. These results indicate that morphologies of Au nanocrystals significantly influence the sensitivity of the biosensors. In addition, the enzyme-free biosensors with Au nanocrystals were stable for 2 months. Au nanocrystal-based enzyme-free system, which is proposed in this study, can be used as a platform for various electrochemical biosensors.

Keywords: Au nanospheres; Nanorods; Biosensors; Electrocatalyst; Enzyme-free

Edith Torres-Chavolla, Evangelyn C. Alocilja. (Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI 48824, USA). Nanoparticle based DNA biosensor for tuberculosis detection using thermophilic helicase-dependent isothermal amplification. Biosensors and Bioelectronics, Volume 26(11) (2011): 4614-4618

The present study describes the development of a DNA based biosensor to detect *Mycobacterium tuberculosis* using thermophilic helicase-dependent isothermal amplification (tHDA) and dextrin coated gold nanoparticles (AuNPs) as electrochemical reporter. The biosensor is composed of gold nanoparticles (AuNPs) and amine-terminated magnetic particles (MPs) each functionalized with a different DNA probe that specifically hybridize with opposite ends of a fragment within the *IS6110* gene, which is *M. tuberculosis* complex (MTC) specific. After hybridization, the formed complex (MP-target-AuNP) is magnetically separated from the solution and the AuNPs are electrochemically detected on a screen printed carbon electrode (SPCE) chip. The obtained detection limit is 0.01 ng/μl of isothermally amplified target (105 bp). This biosensor system can be potentially implemented in peripheral laboratories with the use of a portable, handheld potentiostat.

Keywords: Dextrin coated gold nanoparticles; Tuberculosis; Isothermal DNA amplification; Electrochemical biosensor

Kehua Xu, Huachao Chen, Huixia Wang, Jiangwei Tian, Jing Li, Qingling Li, Na Li, Bo Tang. (College of Chemistry, Chemical Engineering and Materials Science, Key Laboratory of Molecular and Nano Probes, Engineering Research Center of Pesticide and Medicine Intermediate Clean Production, Ministry of Education, Shandong Provincial Key Laboratory of Clean Production of Fine Chemicals, Shandong Normal University, Jinan 250014, PR China). A nanoprobe for nonprotein thiols based on assembling of QDs and 4-

amino-2,2,6,6-tetramethylpiperidine oxide. Biosensors and Bioelectronics, Volume 26(11) (2011): 4632-4636

A new fluorescent nanoprobe, 4-amino-2,2,6,6-tetramethylpiperidine oxide (AT)-functionalized CdTe quantum dots (QDs–AT), was synthesized, for selective detection of nonprotein thiols based on electron transfer (ET). In the presence of nonprotein thiols, the nitroxide radicals in QDs–AT were converted to hydroxylamines, resulting in the fluorescence recovery of the quenched QDs. The detection mechanism of the probe was investigated using Rh-Se-2 probe. The nanoprobe has high sensitivity toward glutathione (GSH) with a detection limit of 7.1×10^{-8} M. The fluorescent imaging of living cells showed that QDs–AT could distinguish the concentration differences of GSH in HL-7702 and HepG2 cells.

Keywords: Fluorescent probe; Nonprotein thiol; Quantum dots; Cell imaging

Bioengineering

Jiao Zhao[1], Timothy D. Scheibe[2], R. Mahadevan[1,3,*]. Model-based analysis of the role of biological, hydrological and geochemical factors affecting uranium bioremediation. Biotechnology and Bioengineering, Volume 108(7) (2011): 1537–1548

Uranium contamination is a serious concern at several sites motivating the development of novel treatment strategies such as the *Geobacter*-mediated reductive immobilization of uranium. However, this bioremediation strategy has not yet been optimized for the sustained uranium removal. While several reactive-transport models have been developed to represent *Geobacter*-mediated bioremediation of uranium, these models often lack the detailed quantitative description of the microbial process (e.g., biomass buildup in both groundwater and sediments, electron transport system, etc.) and the interaction between biogeochemical and hydrological process. In this study, a novel multi-scale model was developed by integrating our recent model on electron capacitance of *Geobacter* (Zhao et al., 2010) with a comprehensive simulator of coupled fluid flow, hydrologic transport, heat transfer, and biogeochemical reactions. This mechanistic reactive-transport model accurately reproduces the experimental data for the bioremediation of uranium with acetate amendment. We subsequently performed global sensitivity analysis with the reactive-transport model in order to identify the main sources of prediction uncertainty caused by synergistic effects of biological, geochemical, and hydrological processes. The proposed approach successfully captured significant contributing factors across time and space, thereby improving the structure and parameterization of the comprehensive reactive-transport model. The global sensitivity analysis also provides a potentially useful tool to evaluate uranium bioremediation strategy. The simulations suggest that under difficult environments (e.g., highly contaminated with U(VI) at a high migration rate of solutes), the efficiency of uranium removal can be improved by adding *Geobacter* species to the contaminated site (bioaugmentation) in conjunction with the addition of electron donor (biostimulation). The simulations also highlight the interactive effect of initial cell concentration and flow rate on U(VI) reduction.

Keywords: bioremediation of uranium; electron capacitance; ***Geobacter***; global sensitivity analysis; reactive-transport model

Adriano Pinto Mariano[1,2], Nasib Qureshi[3], Rubens Maciel Filho[2], Thaddeus Chukwuemeka Ezeji[1,*]. Bioproduction of butanol in bioreactors: New insights from simultaneous in situ butanol recovery to eliminate product toxicity[†]. Biotechnology and Bioengineering, Volume 108(8) (2011): 1757–1765

Simultaneous acetone butanol ethanol (ABE) fermentation by *Clostridium beijerinckii* P260 and in situ product recovery was investigated using a vacuum process operated in two modes: continuous and intermittent. Integrated batch fermentations and ABE recovery were conducted at 37°C using a 14-L bioreactor (7.0L fermentation volume) containing initial substrate (glucose) concentration of 60g/L. The bioreactor was connected in series with a condensation system and vacuum pump. Vacuum was applied continuously or intermittently with 1.5h vacuum sessions separated by 4, 6, and 8 h intervals. A control ABE fermentation experiment was characterized by incomplete glucose utilization due to butanol toxicity to *C. beijerinckii* P260, while fermentation coupled with in situ recovery by both continuous and intermittent vacuum modes resulted in complete utilization of glucose, greater productivity, improved cell growth, and concentrated recovered ABE stream. These results demonstrate that vacuum technology can be applied to integrated ABE fermentation and recovery even though the boiling point of butanol is greater than that of water. Biotechnol.

Keywords: butanol; ABE; *Clostridium beijerinckii* P260; vacuum fermentation; integrated process

Yang Jiang, Leonie Marang, Robbert Kleerebezem, Gerard Muyzer, Mark C.M. van Loosdrecht. Polyhydroxybutyrate production from lactate using a mixed microbial culture[†]. Biotechnology and Bioengineering, Volume 108(9) (2011): 2022–2035

In this study we investigated the use of lactate and a lactate/acetate mixture for enrichment of poly-3-hydroxybutyrate (PHB) producing mixed cultures. The mixed cultures were enriched in sequencing batch reactors (SBR) that established a feast–famine regime. The SBRs were operated under conditions that were previously shown to enable enrichment of a superior PHB producing strain on acetate (i.e., 12 h cycle length, 1 day SRT and 30°C). Two new mixed cultures were eventually enriched from activated sludge. The mixed culture enriched on lactate was dominated by a novel gammaproteobacterium. This enrichment can accumulate over 90wt% PHB within 6h, which is currently the best result reported for a bacterial culture in terms of the final PHB content and the biomass specific PHB production rate. The second mixed culture enriched on a mixture of acetate and lactate can produce up to 84wt% PHB in just over 8h. The predominant bacterial species in this culture were *Plasticicumulans acidivorans* and *Thauera selenatis*, which have both been reported to accumulate large amounts of PHB. The data suggest that *P. acidivorans* is a specialist on acetate conversion, whereas *Thauera* sp. is a specialist on lactate conversion. The main conclusion of this work is that the use of different substrates has a direct impact on microbial composition, but has no significant effect on the functionality of PHB production process.

Keywords: polyhydroxybutyrate; mixed microbial culture; lactate; microbial diversity; selective pressure

Haiying Tang, Meng Chen, M.E.D. Garcia, Nadia Abunasser, K.Y. Simon Ng, Steven O. Salley. Culture of microalgae *Chlorella minutissima* for biodiesel feedstock production. Biotechnology and Bioengineering, Volume 108(10) (2011): 2280–2287

Microalgae are among the most promising of non-food based biomass fuel feedstock alternatives. Algal biofuels production is challenged by limited oil content, growth rate, and economical cultivation. To develop the optimum cultivation conditions for increasing biofuels feedstock production, the effect of light source, light intensity, photoperiod, and nitrogen starvation on the growth rate, cell density, and lipid content of *Chlorella minutissima* were studied. The fatty acid content and composition of *Chlorella minutissima* were also investigated under the above conditions. Fluorescent lights were more effective than red or white light-emitting diodes for algal growth. Increasing light intensity resulted in more rapid algal growth, while increasing the period of light also significantly increased biomass productivity. Our results showed that the lipid and triacylglycerol content were increased under N starvation conditions. Thus, a two-phase strategy with an initial nutrient-sufficient reactor followed by a nutrient deprivation strategy could likely balance the desire for rapid and high biomass generation (124mg/L) with a high oil content (50%) of *Chlorella minutissima* to maximize the total amount of oil produced for biodiesel production. Moreover, methyl palmitate (C16:0), methyl oleate (C18:1), methyl linoleate (C18:2), and methyl linolenate (C18:3) are the major components of *Chlorella minutissima* derived FAME, and choice of light source, intensity, and N starvation impacted the FAME composition of *Chlorella minutissima*. The optimized cultivation conditions resulted in higher growth rate, cell density, and oil content, making *Chlorella minutissima* a potentially suitable organism for biodiesel feedstock production.

Keywords: microalgae; *Chlorella minutissima*; algae cultivation; biodiesel production

Lin Ye, Tong Zhang. Ammonia-oxidizing bacteria dominates over ammonia-oxidizing archaea in a saline nitrification reactor under low DO and high nitrogen loading. Biotechnology and Bioengineering, Volume 108(11) (2011): 2544–2552

A continuous nitrification reactor treating saline wastewater was operated for almost 1 year under low dissolved oxygen (DO) levels (0.15–0.5mg/L) and high nitrogen loadings (0.26–0.52kg-N/(m^3 day)) in four phases. The diversity and abundance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were analyzed by cloning, terminal restriction fragment length polymorphism (T-RFLP) and quantitative polymerase chain reaction (qPCR). The results showed that there were only one dominant AOA species and one dominant AOB species in the reactor in all of the four experimental phases. The *amo*A gene of the dominant AOA only had a similarity of 89.3% with the cultured AOA species *Nitrosopumilus maritimus* SCM1. All of the AOB species detected in the reactor belong to *Nitrosomonas* genus and it was found that the AOB populations changed with the ammonium loadings and DO levels. The abundance of AOB in the reactor was 40 times larger than that of AOA, and the ratio of AOB to AOA increased significantly up to 2,000 to 4,000 with the increase of ammonium loading, indicating that AOB are much more competitive than AOA in high ammonium environments and probably AOA play a less important role than AOB in the nitrification reactors.

Keywords: ammonia-oxidizing archaea; ammonia-oxidizing bacteria; nitrification reactor; saline wastewater

Jenni Rahikainen[1], Saara Mikander[1], Kaisa Marjamaa[1], Tarja Tamminen[1], Angelos Lappas[2], Liisa Viikari[3], Kristiina Kruus[1,*]. Inhibition of enzymatic hydrolysis by residual lignins from softwood—study of enzyme binding and inactivation on lignin-rich surface. Biotechnology and Bioengineering, Volume 108(12) (2011): 2823–2834

Lignin-derived inhibition is a major obstacle restricting the enzymatic hydrolysis of cell wall polysaccharides especially with softwood lignocellulosics. Enzyme adsorption on lignin is suggested to contribute to the inhibitory effect of lignin. The interaction of cellulases with softwood lignin was studied in the present work with commercial *Trichoderma reesei* cellulases (Celluclast) and lignin-rich residues isolated from steam pretreated softwood (SPS) by enzymatic and acid hydrolysis. Both lignin preparations inhibited the hydrolysis of microcrystalline cellulose (Avicel) and adsorbed the major cellulases present in the commercial cellulase mixture. The adsorption phenomenon was studied at low temperature (4°C) and at the typical hydrolysis temperature (45°C) by following activities of free and lignin-bound enzymes. Severe inactivation of the lignin-bound enzymes was observed at 45°C, however at 4°C the enzymes retained well their activity. Furthermore, SDS–PAGE analysis of the lignin-bound enzymes indicated that very strong interactions form between the residue and the enzymes at 45°C, because the enzymes were not released from the residue in the electrophoresis. These results suggest that heat-induced denaturation may take place on the surface of softwood lignin at the hydrolysis temperature.

Keywords: lignocellulose; softwood lignin; enzymatic hydrolysis; cellulase; cellulase adsorption; *Trichoderma reesei*; denaturation

S. Simon[1], H. J. Krause[2], C. Weber[2], W. Peukert[1,*]. Physical degradation of proteins in well-defined fluid flows studied within a four-roll apparatus. Biotechnology and Bioengineering, Volume 108(12) (2011): 2914–2922

In most applications of biotechnology and downstream processing proteins are exposed to fluid stresses in various flow configurations which often lead to the formation of unwanted protein aggregates. In this paper we present physical degradation experiments for proteins under well-defined flow conditions in a four-roll apparatus. The flow field was characterized numerically by computational fluid dynamics (CFD) and experimentally by particle image velocimetry (PIV). The local shear strain rate as well as the local shear and elongation rate was used to characterize the hydrodynamic stress environment acting on the proteins. Lysozyme was used as a model protein and subjected to well-defined fluid stresses in high and low stress environment. By using in situ turbidity measurements during stressing the aggregate formation was monitored directly in the fluid flow. An increase in absorbance at 350 nm was attributed to a higher content of visible particles (>1μm). In addition to lysozyme, the formation of aggregates was confirmed for two larger proteins (bovine serum albumin and alcohol dehydrogenase). Thus, the presented experimental setup is a helpful tool to monitor flow-induced protein aggregation with high reproducibility. For instance, screening experiments for formulation development of biopharmaceuticals for fill and finish operations can be performed in the lab-scale in a short time-period if the stress distributions in the application are transferred and applied in the four-roll mill.

Keywords: denaturation; elongation; monitoring of aggregation; protein adsorption; protein aggregation; shear

Mona Abo-Hashesh[a], Ruofan Wang[a, b], Patrick C. Hallenbeck[a]. ([a]Département de Microbiologie et Immunologie, Université de Montréal, CP 6128 Succursale Centre-ville, Montréal, Québec, Canada H3C 3J7, [b]Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China). Metabolic engineering in dark fermentative hydrogen production; theory and practice. Bioresource Technology, Volume 102(18) (2011): 8414-8422

Dark fermentation is an attractive option for hydrogen production since it could use already existing reactor technology and readily available substrates without requiring a direct input of solar energy. However, a number of improvements are required before the rates and yields of such a process approach those required for a practical process. Among the options for achieving the required advances, metabolic engineering offers some powerful tools for remodeling microbes to increase product production rates and molar yields. Here we review the current metabolic engineering tool box that is available, discuss the current status of engineering efforts as applied to dark hydrogen production, and suggest areas for future improvements.

Keywords: Metabolic engineering; Biohydrogen; Hydrogenase; Anaerobic metabolism

Agricultural Biotechnology

Zinatul A. Zainol[1,2], Latifah Amin[1,3]*, Noor Sharizad Rusly[1,3], Hasrizul Hashim[1,3], Nik Marzuki Sidik[1,4], Frank Akpoviri[1,2] and Rosli Ramli[5]. ([1]Social Impact of Biotechnology Development in Malaysia (SIMBIO) Research Group, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia, [2]Faculty of Law, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia, [3]Centre for General Studies, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia, [4]Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia, [5]Institute of Biological Sciences, Universiti Malaya, 50603 Kuala Lumpur. *Corresponding author. E-mail: nilam@ukm.my. Tel: +0192726182 Fax: +0689252976). The need for biosafety regulation in developing countries: Benefits and controversies. African Journal of Biotechnology Vol. 10 (58)(2011): 12389-1239

Nowadays, the rapid development of biotechnology has become a main concern for a larger part of the world. It has become one of the most promising fields which guarantee returns to businesses and offers benefits to the society. When dealing with biotechnology, the first issue that comes to mind is the safeness of the technology from tip to toe, that is, the safeness of the products of biotechnology, how they can be used on human beings and animal, and their effects on the environment. The objective of this paper is to assess the needs and adequacy of the regulation in developing countries compared to the developed countries. In order to address these concerns, governments have adopted appropriate regulations to ensure the safety of the biotechnology products, and to protect not just human but the environment universally. This paper will discuss those regulations, especially as adopted by developing countries along with

their implications. It is hoped that the paper will recover the lack of the regulations in relation to developed country.

Keywords: Biotechnology, biosafety, developing countries, benefits, risks and controversies.

Abbreviations: GMOs, Genetically modified organisms; **GM,** genetically modified; **NRE,** Natural Resources and Environment; **NBB,** National Biosafety Board.

Latifah Amin[1,2]*, Zinatul Ashiqin Zainol[1,3], Noor Sharizad Rusly[1,2], Frank Akpoviri[1,3] and Nik Marzuki Sidik[1,4]. ([1]Social Impact of Biotechnology Development in Malaysia Research Group (SIMBIO), Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia, [2]Centre for General Studies, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia, [3]Faculty of Law, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia, [4]Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia. *Corresponding author. E-mail: nilam@ukm.my. Tel: +0192726182. Fax: +0689252976). Risk assessment of genetically modified organisms (GMOs). African Journal of Biotechnology Vol. 10 (58)(2011): 12418-12424

Risk assessment is a procedure normally carried out prior to decision-making on the release of genetically modified organisms (GMOs) into the environment. Most countries dealing with the release of GMOs have appropriate guidelines. The objectives of this paper are to critically examine the risk assessment provisions of the Malaysian Biosafety Act (2007), and to compare it with several risk assessment provisions in the Cartagena Protocol, as well as regulations in developed countries. There are inadequacies in the risk assessment provisions of the Malaysian Biosafety Act (2007 Act), compared to those of the Cartagena Protocol, as well as those found in European Commission Directives. Although the central objective of the 2007 Act was similar to the Cartagena Protocol, the Act was found to be very basic with only a brief provision on risk assessment and there is no specific coverage on the socio-economic and ethical aspects as well as the precautionary approach. It is hoped that these inadequacies will be improved upon, in order to bring the Malaysian Biosafety Act closer to the level seen in the biosafety laws of more developed countries and to ensure adequate level of protection for the Malaysian people against any adverse effects of GMOs and products.

Keywords: Risk assessment, biosafety, Malaysian Biosafety Act 2007, genetically modified organisms (GMOs).

Abbreviations: GMOs, genetically modified organisms; **GM,** genetically modified; **EU,** European Union; **LMOs,** living modified organisms; **NBB,** National Biosafety Board; **DNA,** Deoxyribonucleic acid.

Bioenergy

Xiongjun Shao, Babu Raman, Mingjun Zhu, Jonathan R. Mielenz, Steven D. Brown, Adam M. Guss and Lee R. Lynd. Mutant selection and phenotypic and genetic characterization of ethanol-tolerant strains of *Clostridium thermocellum*. Applied Microbiology and Biotechnology, Volume 92(3) (2011): 641-652

Clostridium thermocellum is a model microorganism for converting cellulosic biomass into fuels and chemicals via consolidated bioprocessing. One of the challenges for industrial application of this organism is its low ethanol tolerance, typically 1–2% (*w*/*v*) in wild-type strains. In this study, we report the development and characterization of mutant *C. thermocellum* strains that can grow in the presence of high ethanol concentrations. Starting from a single colony, wild-type *C. thermocellum* ATCC 27405 was sub-cultured and adapted for growth in up to 50 g/L ethanol using either cellobiose or crystalline cellulose as the growth substrate. Both the adapted strains retained their ability to grow on either substrate and displayed a higher growth rate and biomass yield than the wild-type strain in the absence of ethanol. With added ethanol in the media, the mutant strains displayed an inverse correlation between ethanol concentration and growth rate or biomass yield. Genome sequencing revealed six common mutations in the two ethanol-tolerant strains including an alcohol dehydrogenase gene and genes involved in arginine/pyrimidine biosynthetic pathway. The potential role of these mutations in ethanol tolerance phenotype is discussed.

Keywords: *Clostridium thermocellum* – Ethanol tolerance – Genome sequencing – Strain adaptation – Mutations

Claudio Muñoz, Jaime Baeza, Juanita Freer and Regis Teixeira Mendonça. Bioethanol production from tension and opposite wood of *Eucalyptus globulus* using organosolv pretreatment and simultaneous saccharification and fermentation. Journal of Industrial Microbiology & Biotechnology, Volume 38(11) (2011): 1861-1866

During tree growth, hardwoods can initiate the formation of tension wood, which is a strongly stressed wood on the upper side of the stem and branches. In *Eucalyptus globulus*, tension wood presents wider and thicker cell walls with low lignin, similar glucan and high xylan content, as compared to opposite wood. In this work, tension and opposite wood of *E. globulus* trees were separated and evaluated for the production of bioethanol using ethanol/water delignification as pretreatment followed by simultaneous saccharification and fermentation (SSF). Low residual lignin and high glucan retention was obtained in organosolv pulps of tension wood as compared to pulps from opposite wood at the same H-factor of reaction. The faster delignification was associated with the low lignin content in tension wood, which was 15% lower than in opposite wood. Organosolv pulps obtained at low and high H-factor (3,900 and 12,500, respectively) were saccharified by cellulases resulting in glucan-to-glucose yields up to 69 and 77%, respectively. SSF of the pulps resulted in bioethanol yields up to 35 g/l that corresponded to 85–95% of the maximum theoretical yield on wood basis, considering 51% the yield of glucose to ethanol conversion in fermentation, which could be considered a very satisfactory result

compared to previous studies on the conversion of organosolv pulps from hardwoods to bioethanol. Both tension and opposite wood of *E. globulus* were suitable raw materials for organosolv pretreatment and bioethanol production with high conversion yields.

Keywords: Bioethanol – *Eucalyptus globulus* – Tension wood – Organosolv – SSF

Laura Pinilla, Rodrigo Torres and Claudia Ortiz. Bioethanol production in batch mode by a native strain of *Zymomonas mobilis.* World Journal of Microbiology and Biotechnology, Volume 27(11)(2011): 2521-2528

Two wild strains of *Zymomonas mobilis* were isolated (named as ML1 and ML2) from sugar cane molasses obtained from different farms of Santander, Colombia. Initially, selection of the best ethanol-producer strains was carried out using ethanol production parameters obtained with a commercial strain *Z. mobilis* DSM 3580. Three isolated strains were cultivated in a culture medium containing yeast extract, peptone, glucose and salts, at pH 6 and 32°C with stirring rate of 65 rpm during 62 h. The best results of ethanol production were obtained with the native strain ML1, reaching a maximum ethanol concentration of 79.78 g l^{-1}. ML1 and ML2 strains were identified as *Z. mobilis*, according to the morphology, biochemical tests and molecular characterization by PCR of specific DNA sequences from *Z. mobilis*. Subsequently, the effect of different nitrogen sources on production of ethanol was evaluated. The best results were obtained using urea at a 0.73 g/l. In this case, maximum concentration of ethanol was 83.81 g l^{-1}, with kinetic parameters of yield of ethanol on biomass ($Y_{P/X)} = 69.01$(g g^{-1}), maximum volumetric productivity of ethanol (Qp_{max}) = 2.28 (g l^{-1} h^{-1}), specific productivity of ethanol (q_P) = 3.54 (h^{-1}) and specific growth rate (μ) = 0.12 h^{-1}. Finally, we studied the effect of different culture conditions (pH, temperature, stirring, C/N ratio) with a Placket-Burman′s experimental design. This optimization indicated that the most significant variables were temperature and stirring. In the best culture conditions a significant increase in all variables of response was achieved, reaching a maximum ethanol concentration of 93.55 g l^{-1}.

Keywords: Bioethanol – *Zymomonas mobilis* – Biofuels – Batch fermentation

Leonardo Lépiz-Aguilar, Carlos E. Rodríguez-Rodríguez, María Laura Arias, Giselle Lutz and William Ulate. Butanol production by *Clostridium beijerinckii* BA101 using cassava flour as fermentation substrate: enzymatic versus chemical pretreatments. World Journal of Microbiology and Biotechnology, Volume 27(8) (2011): 1933-1939

Cassava flour (CF), a cost-effective source of starch, was employed as a substrate for successful acetone-butanol-ethanol (ABE) production by batch-fermentation with *Clostridium beijerinckii*. The effect of temperature, initial concentration of CF and chemical/enzymatic hydrolysis were studied in a 2^3 factorial design. Results revealed that temperature and initial concentration of substrate exert a significant effect on ABE production, as well as interactions of temperature with the other variables. Solvent production was maximized when working at 40°C, 60 g l^{-1} CF and enzymatic pretreatment. An average of 31.38 g l^{-1} ABE was produced after 96 h, with a productivity of 0.33 g l^{-1} h^{-1}. A posterior randomized block design (3 × 2) showed that enzymatic hydrolysis (with saccharification periods of 6 h at 60°C) enhances both reducing sugar and solvent production if compared to chemical pretreatments. Average ABE production in this case was 27.28 g l^{-1}, with a productivity of 0.28 g l^{-1} h^{-1}. Results suggest that CF may be a suitable substrate for industrial ABE production.

Keywords: Cassava – Butanol – ABE fermentation – Hydrolysis – *Clostridium beijerinckii*

Leonardo Lépiz-Aguilar, Carlos E. Rodríguez-Rodríguez, María Laura Arias, Giselle Lutz and William Ulate. Butanol production by *Clostridium beijerinckii* BA101 using cassava flour as fermentation substrate: enzymatic versus chemical pretreatments. World Journal of Microbiology and Biotechnology, Volume 27(8) (2011): 1933-1939

Cassava flour (CF), a cost-effective source of starch, was employed as a substrate for successful acetone-butanol-ethanol (ABE) production by batch-fermentation with *Clostridium beijerinckii*. The effect of temperature, initial concentration of CF and chemical/enzymatic hydrolysis were studied in a 2^3 factorial design. Results revealed that temperature and initial concentration of substrate exert a significant effect on ABE production, as well as interactions of temperature with the other variables. Solvent production was maximized when working at 40°C, 60 g l^{-1} CF and enzymatic pretreatment. An average of 31.38 g l^{-1} ABE was produced after 96 h, with a productivity of 0.33 g l^{-1} h^{-1}. A posterior randomized block design (3 × 2) showed that enzymatic hydrolysis (with saccharification periods of 6 h at 60°C) enhances both reducing sugar and solvent production if compared to chemical pretreatments. Average ABE production in this case was 27.28 g l^{-1}, with a productivity of 0.28 g l^{-1} h^{-1}. Results suggest that CF may be a suitable substrate for industrial ABE production.

Keywords: Cassava – Butanol – ABE fermentation – Hydrolysis – *Clostridium beijerinckii*

Ackmez Mudhoo[1], Tânia Forster-Carneiro[2], Antoni Sánchez[3]. ([1]*Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Mauritius, Réduit, Mauritius,* [2]*Department Chemical Engineering, Food Technology and Environmental Technologies, Faculty of Sea Sciences and Environmental Sciences, University of Cadiz, Campus Rio San Pedro s/n, 11510-Puerto Real, Cádiz, Spain,* [3]*Departament d'Enginyeria Química, Escola Tècnica Superior d'Enginyeria, Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain. Address for Correspondence*: Ackmez Mudhoo, 4, Rajiv Gandhi Street, Maurel Road, Rivière du Rempart, Mauritius. Tel.: +230 4037772; Fax: +230 4657144; E-mail: ackmezchem@yahoo.co.uk). Biohydrogen production and bioprocess enhancement: A review. Crit Rev Biotechnol. 2011, Vol. 31(3)(2011): 250-263

This paper provides an overview of the recent advances and trends in research in the biological production of hydrogen (biohydrogen). Hydrogen from both fossil and renewable biomass resources is a sustainable source of energy that is not limited and of different applications. The most commonly used techniques of biohydrogen production, including direct biophotolysis, indirect biophotolysis, photo-fermentation and dark-fermentation, conventional or "modern" techniques are examined in this review. The main limitations inherent to biochemical reactions for hydrogen production and design are the constraints in reactor configuration which influence biohydrogen production, and these have been identified. Thereafter, physical pretreatments, modifications in the design of reactors, and biochemical and genetic manipulation techniques that are being developed to enhance the overall rates and yields of biohydrogen generation are revisited.

Keywords: Hydrogen, biofuel, biomass, green chemistry, pretreatment, reactors, bacterial strains, genetic manipulation

Yangmin Gong and Mulan Jiang. Biodiesel production with microalgae as feedstock: from strains to biodiesel. Biotechnology Letters, Volume 33(7) (2011): 1269-1284

Due to negative environmental influence and limited availability, petroleum-derived fuels need to be replaced by renewable biofuels. Biodiesel has attracted intensive attention as an important biofuel. Microalgae have numerous advantages for biodiesel production over many terrestrial plants. There are a series of consecutive processes for biodiesel production with microalgae as feedstock, including selection of adequate microalgal strains, mass culture, cell harvesting, oil extraction and transesterification. To reduce the overall production cost, technology development and process optimization are necessary. Genetic engineering also plays an important role in manipulating lipid biosynthesis in microalgae. Many approaches, such as sequestering carbon dioxide from industrial plants for the carbon source, using wastewater for the nutrient supply, and maximizing the values of by-products, have shown a potential for cost reduction. This review provides a brief overview of the process of biodiesel production with microalgae as feedstock. The methods associated with this process (e.g. lipid determination, mass culture, oil extraction) are also compared and discussed.

Keywords: Biodiesel – Lipid – Microalgae – Transesterification

Zheng Jiang[1]*, Li Zhongbao[1]*, Lu Yinghua[2], Tang Xuemin[1], Lu Bin[1], Li Yuanyue[1], Lu Zhiqiang[1], Lin Yaojiang[3] and Zhou Jixin[3]. ([1]Key Laboratory of Science and Technology for Aquaculture and Food Safety in Fujian Province University, Fisheries College of Jimei University, Xiamen 361021, China, [2]Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China, [3]Shuntian Environmental Technology Limited Company, Longyan 364000, China. *Corresponding authors. E-mail: li2000zb@gmail.com, zhengjiang618@gmail.com). Cultivation of the microalga, *Chlorella pyrenoidosa*, in biogas wastewater. African Journal of Biotechnology, Vol. 10 (61)(2011): 13115-13120

Biogas wastewater is always a problem as a result of its extremely high concentrations of nitrogen and phosphorus, which is the main reason for the eutrophication of the surrounding water. The microalga, *Chlorella pyrenoidosa*, can utilize the nitrogen and phosphorus in wastewater for its growth. Therefore, the microalga was introduced to be cultivated in the biogas wastewater, which could not only bioremediate the wastewater, but also produce plenty of the microalga biomass that could be used for the exploitation of fertilizers, feed additives and biofuels. This study showed that the microalga, *C. pyrenoidosa* could grow well in the biogas wastewater under optimal condition: initial cell density of 0.15 (OD_{680}), pH 8 and illumination intensity of 10000 LX. Under the optimal condition, the dry cell weight of the microalgae reached 0.1 g/L after cultivation in the wastewater for fourteen (14) days; in the meantime, the microalga also removed 71.8% of phosphorus, 100% of ammoniacal nitrogen (NH_4^+-N), 52.8% of nitrate nitrogen (NO_3-N) and 23.0% of nitrite nitrogen (NO_2-N) from the biogas wastewater, suggesting that the cultivation of *C. pyrenoidosa* in biogas wastewater would be efficient for the treatment of wastewater. This study also provided a low-cost way to produce the microalga and its relevant products.

Keywords: *Chlorella pyrenoidosa*, biogas wastewater, cultivation, phosphorus, nitrogen.

Deepa Shenoy, Anjali Pai, R.K. Vikas, H.S. Neeraja, J.S. Deeksha, Chetan Nayak, C. Vaman Rao. (Department of Biotechnology Engineering, NMAM Institute of Technology, Karkala-Mangalore road, Nitte, Karnataka 574110, India). A study on bioethanol production from cashew apple pulp and coffee pulp waste. Biomass and Bioenergy, Volume 35(10) (2011): 4107-4111

Bioethanol production from dry cashew apple pulp and coffee pulp was investigated. The pulp was digested with 2% sulfuric acid and subjected to high pressure (15 psi) cooking at 120 °C for 10 min followed by further 1 and a half hour pressure cooking at 90 °C to solubilize the pulp. Solubilized pulp was filtered and the debris on the filter paper was washed with minimum quantity of distilled water and then oven dried to find the weight of the insoluble lignin mass. Total sugar content in squeezed and dried cashew apple pulp (CAP), dry coffee pulp (DCP) and wet coffee pulp (WCP) was found to be 2.12, 1.62 and 0.62 g/100 ml of hydrolyzate. Reducing sugar content in squeezed CAP, DCP and WCP was found to be 0.14, 0.71and 0.23g/100 ml of hydrolyzate. Filtrate was neutralized with thick suspension of calcium hydroxide slurry until the pH reaches to 6.0. Neutralized slurry was kept at lab temperature overnight and the supernatant was decanted through filter paper. To 150 ml of filtrate yeast (*Saccharomyces creviciae*) was added at a concentration of 5.0 g/l concentration and subjected to fermentation for 48 h at 30 °C in a shaker incubator at 120 rpm. Ethanol content in the fermented broth was estimated by titrimetric and gas chromatographic method. Ethanol yield in the fermented broth was found to be 0.5, 0.46 and 0.46 g/g of sugar in squeezed CAP, DCP and WCP. Theoretical ethanol yield (Y_{max}%) of squeezed CAP, DCP and WCP was found to be 46, 9.35 and 40% respectively.

Keywords: Dry cashew apple pulp; Dry coffee pulp; Wet coffee pulp; Hydrolyzate; Sulfuric acid; Ethanol

Jegannathan Kenthorai Raman, Vanessa Foo Wang Ting, Ravindra Pogaku. (Centre of Materials and Minerals, Department of Chemical Engineering, School of Engineering and Information Technology, University Malaysia Sabah, 88999 Kota Kinabalu, Sabah, Malaysia). Life cycle assessment of biodiesel production using alkali, soluble and immobilized enzyme catalyst processes. Biomass and Bioenergy, Volume 35(10) (2011): 4221-4229

This study deals with the Life Cycle Assessment (LCA) of three different catalytic processes for biodiesel production. In the LCA study, a “cradle to gate” approach was adopted to estimate the environmental impact of different catalytic processes such as immobilized, soluble biocatalyst and alkali catalyst. The results revealed that, biodiesel production using immobilized biocatalyst has less environmental impact compared to alkali and soluble biocatalyst. The environmental impact of the immobilized biocatalyst depends on the reusability factor.

Keywords: Life cycle assessment; Biodiesel; Alkali catalyst; Biocatalyst; Encapsulation; Lipase

Jovana A. Grahovac, Jelena M. Dodić, Siniša N. Dodić, Stevan D. Popov, Aleksandar I. Jokić, Zoltan Z. Zavargo. (Faculty of Technology, Department of Biotechnology and Pharmaceutical Engineering, University of Novi Sad, Bul. cara Lazara 1, 21000 Novi Sad, Serbia) Optimization of bioethanol production from intermediates of sugar beet processing

by response surface methodology. Biomass and Bioenergy, Volume 35(10) (2011): 4290-4296

Bioethanol production in batch culture by free *Saccharomyces cerevisiae* cells from intermediates of sugar beet processing was investigated and compared with molasses, traditionally used substrate. The overall fermentation efficiency of different yeasts was examined and there is no statistical difference between them indicating that the criteria for yeast strain choice should be based on availability and cost-effective factor. When raw, thin and thick juices and molasses were used, under anaerobic conditions for 60 h, at the temperature of 30 °C and agitation rate 3.3 Hz, obtained ethanol contents were 82.87 $dm^3\ m^{-3}$, 80.31 $dm^3\ m^{-3}$, 80.70 $dm^3\ m^{-3}$ and 73.96 $dm^3\ m^{-3}$, respectively. The ethanol yield amounted 490 g kg^{-1} for media based on intermediates and 450 g kg^{-1} for mash prepared with molasses. Response surface methodology was used for description of ethanol production. By obtained mathematical model (second degree polynomial), optimal fermentation duration is estimated on 36 h for intermediates, while for molasses it reaches 50 h. Bioethanol production from intermediates of sugar beet processing is technically possible and enables higher effectiveness of process.

Keywords: Optimization; Bioethanol; Sugar beet; *Saccharomyces cerevisiae*; RSM

Ana Evangelista Marques[a], Ana Teresa Barbosa[b], Joana Jotta[b], Manuel Caldeira Coelho[c], Paula Tamagnini[d], Luísa Gouveia[a]. ([a]Laboratório Nacional de Energia e Geologia (LNEG), Unidade de Bioenergia, Estrada do Paço do Lumiar, 1649-038 Lisboa, Portugal, [b]Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal, [c]Laboratório Nacional de Energia e Geologia (LNEG), Unidade de Produção e Consumo Sustentável, Estrada do Paço do Lumiar, 1649-038 Lisboa, Portugal, [d]Instituto de Biologia Molecular e Celular, Faculdade de Ciências, Dept. Botânica, Universidade do Porto, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal). **Biohydrogen production by *Anabaena* sp. PCC 7120 wild-type and mutants under different conditions: Light, nickel, propane, carbon dioxide and nitrogen. Biomass and Bioenergy, Volume 35(10) (2011): 4426-4434**

Increasing awareness of environmental problems caused by the current use of fossil fuel-based energy, has led to the search for alternatives. Hydrogen is a good alternative and the cyanobacterium *Anabaena* sp. PCC 7120 is naturally able to produce molecular hydrogen, photosynthetically from water and light. However, this H_2 is rapidly consumed by the uptake hydrogenase.

This study evaluated the hydrogen production of *Anabaena* sp. PCC 7120 wild-type and mutants: *hupL*$^-$ (deficient in the uptake hydrogenase), *hoxH*$^-$ (deficient in the bidirectional hydrogenase) and *hupL*$^-$*/hoxH*$^-$ (deficient in both hydrogenases) on several experimental conditions, such as gas atmosphere (argon and propane with or without N_2 and/or CO_2 addition), light intensity (54 and 152 $\mu Em^{-2}s^{-1}$), light regime (continuous and light/dark cycles 16 h/8 h) and nickel concentrations in the culture medium.

In every assay, the *hupL*$^-$ and *hupL*$^-$*/hoxH*$^-$ mutants stood out over wild-type cells and the *hoxH*$^-$ mutant. Nevertheless, the *hupL*$^-$ mutant showed the best hydrogen production except in an argon atmosphere under 16 h light/8 h dark cycles at 54 $\mu Em^{-2}s^{-1}$ in the light period, with 1 μM of $NiCl_2$ supplementation in the culture medium, and under a propane atmosphere.

In all strains, higher light intensity leads to higher hydrogen production and if there is a daily 1% of CO_2 addition in the gas atmosphere, hydrogen production could increase 5.8 times, related to the great increase in heterocysts differentiation (5 times more, approximately), whereas nickel supplementation in the culture medium was not shown to increase hydrogen production. The daily incorporation of 1% of CO_2 plus 1% of N_2 did not affect positively hydrogen production rate.

Keywords: *Anabaena* sp. PCC 7120; Biohydrogen; *hoxH*$^-$ mutant; *hupL*$^-$ mutant; *hupL*$^-$*/hoxH*$^-$ mutant; Propane

Heungjo An[a], Wilbert E. Wilhelm[a], Stephen W. Searcy[b]. ([a]Department of Industrial and Systems Engineering, Texas A&M University, TAMUS 3131, College Station, TX 77843-3131, United States, [b]Department of Biological and Agricultural Engineering, Texas A&M University, TAMUS 2117, College Station, TX 77843-3131, United States). Biofuel and petroleum-based fuel supply chain research: A literature review. Biomass and Bioenergy, Volume 35(9) (2011): 3763-3774

During the last decade, countries around the world - especially the U.S., Brazil, and many in Europe - have worked to accelerate the commercialization of a biofuel industry. As pilot plant studies for the second-generation biofuel (e.g., cellulosic biofuel) currently seek to determine the most viable feedstocks and processing technologies, it is an opportune time to formulate operations research (OR) models of the biofuel supply chain (SC) so they might be used to implement the technologies that prove to be most promising. This paper provides a literature review of research on the biofuel SC. It classifies prior research according to decision time frame (i.e., strategic, tactical, operational, and integrated) as well as level in the supply chain (i.e., upstream, midstream, and downstream). In addition, it reviews related research on agri-products, which have some commonalities relative to harvesting and perishability; petroleum-based fuels, which have some commonalities related to distribution (some biofuels can be mixed with gasoline but others cannot); and generic supply chains, which provide some applicable modeling structures. Finally, this paper emphasizes unique needs to support decisions that integrate the farm with commercial levels (e.g., storage, pre-processing, refining, and distribution) and identifies fertile avenues for future research on the biofuel supply chain. OR models are needed to help assure the economic viability of the biofuel industry. They can be used by growers, processors, and distributors to design and manage an integrated system and by government to inform policies needed to stimulate the growth of the industry and, perhaps, subsidize it.

Keywords: Biofuel; Biomass; Petroleum-based fuel; Logistics; Supply chain; Operations research

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carbon dioxide sequestration from an integrated ethanol biorefinery in Iowa: A technical appraisal and economic feasibility evaluation. Biomass and Bioenergy, Volume 35(9) (2011): 3865-3876

Microalgae present some advantageous qualities for reducing carbon dioxide (CO_2) emissions from ethanol biorefineries. As photosynthetic organisms, microalgae utilize sunlight and CO_2 to generate biomass. By integrating large-scale microalgal cultivation with ethanol biorefineries, CO_2 sequestration can be coupled with the growth of algae, which can then be used as feedstock for biodiesel production. In this case study, a 50-mgy ethanol biorefinery in Iowa was evaluated as a candidate for this process. Theoretical projections for the amount of land needed to grow algae in raceway ponds and the oil yields of this operation were based on the amount of CO_2 from the ethanol plant. A practical algal productivity of 20 g m^{-2} d^{-1} would require over 2,000 acres of ponds for complete CO_2 abatement, but with an aggressive productivity of 40–60 g m^{-2} d^{-1}, a significant portion of the CO_2 could be consumed using less than 1,000 acres. Due to the cold temperatures in Iowa, a greenhouse covering and a method to recover waste heat from the biorefinery were devised. While an algal strain, such as *Chlorella vulgaris*, would be able to withstand some temperature fluctuations, it was concluded that this process is limited by the amount of available heat, which could maintain only 41 acres at 73 °F. Additional heating requirements result in a cost of 10–40 USD per gallon of algal oil, which is prohibitively expensive for biodiesel production, but could be profitable with the incorporation of high-value algal coproducts.

Keywords: Microalgae; Ethanol; Biodiesel; CO_2 sequestration; Midwest; Biorefinery

Umer Rashid[a, b], Farooq Anwar[a, c], Gerhard Knothe[d]. ([a]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad 38040, Pakistan, [b]Chemical Engineering Department, Universiti Technologi PETRONAS, Bandar Sari Iskandar, 31750 Tronoh, Perak, Malaysia, [c]Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, [d]United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL 61604, USA). Biodiesel from Milo (*Thespesia populnea* L.) seed oil. Biomass and Bioenergy, Volume 35(9) (2011): 4034-4039

There is a need to seek non-conventional seed oil sources for biodiesel production due to issues such as supply and availability as well as food versus fuel. In this context, Milo (*Thespesia populnea* L.) seed oil was investigated for the first time as a potential non-conventional feedstock for preparation of biodiesel. This is also the first report of a biodiesel fuel produced from a feedstock containing cyclic fatty acids as *T. populnea* contains 8,9-methylene-8-heptadecenoic (malvalic) and smaller amounts of two cyclopropane fatty acids besides greater amounts of linoleic, oleic and palmitic acids. The crude oil extracted from *T. populnea* seed was transesterified under standard conditions with sodium methoxide as catalyst. Biodiesel derived from *T. populnea* seed oil exhibited fuel properties of density 880 kg m^{-3}, kinematic viscosity 4.25 mm^2/s; cetane number 59.8; flash point 176 °C; cloud point 9 °C; pour point 8 °C; cold filter plugging point 9 °C; sulfur content 11 mg kg^{-1}; water content 150 mg kg^{-1}; ash content 15 mg kg^{-1}; and acid value as KOH 250 mg kg^{-1}. The oxidative stability of 2.91 h would require the use of antioxidants to meet specifications in standards. Generally, most results compared well with ASTM D6751 and EN 14214 specifications.

Keywords: Biodiesel; Fuel properties; Milo seed oil; *Thespesia populnea*; Transesterification

Thi Thai Yen Doan[a], Balasubramanian Sivaloganathan[b], Jeffrey Philip Obbard[a, b]. ([a]Division of Environmental Science and Engineering, Faculty of Engineering, National University of Singapore, E1A #02-19, 1 Engineering Drive 2, Singapore 117576, Singapore, [b]Tropical Marine Science Institute, National University of Singapore, S2S, 18 Kent Ridge Road, Singapore 119227, Singapore). Screening of marine microalgae for biodiesel feedstock. Biomass and Bioenergy, Volume 35(7) (2011): 2534-2544

Biodiesel production from microalgae lipids is increasingly regarded as a more sustainable and feasible alternative to conventional biodiesel feedstocks derived from terrestrial bioenergy crops. A total of ninety-six strains of marine microalgae, with an elevated biomass productivity and intracellular lipid content, were isolated from the coastal waters of Singapore using an automated flow cytometric cell-sorting technique. Cell sorting was based on the two-dimensional distribution of algal cells for red fluorescence (representing chlorophyll auto-fluorescence) against forward-light scatter (representing cell size) and red vs. green fluorescence. Twenty-one of the strains were further characterized with respect to cell growth rate, biomass concentration, lipid content (total and neutral lipid) and fatty acid profile. The growth rates of *Skeletonema costatum*, *Chaetoceros* and *Thalassiosira* species were greatest among the entire strains, but in terms of absolute lipid yield *Nannochloropsis* strains predominated. *Nannochloropsis* strains had a lipid content ranging from 39.4% to 44.9% of dry weight biomass. Transesterification of the lipids yielded 25–51% of fatty acid methyl ester (FAME) i.e. biodiesel, where total FAME content ranged between 11 and 21% of dry weight biomass. This study describes the microalgae screening process and demonstrates that *Nannochloropsis* is a promising species for biodiesel feedstock.

Keywords: Microalgae; Biodiesel; Flow cytometric cell sorting; Screening; Neutral lipid

Mairan Guigou[a], Claudia Lareo[a], Leticia Verónica Pérez[a], María Elena Lluberas[a], Daniel Vázquez[b], Mario Daniel Ferrari[a]. ([a]Depto. Bioingeniería, Facultad de Ingeniería, Universidad de la República, J. Herrera y Reissig 565, CP 11300 Montevideo, Uruguay, [b]Instituto Nacional de Investigaciones Agronómicas (INIA), La Estanzuela, Colonia, Uruguay). Bioethanol production from sweet sorghum: Evaluation of post-harvest treatments on sugar extraction and fermentation. Biomass and Bioenergy, Volume 35(7) (2011): 3058-3062

Three experimental sweet sorghum varieties (M81, Topper and Theis) and three post-harvest conditions were evaluated for ethanol production: juices extracted by milling were obtained from the whole plant, plant without panicle, and stalk (plant without panicle and leaves), respectively. A linear relationship was found between the total fermentable sugar concentrations and Brix degrees of the juices, which can predict the potential ethanol yield by field analytical tests. The juice extractability presented different behavior among the sweet sorghum varieties with respect to the treatments studied. However such treatments did not affect the level of sugar concentration of the juices obtained and the fermentation efficiency. Topper and Theis showed the best performance in terms of ethanol concentration, fermentation efficiency and ethanol yield. The variety used and its post-harvest treatment should be appropriately selected in order to improve the ethanol production from sweet sorghum.

Keywords: Ethanol; Sweet sorghum; *Saccharomyces cerevisiae*; Alcoholic fermentation; Juice extractability

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The excess of sugarcane bagasse (SCB) from the sugar-alcohol industry is considered a by-product with great potential for many bioproducts production. This work had as objective to verify the performance of sugarcane bagasse hemicellulosic hydrolysate (SCBHH) as source of sugars for enzymatic or *in vitro* xylitol production. For this purpose, xylitol enzymatic production was evaluated using different concentrations of treated SCBHH in the commercial reaction media. The weak acid hydrolysis of SCB provided a hydrolysate with 18 g L^{-1} and 6 g L^{-1} of xylose and glucose, respectively. Considering the reactions, changes at xylose–xylitol conversion efficiency and volumetric productivity in xylitol were not observed for the control experiment and using 20 and 40% $v.v^{-1}$ of SCBHH in the reaction media. The conversion efficiency achieved 100% in all the experiments tested. The results showed that treated SCBHH is suitable as xylose and glucose source for the enzymatic xylitol production and that this process has potential as an alternative for traditional xylitol production ways.

Keywords: Lignocellulosic residues; Sugarcane bagasse; Hemicellulose; Xylitol; Enzymatic process; Sustainability

Young Sun Hong, Hyon Hee Yoon. (Department of Chemical Engineering, Kyungwon University, Seongnam, Gyeonggi-do 461 701, Republic of Korea). Ethanol production from food residues. Biomass and Bioenergy, Volume 35(7) (2011): 3271-3275

Food residues were converted to ethanol by simultaneous saccharification with an amylolytic enzyme complex (a mixture of amyloglucosidase, α-amylase, and protease), and fermentation (SSF) with the yeast, *Saccharomyces cerevisiae*. About 36 g dm^{-3} of ethanol was obtained from 100 g dm^{-3} food residue in 48 h of fermentation. In the SSF with no nitrogen supplements, 25 g dm^{-3} of ethanol was produced from 100 g dm^{-3} food residues. In addition, none of the nutrient components except yeast extract from the SSF medium were found to affect ethanol production from food residues. This result indicates that food residues could be a good economic bioresource for ethanol production.

Keywords: Amylase; Ethanol; Fermentation; Food residues; Saccharification; *Saccharomyces cerevisiae*

Wei Qiao[a, b], Chong Peng[a, b], Wei Wang[c], ZhongZhi Zhang[a, b]. ([a]College of Chemical Engineering, China University of Petroleum, Beijing 102249, China, [b]State Key Laboratory of Heavy Oil Processing, China University of Petroleum, Beijing 102249, China, [c]School of Environment, Tsinghua University, Beijing 100084, China). Biogas production from supernatant of hydrothermally treated municipal sludge by upflow anaerobic sludge blanket reactor. Bioresource Technology, Volume 102(21) (2011): 9904-9911

The supernatant of hydrothermally treated sludge was treated by an upflow anaerobic sludge blanket (UASB) reactor for a 550-days running test. The hydrothermal parameter was 170 °C for 60 min. An mesophilic 8.6 L UASB reactor was seeded with floc sludge. The final organic loading rate (OLR) could reach 18 kg COD/m^3 d. At the initial stage running for 189 days, the feed supernatant was diluted, and the OLR reached 11 kg COD/m^3 d. After 218 days, the reactor achieved a high OLR, and the supernatant was pumped into the reactor without dilution. The influent COD fluctuated from 20,000 to 30,000 mg/L and the COD removal rate remained at approximately 70%. After 150 days, granular sludge was observed. The energy balance calculation show that heating 1.0 kg sludge needs 0.34 MJ of energy, whereas biogas energy from the supernatant of the heated sludge is 0.43 MJ.

Keywords: Biogas; Municipal sludge; Hydrothermal treatment; UASB

Paramjit K. Bajwa[a], Chetsada Phaenark[a, 1], Nicola Grant[b, 2], Xiao Zhang[c, 3], Michael Paice[c], Vincent J.J. Martin[b], Jack T. Trevors[a], Hung Lee[a]. ([a]School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1, [b]Department of Biology, Concordia University, Montréal, Québec, Canada H4B 1R6, [c]FPInnovations – Pulp and Paper Division, Pointe Claire, Québec, Canada H9R 3J9). **Ethanol production from selected lignocellulosic hydrolysates by genome shuffled strains of *Scheffersomyces stipitis*. Bioresource Technology, Volume 102(21) (2011): 9965-9969**

Two genome-shuffled *Scheffersomyces stipitis* strains, GS301 and GS302, exhibiting improved tolerance to hardwood spent sulphite liquor, were tested for growth and fermentation performance on three wood hydrolysates: (a) steam-pretreated enzymatically hydrolyzed poplar hydrolysate from Mascoma Canada, (b) steam pretreated poplar hydrolysate from University of British Columbia Forest Products Biotechnology Laboratory, and (c) mixed hardwoods pre-hydrolysate from FPInnovations (FPI). In the FPI hydrolysate, the wild type (WT) died off within 25 h, while GS301 and GS302 survived beyond 100 h. In fermentation tests, GS301 and GS302 completely utilized glucose and xylose in each hydrolysate and produced 0.39–1.4% (w/v) ethanol. In contrast, the WT did not utilize or poorly utilized glucose and xylose and produced non-detectable to trace amounts of ethanol. The results demonstrated cross tolerance of the mutants to inhibitors in three different wood hydrolysates and reinforced the utility of mating-based genome shuffling approach in industrial yeast strain improvement.

Keywords: Genome shuffling; Inhibitors; Lignocellulose; Pentose-fermenting; *Scheffersomyces stipitis*

Aftab Ahamed, Birgitte K. Ahring. (Center for Bioproducts and Bioenergy, Bioproducts Sciences and Engineering Laboratory, Washington State University, Richland, WA 99354-1671, USA). **Production of hydrocarbon compounds by endophytic fungi *Gliocladium* species grown on cellulose. Bioresource Technology, Volume 102(20) (2011): 9718-9722**

Endophytic fungi belonging to the genus *Gliocladium* are able to degrade plant cellulose and synthesize complex hydrocarbons under microaerophilic conditions. These fungi could thus be used to produce biofuels from cellulosics without the need for hydrolytic pretreatments. Gas chromatography–mass spectrometry–solid-phase micro-extraction (GC–MS–SPME) of head space gases from *Gliocladium* cultures demonstrated the production of C_6–C_{19} hydrocarbons

including hexane, benzene, heptane, 3,4-dimethyl hexane, 1-octene, m-xylene, 3-methyl nonane, dodecane, tridecane, hexadecane and nonadecane directly from the cellulosic biomass. Hydrocarbon production was 100-fold higher in co-cultures of *Gliocladium* and *Escherichia coli* than in pure *Gliocladium* cultures. The dry mycelia weight is stable at stationary period in co-culture condition which may lead to synthesize more hydrocarbons. These fungi could potentially be developed into cost-effective biocatalysts for production of biofuels.

Keywords: Biofuel; Hydrocarbons; Green energy; Bio-products; Endophyte

Xianzheng Yuan[a, c], Xiaoshuang Shi[a, c], Peidong Zhang[a], Yueli Wei[b], Rongbo Guo[a,], Lisheng Wang[a]. ([a]Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, Shandong Province 266101, PR China, [b]College of Environment and Safety Engineering, Qingdao University of Science and Technology, Qingdao, Shandong Province 266042, PR China, [c]Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China). Anaerobic biohydrogen production from wheat stalk by mixed microflora: Kinetic model and particle size influence. Bioresource Technology, Volume 102(19) (2011): 9007-9012

This study investigated the influence of particle size on anaerobic biohydrogen production from wheat stalk by mixed microflora. In addition, the kinetic model for the formation of main products was also mentioned. The results demonstrated that all the cumulative productions of hydrogen, acetate and butyrate decreased as the particle size increasing from 1 to 10 mm at a constant TS value of 2%, 5% and 8%, respectively. However, this difference for aqueous products was not very obvious compared with hydrogen. A modified Gompertz equation was able to adequately describe the cumulative production of hydrogen, acetate and butyrate (R^2 higher than 0.989). The results also indicated that the formation of the main products were all associated with the degradation of cellulose and hemicellulose (R^2 higher than 0.855).

Keywords: Wheat stalk; Anaerobic biohydrogen; Particle size; Kinetic model

G. Venkata Subhash, S. Venkata Mohan. (Bioengineering and Environmental Centre (BEEC), Indian Institute of Chemical Technology (IICT), Hyderabad 500 607, India). Biodiesel production from isolated oleaginous fungi *Aspergillus sp.* using corncob waste liquor as a substrate. Bioresource Technology, Volume 102(19) (2011): 9286-9290

The study documented the potential of isolated filamentous fungus *Aspergillus sp.* as whole cell biocatalyst for biodiesel production using Sabourauds dextrose broth medium (SDBM) and corncob waste liquor (CWL) as substrates. SDBM showed improvement in both biomass production (13.6 g dry weight/1000 ml) and lipid productivity (23.3%) with time. Lipid extraction was performed by direct (DTE) and indirect (IDTE) transesterification methods. DTE showed higher transesterification efficiency with broad spectrum of fatty acids profile over IDTE. CWL as substrate showed good lipid productivity (22.1%; 2 g dry biomass; 48 h) along with efficient substrate degradation. Lipids derived from both substrates depicted high fraction of saturated fatty acids than unsaturated ones. Physical characteristics of fungal based biodiesel correlated well with prescribed standards. CWL derived biodiesel showed relatively good fuel properties (acid number, 0.40 mg KOH/g of acid; iodine value, 11 g I_2/100 g oil; density, 0.8342 g/cm^3) than SDBM derived biodiesel.

Keywords: Transesterification; Fatty acid methyl ester (FAME); Saturated fatty acid (SFA); Substrate degradation

Chi-Mei Lee, Guo-Jan Hung, Chu-Fang Yang. (Department of Environmental Engineering, National Chung-Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan). Hydrogen production by *Rhodopseudomonas palustris* WP 3-5 in a serial photobioreactor fed with hydrogen fermentation effluent. Bioresource Technology, Volume 102(18) (2011): 8350-8356

In this study, a lab-scale serial photobioreactor composed of three column reactors was constructed and continuously operated to investigate several parameters influencing photohydrogen production when using the synthetic wastewater and the anaerobic hydrogen fermentation effluents as the influents. The results indicated that better hydrogen production rate was obtained when the serial photobioreactor was operated under cellular recycling at a short HRT of 8 h. The serial photobioreactor maintained high hydrogen content ca. 80% in the produced gas and 0.4× dilution ratio was the suitable ratio for hydrogen production. When the photobioreactor fed with the real wastewater (Effluent 1) containing 100 mg/L NH_4Cl, Column 1 reactor successfully reduced ammonia concentration to about 60 mg/L for cell synthesis, resulting in a steady hydrogen production in the following two column reactors. The average hydrogen production rate was 205 mL-H_2/L/d.

Keywords: Hydrogen production; Purple nonsulfur photosynthetic bacterium *Rhodopseudomonas palustris* WP 3-5; Ammonia; Serial photobioreactor

Ela Eroglu[a], Anastasios Melis[b]. ([a]Centre for Energy, School of Mechanical and Chemical Engineering, The University of Western Australia, Crawley, WA 6009, Australia, [b]University of California, Dept. of Plant and Microbial Biology, Berkeley, CA 94720-3102, USA). Photobiological hydrogen production: Recent advances and state of the art. Bioresource Technology, Volume 102(18) (2011): 8403-8413

Photobiological hydrogen production has advanced significantly in recent years, and on the way to becoming a mature technology. A variety of photosynthetic and non-photosynthetic microorganisms, including unicellular green algae, cyanobacteria, anoxygenic photosynthetic bacteria, obligate anaerobic, and nitrogen-fixing bacteria are endowed with genes and proteins for H_2-production. Enzymes, mechanisms, and the underlying biochemistry may vary among these systems; however, they are all promising catalysts in hydrogen production. Integration of hydrogen production among these organisms and enzymatic systems is a recent concept and a rather interesting development in the field, as it may minimize feedstock utilization and lower the associated costs, while improving yields of hydrogen production. Photobioreactor development and genetic manipulation of the hydrogen-producing microorganisms is also outlined in this review, as these contribute to improvement in the yield of the respective processes.

Keywords: Biohydrogen; Biophotolysis; Bioreactor; Photosynthesis; Photo-fermentation

Abbreviations: ATP, adenosine triphosphate; ETS, electron transport system; Fd, ferredoxin; hup^-, uptake hydrogenase deficient; LHC, light harvesting complex; PAR, photosynthetically

active radiation; PHB, polyhydroxybutyrate; PHB^-, PHB synthase deficient; PNS, purple non-sulphur; PS1, photosystem 1; PS2, photosystem 2; TAP, Tris–acetate–phosphate medium; TAP-S, (sulphur deprived) Tris–acetate–phosphate medium; η, light conversion efficiency

Dong-Hoon Kim, Mi-Sun Kim.. (Wastes Energy Research Center, Korea Institute of Energy Research, 102 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea). Hydrogenases for biological hydrogen production. Bioresource Technology, Volume 102(18) (2011): 8423-8431

Biological H_2 production offers distinctive advantages for environmental protection over existing physico-chemical methods. This study focuses specifically on hydrogenases, a class of enzymes that serves to effectively catalyze H_2 formation from protons or oxidation to protons. It reviews the classification schemes (i.e. [NiFe]-, [FeFe]-, and [Fe]-hydrogenases) and properties of these enzymes, which are essential to understand the mechanisms for H_2 production, the control of cell metabolism, and subsequent increases in H_2 production. There are five kinds of biological hydrogen production methods, categorized based upon the light energy requirement, and feedstock sources. The genetic engineering work on hydrogenase to enhance H_2 production is reviewed here. Further discussions in this study include nitrogenase, an enzyme that normally catalyzes the reduction of N_2 to ammonia but is also able to produce H_2 under photo-heterotrophic conditions, as well as other applicable fields of hydrogenase other than H_2 production.

Keywords: Hydrogenase; Biological hydrogen production; Nitrogenase; Fermentation; Genetic engineering

Xin Zhao, Defeng Xing, Na Fu, Bingfeng Liu, Nanqi Ren. (State Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, PR China). Hydrogen production by the newly isolated *Clostridium beijerinckii* RZF-1108. Bioresource Technology, Volume 102(18) (2011): 8432-8436

A fermentative hydrogen-producing strain, RZF-1108, was isolated from a biohydrogen reactor, and identified as *Clostridium beijerinckii* on the basis of the 16S rRNA gene analysis and physiobiochemical characteristics. The effects of culture conditions on hydrogen production by *C. beijerinckii* RZF-1108 were investigated in batch cultures. The hydrogen production and growth of strain RZF-1108 were highly dependent on temperature, initial pH and substrate concentration. Yeast extract was a favorable nitrogen source for hydrogen production and growth of RZF-1108. Hydrogen production corresponded to cell biomass yield in different culture conditions. The maximum hydrogen evolution, yield and production rate of 2209 ml H_2/l medium, 1.97 mol H_2/mol glucose and 104.20 ml H_2/g CDW h^{-1} were obtained at 9 g/l of glucose, initial pH of 7.0, inoculum volume of 8% and temperature of 35 °C, respectively. These results demonstrate that *C. beijerinckii* can efficiently produce H_2, and is another model microorganism for biohydrogen investigations.

Keywords: Biohydrogen production; Hydrogen-producing bacterium; C*lostridium beijerinckii*; Isolation; Culture condition

Chun-Hsiung Hung[a], Yi-Tang Chang[b], Yu-Jie Chang[c]. ([a]Department of Environmental Engineering, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan, [b]Department of Microbiology, Soochow University, 70, Linxi Road, Shilin District, Taipei 111, Taiwan, [c]Graduate School of Environmental Education and Resources, Taipei Municipal University of Education, 1, Ai-Guo West Road, Taipei 100, Taiwan). Roles of microorganisms other than *Clostridium* and *Enterobacter* in anaerobic fermentative biohydrogen production systems – A review. Bioresource Technology, Volume 102(18) (2011): 8437-8444

Anaerobic fermentative biohydrogen production, the conversion of organic substances especially from organic wastes to hydrogen gas, has become a viable and promising means of producing sustainable energy. Successful biological hydrogen production depends on the overall performance (results of interactions) of bacterial communities, i.e., mixed cultures in reactors. Mixed cultures might provide useful combinations of metabolic pathways for the processing of complex waste material ingredients, thereby supporting the more efficient decomposition and hydrogenation of biomass than pure bacteria species would. Therefore, understanding the relationships between variations in microbial composition and hydrogen production efficiency is the first step in constructing more efficient hydrogen-producing consortia, especially when complex and non-sterilized organic wastes are used as feeding substrates. In this review, we describe recent discoveries on bacterial community composition obtained from dark fermentation biohydrogen production systems, with emphasis on the possible roles of microorganisms that co-exist with common hydrogen producers.

Keywords: Anaerobic; Dark fermentation; Co-existed; Bacterial community; Hydrogen

R.Y. Li[b], T. Zhang[a], H.H.P. Fang[a]. ([a]Department of Civil Engineering, The University of Hong Kong, Hong Kong, China, [b]School of Environmental Science and Engineering, Tianjin University, Tianjin, China). Application of molecular techniques on heterotrophic hydrogen production research. Bioresource Technology, Volume 102(18) (2011): 8445-8456

This paper reviews the application of molecular techniques in heterotrophic hydrogen production studies. Commonly used molecular techniques are introduced briefly first, including cloning-sequencing after polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), terminal-restriction fragment length polymorphism (T-RFLP), fluorescence *in situ* hybridization (FISH) and quantitative real-time PCR. Application of the molecular techniques in heterotrophic hydrogen production studies are discussed in details, focusing on identification of new isolates for hydrogen production, characterization of microbial compositions in bioreactors, monitoring microbial diversity variation, visualization of microbial distribution in hydrogen-producing granular sludge, and quantification of various microbial populations. Some significant findings in recent hydrogen production studies with the application of molecular techniques are discussed, followed by a research outlook of the heterotrophic biohydrogen field.

Keywords: Hydrogen production; Molecular techniques; 16S rDNA; Fe-hydrogenase gene; PCR

S. Venkata Mohan, M. Lenin Babu. (Bioengineering and Environmental Centre (BEEC), Indian Institute of Chemical Technology (IICT), Hyderabad 500 607, India). Dehydrogenase activity in association with poised potential during biohydrogen production in single chamber microbial electrolysis cell. Bioresource Technology. Volume 102(18) (2011): 8457-8465

Variation in the dehydrogenase (DH) activity and its simultaneous influence on hydrogen (H_2) production, substrate degradation rate (SDR) and volatile fatty acid (VFA) generation was investigated with respect to varying poised potential in single chambered membrane-less microbial electrolysis cell (MEC) using anaerobic consortia as biocatalyst. Poised potential showed significant influence on H_2 production and DH activity. Maximum H_2 production was observed at 1.0 V whereas the control system showed least H_2 production among the experimental variations studied. DH activity was observed maximum at 0.6 V followed by 0.8, 0.9 and 1.0 V, suggests the influence of poised potential on the microbial metabolism. Almost complete degradation of substrate was observed in all the experimental conditions studied irrespective of the applied potential. Experimental data was also analysed employing multiple regression analysis and 3D-surface plots to find out the best theoretical poised potential for maximum H_2 production and DH activity.

Keywords: Anaerobic consortia; Bio-potential; Microbial electrolysis; Microbial fuel cell; Multiple regression analysis

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Biohydrogen is considered as an attractive clean energy source due to its high energy content and environmental-friendly conversion. Analyzing various economic scenarios can help decision makers to optimize development strategies for the biohydrogen sector. This study surveys econometric models of biohydrogen development, including input-out models, life-cycle assessment approach, computable general equilibrium models, linear programming models and impact pathway approach. Fundamentals of each model were briefly reviewed to highlight their advantages and disadvantages. The input–output model and the simplified economic input–output life-cycle assessment model proved most suitable for economic analysis of biohydrogen energy development. A sample analysis using input–output model for forecasting biohydrogen development in the United States is given.

Keywords: Biohydrogen; Linear programming model; Input–output model; Computational general equilibrium models

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Photobiological hydrogen production has recently attracted interest in terms of being a potential source for an alternative energy carrier. Especially the natural light driven hydrogen metabolism of unicellular green algae appears as an attractive blueprint for a clean and potentially unlimited dihydrogen source. However, the efficiency of *in vivo* systems is limited by physiological and evolutionary constraints and scientists only begin to understand the regulatory networks influencing cellular hydrogen production. A growing number of projects aim at circumventing these limitations by focusing on semi-artificial systems. They reconstitute parts of the native electron transfer chains *in vitro,* combining photosystem I as a photoactive element with a proton reducing catalytic element such as hydrogenase enzymes or noble metal nanoparticles. This review summarizes various approaches and discusses limitations that have to be overcome in order to establish economically applicable systems.

Keywords: Biohydrogen; Photosystem I; Electron transfer; Hydrogenase; Semi-artificial

Cheng-Long Guo[a, b], Xun Zhu[a, b], Qiang Liao[a, b], Yong-Zhong Wang[a, b], Rong Chen[a, b], Duu-Jong Lee[a, c]. ([a]Institute of Engineering Thermophysics, Chongqing University, Chongqing 400030, China, [b]Key Laboratory of Low-grade Energy Utilization Technologies and Systems, Ministry of Education, China, [c]Department of Chemical Engineering, National Taiwan University, Taipei, Taiwan). Enhancement of photo-hydrogen production in a biofilm photobioreactor using optical fiber with additional rough surface. Bioresource Technology, Volume 102(18) (2011): 8507-8513

In this study, a biofilm photobioreactor with optical fibers that have additional rough surface (OFBP-R) was developed and it was shown that additional rough surface greatly enhanced the biofilm formation and thus increased the cell concentration, leading to an improvement in the hydrogen production performance. The effects of operational conditions, including the influent substrate concentration, flow rate, temperature and influent medium pH, on the performance of OFBP-R were also investigated. The experimental results showed that the optimum operational conditions for hydrogen production were: the influent substrate concentration 60 mM, flow rate 30 mL/h, temperature 30 °C and influent medium pH 7. Under the optimal operation conditions discovered in this work, the OFBP-R yielded fairly good and stable long-term performance with hydrogen production rate of 1.75 mmol/L/h, light conversion efficiency of 9.3% and substrate degradation efficiency of 75%.

Keywords: Photo-hydrogen production; Optical fiber; Cell immobilization; Biofilm; Photobioreactor

Chieh-Lun Cheng[a], Yung-Chung Lo[a], Kuo-Shing Lee[b], Duu-Jong Lee[c], Chiu-Yue Lin[d], Jo-Shu Chang[a, e, f, g]. ([a]Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan, [b]Department of Safety Health and Environmental Engineering, Central Taiwan University of Science and Technology, Taichung, Taiwan, [c]Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei, Taiwan, [d]Department of Environmental Engineering and Science, Feng Chia University, Taichung, Taiwan, [e]Center for Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan, [f]Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan, Taiwan, [g]Sustainable Environment Research Center, National Cheng Kung University, Tainan, Taiwan). **Biohydrogen production from lignocellulosic feedstock. Bioresource Technology, Volume 102(18) (2011): 8514-8523**

Due to the recent energy crisis and rising concern over climate change, the development of clean alternative energy sources is of significant interest. Biohydrogen produced from cellulosic feedstock, such as second generation feedstock (lignocellulosic biomass) and third generation feedstock (carbohydrate-rich microalgae), is a promising candidate as a clean, CO_2-neutral, non-polluting and high efficiency energy carrier to meet the future needs. This article reviews state-of-the-art technology on lignocellulosic biohydrogen production in terms of feedstock pretreatment, saccharification strategy, and fermentation technology. Future developments of integrated biohydrogen processes leading to efficient waste reduction, low CO_2 emission and high overall hydrogen yield is discussed.

Keywords: Biohydrogen; Cellulases; Lignocellulosic feedstock; Saccharification; Pretreatment

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Biohydrogen is regarded as an attractive future clean energy carrier due to its high energy content and environmental-friendly conversion. It has the potential for renewable biofuel to replace current hydrogen production which rely heavily on fossil fuels. While biohydrogen production is still in the early stage of development, there have been a variety of laboratory- and pilot-scale systems developed with promising potential. This work presents a review of advances in bioreactor and bioprocess design for biohydrogen production. The state-of-the art of biohydrogen production is discussed emphasizing on production pathways, factors affecting biohydrogen production, as well as bioreactor configuration and operation. Challenges and prospects of biohydrogen production are also outlined.

Keywords: Biohydrogen; Biophotolysis; Bioreactor; Dark fermentation; Photo-fermentation

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Acidogenic fermentation can be used to produce hydrogen from a range of biomass sources. The effluent from this process can be utilised in a number of biological processes enabling further recovery of energy from the biomass. In this review a number of candidate technologies are assessed including conventional methanogenic anaerobic digestion, dark fermentative hydrogen production, photo-fermentation, and bioelectrochemical systems. The principles, benefits and challenges associated with integrating these technologies are discussed, with particular emphasis on integration with fermentative hydrogen production, and the current state of integrative development is presented. The various system configurations for potential integrations presented here may simultaneously permit an increase in the conversion efficiency of biomass to energy, improved adaptability to varying operating conditions, and improved stability. Such integration, while increasing system complexity, may mean that these bioprocesses could be deployed in a wider range of scenarios and be used with a greater range of substrates.

Keywords: Biohydrogen; Methanogenic; Photo fermentation; Bioelectrical; Integration

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In many respects, hydrogen is an ideal biofuel. However, practical, sustainable means of its production are presently lacking. Here we review recent efforts to apply the capacity of photosynthetic bacteria to capture solar energy and use it to drive the nearly complete conversion of substrates to hydrogen and carbon dioxide. This process, called photofermentation, has the potential capacity to use a variety of feedstocks, including the effluents of dark fermentations, leading to the development of various configurations of two-stage systems, or various industrial and agricultural waste streams rich in sugars or organic acids. The metabolic and enzymatic properties of this system are presented and the possible waste streams that might be successfully used are discussed. Recently, various immobilized systems have been developed and their advantages and disadvantages are examined.

Keywords: Biohydrogen; Photofermentation; Photosynthetic bacteria; Biomass resources

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Hydrogen production by dark fermentation may suffer of inhibition or instability due to pH deviations from optimality. The co-fermentation of promptly degradable feedstock with alkali-rich materials, such as livestock wastes, may represent a feasible and easy to implement approach to avoid external adjustments of pH.

Experiments were designed to investigate the effect of the mixing ratio of fruit–vegetable waste with swine manure with the aim of maximizing biohydrogen production while obtaining process stability through the endogenous alkalinity of manure.

Fruit–vegetable/swine manure ratio of 35/65 and HRT of 2 d resulted to give the highest production rate of 3.27 ± 0.51 L_{H2} L^{-1} d^{-1}, with a corresponding hydrogen yield of 126 ± 22 mL_{H2} $g^{-1}_{VS\text{-}added}$ and H_2 content in the biogas of 42 ± 5%. At these operating conditions the process exhibited also one of the highest measured stability, with daily productions deviating for less than 14% from the average.

Keywords: Biohydrogen; Swine manure; Fruit and vegetables waste; Co-fermentation; Response surface analysis

X. Gómez, C. Fernández, J. Fierro, M.E. Sánchez, A. Escapa, A. Morán. (Chemical Engineering Department, University of Leon, IRENA, Avda. de Portugal 41, Leon 24071, Spain). Hydrogen production: Two stage processes for waste degradation. Bioresource Technology, Volume 102(18) (2011): 8621-8627

The dark fermentation process generates hydrogen by biological means. It presents two main advantages: fulfilling requirements for mild operational conditions and gaining benefit from the residual biomass. The process itself may be seen as a pre-treatment step in a complete stabilisation chain, with the aim of attaining the valorisation of residual biomass. However, increasing the yield of H_2 production is an imperative task. In this manuscript, a review of recent work in the field of fermentative hydrogen production is presented. As dark fermentation has a maximum yield of 33% (on sugars), a description is also presented of possible second stage processes for the degradation of dark fermentation effluents. Alternatives considered were photofermentation and bioelectrochemical systems (BES) as processes capable of converting fermentation sub-products into H_2. Anaerobic digestion as a final stabilisation stage was also considered owing to the wide application of this technology in the treatment of bio-wastes.

Keywords: Hydrogen production; Dark fermentation; Scale-up experiences; Photo-fermentation; Bioelectrochemical systems

Wei Song, Naim Rashid, Wookjin Choi, Kisay Lee. (Department of Environmental Engineering and Biotechnology, Myongji University, Yongin 449-728, Republic of Korea). Biohydrogen production by immobilized *Chlorella* sp. using cycles of oxygenic photosynthesis and anaerobiosis. Bioresource Technology, Volume 102(18) (2011): 8676-8681

Hydrogen production was studied using immobilized green alga *Chlorella* sp. through a two-stage cyclic process where immobilized cells were first incubated in oxygenic photosynthesis followed by anaerobic incubation for H_2 production in the absence of sulfur. *Chlorella* sp. used in this study was capable of generating H_2 under immobilized state in agar. The externally added glucose enhanced H_2 production rates and total produced volume while shortened the lag time required for cell adaptation prior to H_2 evolution. The rate of hydrogen evolution was increased as temperature increased, and the maximum evolution rate under 30 mM glucose was 183 mL/h/L and 238 mL/h/L at 37 °C and 40 °C, respectively. In order to continue repeated cycles of H_2 production, at least two days of photosynthesis stage should be allowed for cells to recover H_2 production potential and cell viability before returning to H_2 production stage again.

Keywords: Hydrogen production; *Chlorella*; Immobilization; Photosynthesis; Anaerobiosis

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The present study investigated a two-stage anaerobic hydrogen and methane process for increasing bioenergy production from organic wastes. A two-stage process with hydraulic retention time (HRT) 3 d for hydrogen reactor and 12 d for methane reactor, obtained 11% higher energy compared to a single-stage methanogenic process (HRT 15 d) under organic loading rate (OLR) 3 gVS/(L d). The two-stage process was still stable when the OLR was increased to 4.5 gVS/(L d), while the single-stage process failed. The study further revealed that by changing the $HRT_{hydrogen}$:$HRT_{methane}$ ratio of the two-stage process from 3:12 to 1:14, 6.7%, more energy could be obtained. Microbial community analysis indicated that the dominant bacterial species were different in the hydrogen reactors (*Thermoanaerobacterium thermosaccharolyticum*-like species) and methane reactors (*Clostridiumthermocellum*-like species). The changes of substrates and HRT did not change the dominant species. The archaeal community structures in methane reactors were similar both in single- and two- stage reactors, with acetoclastic methanogens *Methanosarcina acetivorans*-like organisms as the dominant species.

Keywords: Anaerobic digestion; Hydrogen; Methane; Two-stage process

Alessandro Ciranna, Ville Santala, Matti Karp. (Department of Chemistry and Bioengineering, Tampere University of Technology, Tampere, Finland). Biohydrogen production in alkalithermophilic conditions: *Thermobrachium celere* as a case study. Bioresource Technology, Volume 102(18) (2011): 8714-8722

In the present work the hydrogenesis in the anaerobic alkalithermophilic bacterium *Thermobrachium celere* was studied. The impact of several factors on hydrogen production during glucose fermentation was investigated in batch conditions. The optimal hydrogen production occurred at $pH_{67\ ^{\circ}C}$ 8.2 with phosphate buffer concentration of 50 mM. Hydrogen yield reached the highest value of 3.36 mol H_2/mol glucose when the partial pressure in the gas headspace was reduced. Supplementation of nitrogen sources and iron affected hydrogen production. Under optimized conditions, the maximum H_2 accumulation and H_2 production rate were estimated to be respectively 124.3 mmol H_2/l culture and 20.7 mmol H_2/l/h. Considering the efficient and rapid hydrogen evolution, and the ability to grow in extreme environments, *T. celere* might be a good candidate for biohydrogen production in open (non-sterile) bioprocess system.

Keywords: Anaerobic bacteria; Alkalithermophilic bacteria; H_2 partial pressure; Dark fermentation; VFA

Jiele Xu, Ziyu Wang, Jay J. Cheng. (Department of Biological and Agricultural Engineering, Campus Box 7625, North Carolina State University, Raleigh, NC 27695, USA). Bermuda grass as feedstock for biofuel production: A review. Bioresource Technology, Volume 102(17) (2011): 7613-7620

Bermuda grass is a promising feedstock for the production of fuel ethanol in the Southern United States. This paper presents a review of the significant amount of research on the conversion of Bermuda grass to ethanol and a brief discussion on the factors affecting the biomass production in the field. The biggest challenge of biomass conversion comes from the recalcitrance of lignocellulose. A variety of chemical, physico-chemical, and biological pretreatment methods have been investigated to improve the digestibility of Bermuda grass with encouraging results reported. The subsequent enzymatic hydrolysis and fermentation steps have also been extensively studied and effectively optimized. It is expected that the development of genetic engineering technologies for the grass and fermenting organisms has the potential to greatly improve the economic viability of Bermuda grass-based fuel ethanol production systems. Other energy applications of Bermuda grass include anaerobic digestion for biogas generation and pyrolysis for syngas production.

Keywords: Bermuda grass; Enzymatic hydrolysis; Ethanol; Fermentation; Pretreatment

Sonia Heaven, Andrew M. Salter, Charles J. Banks. (School of Civil Engineering and the Environment, University of Southampton, Southampton SO17 1BJ, UK). Integration of on-farm biodiesel production with anaerobic digestion to maximise energy yield and greenhouse gas savings from process and farm residues. Bioresource Technology, Volume 102(17) (2011): 7784-7793

Anaerobic co-digestion of residues from the cold pressing and trans-esterification of oilseed rape (OSR) with other farm wastes was considered as a means of enhancing the sustainability of on-farm biodiesel production. The study verified the process energy yields using biochemical methane potential (BMP) tests and semi-continuous digestion trials. The results indicated that high proportions of OSR cake in the feedstock led to a decrease in volatile solids destruction and instability of the digestion process. Co-digestion with cattle slurry or with vegetable waste led to acceptable specific and volumetric methane productions, and a digestate low in potentially toxic elements (PTE). The results were used to evaluate energy balances and greenhouse gas emissions of the integrated process compared with biodiesel production alone. Co-digestion was shown to provide energy self-sufficiency and security of supply to farms, with sufficient surplus for export as fuel and electricity.

Keywords: Anaerobic digestion; Biodiesel production; Oilseed rape; Renewable energy; Agro-wastes

Kasirajan Ramachandran, Pandian Sivakumar, Tamilarasan Suganya, Sahadevan Renganathan. (Department of Chemical Engineering, Anna University Chennai, Chennai 600 025, India). Production of biodiesel from mixed waste vegetable oil using an aluminium hydrogen sulphate as a heterogeneous acid catalyst. Bioresource Technology, Volume 102(15) (2011): 7289-7293

$Al(HSO_4)_3$ heterogeneous acid catalyst was prepared by the sulfonation of anhydrous $AlCl_3$. This catalyst was employed to catalyze transesterification reaction to synthesis methyl ester when a mixed waste vegetable oil was used as feedstock. The physical and chemical properties of aluminum hydrogen sulphate catalyst were characterized by scanning electron microscopy (SEM) measurements, energy dispersive X-ray (EDAX) analysis and titration method. The maximum conversion of triglyceride was achieved as 81 wt.% with 50 min reaction time at 220 °C, 16:1 molar ratio of methanol to oil and 0.5 wt.% of catalyst. The high catalytic activity and stability of this catalyst was related to its high acid site density (–OH, Brönsted acid sites), hydrophobicity that prevented the hydration of –OH group, hydrophilic functional groups (–SO_3H) that gave improved accessibility of methanol to the triglyceride. The fuel properties of methyl ester were analyzed. The fuel properties were found to be observed within the limits of ASTM D6751.

Keywords: Methyl ester; Aluminium hydrogen sulphate; Transesterification; Mixed waste vegetable oil

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