Abstract Vol. XXV

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MINISTRY OF ENVIRONMENT, FORESTS AND CLIMATE CHANGE
GOVERNMENT OF INDIA
NEW DELHI

Department of Environmental Science
University of Kalyani
Nadia, West Bengal
December, 2014
Published by:

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ENVIS CENTRE

on

ENVIRONMENTAL BIOTECHNOLOGY

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BACKGROUND

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

This ENVIS Centre is established in the focal theme area - Environmental Biotechnology at the Department of Environmental Science, University of Kalyani, Nadia-741235, West Bengal in the year 2002.

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 fifteen sub-heads. The volume contains abstracts of scientific articles from relevant national and international journals. Viewpoint of this abstract volume is to help the interested research workers, scientists, administrators and the general people.

This is the 25th publication of Abstract Volume of this ENVIS Centre. This contains the abstracts of research papers collected from the various areas of Environmental Biotechnology from different journals published in last six months upto December, 2014. In this issue, various topics like Bioenergy, Bioengineering, Bio-degradation, Bio-remediation, Bio-transformation etc. have been covered. We are grateful to the various libraries and their staff for their cooperation extended to us during the collection of the articles.
Abstract Format

The format of the abstract is as follows:

**Abstract:** The abstracts are arranged in different subheads.

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

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

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

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

Abstract are broadly classified and arranged under the following 16 heads:

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

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

1. Ingest and degrade organic substances as their food and energy source,

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

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

**Bio-Transformation**: This is a process of Biological changes of complex compounds to simpler one or toxic to non-toxic and vice-versa. Several microorganisms are capable of transforming a 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 worm. Biological insecticides against both these insects are being prepared by transfer of a gene from *Bacillus thuringiensis*

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

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

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

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

**Bioengineering:** It is a developing speciality featuring a multidisciplinary approach to the solution of problems in medicine and biology, based on the application of advances in science, engineering and technology. It generally engineers the biological processes through biotechnological or genetic engineering interventions. It may also be a broad-based engineering discipline that involve product design, sustainability and analysis of biological systems.
Pollen-Biotechnology: This is a new field of science dealing with the pollen chemistry and allergenicity of aerospora. This subject also covers genetic manipulation of pollen development of haploid culture. Such haploid plants have immense values in genetic research.

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

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

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

Nano Biotechnology: Bionanotechnology, nanobiotechnology, and nanobiology are terms that refer to the intersection of nanotechnology and biology. Given that the subject is one that has only emerged very recently, bionanotechnology and nanobiotechnology serve as blanket terms for various related technologies. This discipline helps to indicate the merger of biological research with various fields of nanotechnology. Concepts that are enhanced through nanobiology include: nanodevices, nanoparticles, and nanoscale phenomena that occurs within the disciple of nanotechnology. This technical approach to biology allows scientists to imagine and create systems that can be used for biological research.

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

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Bioaccumulation

Wenhao Yang1, He Li1, Taoxiong Zhang1, Lin Sen1, Wuzhong Ni1. (1 College of Environmental and Resource Sciences, MOE Key Laboratory of Environment Remediation and Ecosystem Health, Zhejiang University, Hangzhou, 310058, People’s Republic of China). Classification and identification of metal-accumulating plant species by cluster analysis. Environmental Science and Pollution Research, Volume 21 (18) (2014): 10626-10637

Identification and classification of metal-accumulating plant species is essential for phytoextraction. Cluster analysis is used for classifying individuals based on measured characteristics. In this study, classification of plant species for metal accumulation was conducted using cluster analysis based on a practical survey. Forty plant samples belonging to 21 species were collected from an ancient silver-mining site. Five groups such as hyperaccumulator, potential hyperaccumulator, accumulator, potential accumulator, and normal accumulating plant were graded. For Cd accumulation, the ancient silver-mining ecotype of Sedum alfredii was treated as a Cd hyperaccumulator, and the others were normal Cd-accumulating plants. For Zn accumulation, S. alfredii was considered as a potential Zn hyperaccumulator, Conyza canadensis and Artemisia lavandulaefolia were Zn accumulators, and the others were normal Zn-accumulating plants. For Pb accumulation, S. alfredii and Elatostema lineolatum were potential Pb hyperaccumulators, Rubus hunanensis, Ajuga decumbens, and Erigeron annuus were Pb accumulators, C. canadensis and A. lavandulaefolia were potential Pb accumulators, and the others were normal Pb-accumulating plants. Plant species with the potential for phytoextraction were identified such as S. alfredii for Cd and Zn, C. canadensis and A. lavandulaefolia for Zn and Pb, and E. lineolatum, R. hunanensis, A. decumbens, and E. annuus for Pb. Cluster analysis is effective in the classification of plant species for metal accumulation and identification of potential species for phytoextraction.

Keywords: Cd; Zn; Pb; Accumulation; Cluster analysis; Shoot concentration; Bioconcentration factor; Translocation factor


The potential bioaccumulation and distribution of antibiotics in non-target organisms have been inadequately studied in spite of their widespread occurrence in aquatic systems. We investigated the ability of tetracycline to bioaccumulate through aqueous and dietary routes in an aquatic
The freshwater crustacean *Daphnia magna* was exposed to algal food (*Pseudokirchneriella subcapitata*) contaminated with tetracycline for dietary uptake. Tetracycline was transferred to *D. magna* more through aqueous uptake than through dietary uptake. The uptake rate constant of tetracycline for *D. magna* was \( k_{\text{in, water}} = 0.33 \pm 0.045 \) via the aqueous route and \( k_{\text{in, food}} = 0.16 \pm 0.012 \) via the dietary route for 1.0 mg L\(^{-1}\) tetracycline. Bioconcentration factors of 4.40 ± 0.91 L kg\(^{-1}\) and 3.66 ± 0.50 L kg\(^{-1}\) for 0.1 and 1.0 mg L\(^{-1}\) tetracycline were found for *D. magna*. The biomagnification factor of 0.19 ± 0.04 indicates that magnification of tetracycline through the food web will not occur. The change in the internal concentration of the target compound was also studied for multigenerational (F1-F4) exposure. The internal concentration in *D. magna* showed a decreasing trend with increasing generations except for the parent generation. The bioaccumulation tendency showed a biphasic change in multigenerational exposure.

**Keywords:** Tetracycline; *D. magna*; Bioaccumulation; Biomagnification; Multigenerational exposure

Fung Chi Ko\(^a\), Nien-Ying Wei\(^b\), Lien-Siang Chou\(^c\). (*\(^a\) Institute of Marine Biodiversity and Evolutionary Biology, National Dong-Hwa University, Checheng, Pingtung, Taiwan, \(^b\) National Museum of Marine Biology and Aquarium, Checheng, Pingtung, Taiwan, \(^c\) Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, Taiwan). Bioaccumulation of persistent organic pollutants in stranded cetaceans from Taiwan coastal waters. *Journal of Hazardous Materials*, Volume 277(2014): 127–133

This study focuses on analyzing PBDEs in the liver, muscle, and blubber tissues of stranded dolphins (*Stenella attenuata*) on the Taiwan coast to determine and compare the PBDE levels and distributions among tissue types. Total concentrations of 19 PBDEs (\( \Sigma \)PBDE) in male dolphins (9.97 to 436 ng/g fat) were significantly higher than in female animals (2.73 to 89.5 ng/g fat), implying gender variation in bioaccumulation and the possibility of generation transfer from mother to fetus during pregnancy. The levels of contamination varied among tissue type; contamination was higher in blubber than that in muscle or liver, suggesting a possible transformation and redistribution of these compounds in body burden. Aside from gender and tissue type, \( \Sigma \)PBDE concentrations also significantly correlated with body length, an indicator of dolphin age. PCA analysis results showed no significant difference in PBDE congener pattern distributions in blubber tissues, indicating that blubber may be the final storage of contaminants in cetaceans, and that bioaccumulation of PBDEs may be dependent on chemical properties. BDE-154 and BDE-47 were the predominant PBDE congeners in stranded dolphins, and their correlation with body length suggests the significant metabolic depletion of BDE-154 in this species and possible exposure to both penta-BDE and octa-BDE mixtures.

**Keywords:** Dolphins; PBDEs; Bioaccumulation; Tissue type; Blubber

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N. Suchkova\(^a\), I. Tsiripidis\(^b\), D. Alifragkis\(^c\), J. Ganoulis\(^a\), E. Darakas\(^a\), Th. Sawidis\(^b\). (*\(^a\) School of Civil Engineering, Aristotle University of Thessaloniki, Gr-54124 Thessaloniki, Greece, \(^b\) School of Biology, Aristotle University of Thessaloniki, Gr-54124 Thessaloniki, Greece, \(^c\) Faculty of Forestry and Natural Environment, Aristotle University of Thessaloniki, Gr-54124 Thessaloniki, Greece). Assessment of phytoremediation potential of...
native plants during the reclamation of an area affected by sewage sludge on potential of native plants during the reclamation of an area affected by sewage sludge. Ecological Engineering, Volume 69(2014): 160–169

In this research paper the natural establishment of plants and their contribution to the reclamation of sewage sludge deposits through their uptake of nutrients and their bioconcentration factor (BCF) were investigated and assessed. The main research started in March 2012, and the plants growing naturally at the site under investigation during the first growing season (June 2012) were identified and recorded. On the basis of their occurrence and cover samples from ten species, namely *Amaranthus albus* L., *Amaranthus viridis* L., *Cardaria draba* (L.), *Chenopodium album* L., *Cynodon dactylon* (L.) Pers., *Cyperus rotundus* L., *Lolium perenne* L., *Lycopersicon esculentum* Mill., *Malva parviflora* L., *Portulaca oleracea* L., were collected for chemical analyses. Macronutrients (Ca, Mg, K, P), trace elements (Mn, Cu, Zn, Fe, Cr, Ni, Pb) and Na were measured. The results showed that plants took up macronutrients at relatively high rates, in some cases reducing their excessive levels of concentration in the sludge by as much as 95%. Trace elements were removed at a lower rate compared to hyperaccumulators, this was possibly affected by their reduced bioavailability in the substrate, but the BCF, which was greater than one for most of the species and for most of the trace elements, indicated that the plants were capable of phytoextraction. It was concluded that selected native plants are not only tolerant to adverse environmental conditions, but also contribute by various processes to the reclamation of sites affected by sewage sludge.

**Keywords:** Native plants; Phytoremediation; Sewage sludge; Bioconcentration factor.

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The effect of inorganic pollutants on the treatment of organic pollutants using algal/bacterial microcosm was investigated in a continuous photobioreactor. The microcosm was composed of *Chlorella vulgaris* MM1 and *Pseudomonas* MT1 and was able to efficiently treat artificial waste-water contaminated with 6.4 salicylate and 2.2 mM phenol at a hydraulic retention time of 4 days. No negative effect was recorded when the waste-water was supplemented with 1.6 mM thiocyanate; however, the treatment efficiency severely deteriorated when the system was challenged with 0.74 mM cyanide. Addition of 2 g NaHCO₃ l⁻¹ did not improve the efficiency of the treatment. Toxicity of the pollutants to the alga was cyanide > thiocyanate > phenol > salicylate. The high toxicity of the waste-water was eliminated either by a 25-fold dilution or by photocatalytic pre-treatment which allowed the subsequent efficient biological treatment.

**Keywords:** Chlorella, Cyanide, Microcosm, Photosynthesis, Photocatalytic pre-treatment, Phenol, Pseudomonas, Salicylate, Thiocyanate
Groundwater systems are important sources of water for drinking and irrigation purposes. Unfortunately, human activities have led to widespread groundwater contamination by chlorinated compounds such as tetrachloroethene (PCE). Chloroethenes are extremely harmful to humans and the environment due to their carcinogenic properties. Therefore, this study investigated the potential for bioremediating PCE-contaminated groundwater using laboratory-based biostimulation (BS) and biostimulation–bioaugmentation (BS-BA) assays. This was carried out on groundwater samples obtained from a PCE-contaminated site which had been unsuccessfully treated using chemical oxidation. BS resulted in complete dechlorination by week 21 compared to controls which had only 30% PCE degradation. BS also led to an approximately threefold increase in 16S rRNA gene copies compared to the controls. However, the major bacterial dechlorinating group, Dehalococcoides (Dhc), was undetectable in PCE-contaminated groundwater. This suggested that dechlorination in BS samples was due to indigenous non-Dhc dechlorinators. Application of the BS-BA strategy with Dhc as the augmenting organism resulted in complete dechlorination by week 17 with approximately twofold to threefold increase in 16S rRNA and Dhc gene abundance. Live/dead cell counts (LDCC) showed 70–80% viability in both treatments indicating active growth of potential dechlorinators. The LDCC was strongly correlated with cell copy numbers (r > 0.95) suggesting its potential use for low-cost monitoring of bioremediation. This study also shows the dechlorinating potential of indigenous non-Dhc groups can be successfully exploited for PCE decontamination while demonstrating the applicability of microbiological and chemical methodologies for preliminary site assessments prior to field-based studies.

Enzymatic and alkali pretreatments were employed to improve nickel biosorption capacity of Rhizomucor pusillus biomass. Pretreatment with 0.002–80 g l⁻¹ NaOH and 0.0001–0.1 Anson Unit (AU) g⁻¹ protease enhanced the biosorption capacity of fungal biomass. Increasing the concentration of NaOH from 0.002 to 5 g l⁻¹ improved nickel removal from 93.2 to 100.0% while untreated biomass showed 64.6% Ni(II) removal. Pretreatment with higher concentrations of NaOH, 5–80 g l⁻¹ resulted in nearly complete removal of nickel ions. Pretreatment of the biomass with 0.0001 AU g⁻¹ protease improved the nickel removal to over 91%, while increasing the enzyme loading to 0.1 AU g⁻¹ improved the removal to 93%. Untreated biomass removed 78.4, 63.0, and 96.3% of chromium, copper, and lead ions, respectively, from a mixture solution of the ions. Respective metal removals were increased to 100, 98.9, and 100% after pretreatment with 0.2 g l⁻¹ NaOH solution and to 87.8, 86.7, and 100% after the enzymatic pretreatment with 0.1 AU g⁻¹ protease. Scanning electron microscopy analysis indicated that
alkali and enzymatic pretreatments enhanced the porosity of the biomass. Furthermore, compositional analysis showed that both of the pretreatments removed a major part of fungal proteins (2.1–95.8 % removal). Glucosamine, N-acetyl glucosamine, and phosphates were the major ingredients of the pretreated biomass.

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The exceptional ability of dendrimers to coordinate metal ions yields the potential for many applications including wastewater remediation, which is the focus of this study. Here, the comparison of metal ion removal rate from simulated wastewater by generation 4 dendrimers with external hydroxyl functional groups (G4-OH) is evaluated for Ni^{2+}, Fe^{2+}, and Fe^{3+} ions. Ni^{2+} to amine complexation occurred more rapidly than Fe^{3+}, which was more rapid than Fe^{2+} complexation. These results indicate that both charge density and d-electron configuration are important toward the chelation rate. The impact of both factors is discussed in light of existing models in which precursor aquation rates have been proposed as a key intermediate step. Additionally, the application of the dendrimers as chelation agents is further advanced by immobilizing the dendrimer to titania and re-evaluating its chelation ability for Ni^{2+} removal. The dendrimer immobilization decreased the pseudo-first-order rate coefficient for Ni^{2+}—amine complexation at a pH of 7 by a factor of 7.5. This result is significant as it suggests that mass transfer becomes important following immobilization of the dendrimer to titania.

Keywords: Wastewater remediation; Polyamidoamine dendrimer; Iron; Nickel; Chelation


The Pb(II) and Ni(II) biosorption of a fungal biomass isolated from mine drainage of metal-processing industries in Balya (Balkesir province, Turkey) was optimized using a response surface methodology by altering parameters such as pH, initial metal concentration, contact time and biosorbent dosage. This strain was shown to be highly similar to Penicillium sp. Furthermore, zeta potential measurements and Fourier transform infrared spectroscopy were performed to understand the adsorption mechanism. A Box–Behnken design with 29 experiments was used to evaluate the interactions between independent variables. The results
showed that the fungal biomass isolated from the metal mine drainage could have a significant environmental impact through the biosorption of Pb(II) and Ni(II) in waters polluted with heavy metals, particularly in the drainage from metal mines. The maximum removal values were 76 and 47 % at pH 4.5 for both Pb(II) and Ni(II), with 123 and 33 mg/L initial metal concentrations, 65 and 89 min contact times and 0.2 and 1.6 g/L biosorbent, respectively.

**Keywords:** Response surface methodology (RSM); Heavy metal; Biosorbent; Penicillium janthinellum

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The purpose of this study was to investigate the effect of phytoremediation on soils contaminated with heavy crude oil using plants infected by mycorrhizal fungi. Five plant species, Vetiveria zizanioides, Bidens pilosa, Chloris barbata, Eleusine indica, and Imperata cylindrica, infected with the species of mycorrhizal fungi Glomus mosseae, were selected for this study. The degradation of total petroleum hydrocarbons in soils and several physiological parameters of plants such as shoot length and biomass were analyzed. Out of the 5 plant species tested, only V. zizanioides, B. pilosa, and E. indica could take up the G. mosseae. Out of these three, V. zizanioides showed the greatest growth (biomass) in soils with 100,000 mg kg\(^{-1}\) total petroleum hydrocarbons. In addition, B. pilosainfected with G. mosseae was found to be able to increase degradation by 9% under an initial total petroleum hydrocarbons concentration of 30,000 mg kg\(^{-1}\) in soils after 64 days. We conclude that plants infected with mycorrhizal fungi can enhance the phytoremediation efficiency of soils contaminated with high concentrations of heavy oil.

**Keywords:** Crude oil; Mycorrhiza; Rhizoremediation; Total petroleum hydrocarbons

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Biodegradation of petroleum hydrocarbons as a decontamination mechanism is a relatively slow process. This study aimed to investigate the impact of a tailored consortium of bacteria with higher capacities in biosurfactant production and biodegradation on the acceleration of the biodecontamination process. To this end, 18 biosurfactant producing bacteria were isolated from the crude oil-contaminated soil samples of Isfahan refinery, and the activity of the produced biosurfactants was measured in terms of surface tension reduction and emulsification E24 test. Then, the isolates screened for the biodegradation of kerosene hydrocarbons and chemical
structure of the purified biosurfactants produced by the most efficient isolates were partially characterized. Next, the isolates were sorted based on their surfactant activity and biodegradation efficiency, and the higher ranked bacteria thus selected were utilized to form an efficient consortium removing hydrocarbons from the oil-contaminated soil samples in a slurry phase system. The consortium consisted of *Bacillus subtilis* tb1 and *Pseudomonas aeruginosa* species having the highest biodegradation capabilities and surface activities. The results revealed that the hydrocarbon removal efficiency of the consortium was at least 25 % higher than single species, and the final removal efficiency for the consortium could be reached in a considerably shorter time.

**Keywords:** Fast bioremediation; Bacterial consortium; Biosurfactant; Bioemulsifier; Hydrocarbon


The biosorption of ammoniacal nitrogen (N-NH$_4^+$) from aqueous solutions by dead biomass of brown seaweed *Cystoseira indica* and Jatropha oil cake (JOC), which is generated in the process of biodiesel recovery from its seeds, was studied under diverse experimental conditions. The N-NH$_4^+$biosorption was strictly pH dependent, and maximum uptake capacity of *C. indica* (15.21 mg/g) and JOC (13.59 mg/g) was observed at initial pH 7 and 3, respectively. For each biosorbent–N-NH$_4^+$system, kinetic models were applied to the experimental data to examine the mechanisms of sorption and potential rate-controlling steps. The generalized rate model and pseudo-second-order kinetic models described the biosorption kinetics accurately, and the sorption process was found to be controlled by pore and surface diffusion for these biosorbents. Results of four-stage batch biosorber design analysis revealed that the required time for the 99 % efficiency removal of 40 mg/L N-NH$_4^+$ from 500 L of aqueous solution were 76 and 96 min for *C. indica* and JOC, respectively. The Fourier transform infrared spectroscopy analysis before and after biosorption of ammonium onto *C. indica* and JOC revealed involvement of carboxylic and hydroxyl functional groups.

**Keywords:** Cystoseira indica; Jatropha oil cake; Biosorption; Ammoniacal nitrogen kinetics; Diffusion models; Optimization

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Irrational and rapid global human societal development has culminated to a condition of environmental deterioration. Accidental leakage and deliberate use of organic and inorganic chemicals have contaminated the environment up to the level of ecosystem. Advancements have been made in the field of research on bioremediation of the hazardous contaminants especially in last three decades. Microbial bioremediation has been the most understood biotechnological process of environmental restoration. Bacteria and fungi because of their inherent ability to adapt and grow in extreme environments have been employed for either removal or degradation of the chemical contaminants. Researchers all over the world are getting breakthroughs in finding new bacterial strains having plasmid linked degradation/reduction ability. Molecular biology and genetic engineering helped in crafting the microbes for the desired results on environment. Despite having favorable conditions, microbial remediation largely depends on environmental factors and on the basic biological characters of microbes, especially bacteria being Gram-positive or Gram-negative. Metagenomic studies revealed the importance of microbial ecology as microbes work well in community, i.e., consortia. This review along with several other studies suggests the need of precision during microbial community identification, substrate specificity and the designing of microbes.

Keywords: Environmental contamination; Climate change; Bioremediation; Plasmids; Metagenomics; Gram-positive bacteria; Gram-negative bacteria

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Halotolerant bacteria are regarded as effective oil-scavengers in the polluted saltern and seawater. In this regard, a halotolerant Planococcus was isolated from oil-contaminated area of Dezful north springs, Iran, due to its capacity in biosurfactant (BS) production. To facilitate hydrocarbons degradation, in the current study, the efficiency of BS production as function of growth rate of the halotolerant Planococcus was investigated in the vicinity of heavy crude oil by emulsification index (E24). Subsequently, the BS characterization was made by thin-layer chromatography (TLC), gas chromatography (GC) and infrared spectra analysis, and the stability was determined by E24 value measurement over a certain pH (5–9), temperature (20–100 °C) and salt concentration (0–10 % w/v) ranges. The BS production was found to be growth-associated. Detection of a unique band on TLC and GC chromatogram showed the extensive refining capacity of the BS purification, using the medium supernatant under acetone alkaline precipitation followed by oil dissolution from the sediment by carbon tetrachloride. Accordingly, it was clarified that the BS ultimately accumulated outside the cells. The glycolipid quality of the BS was further determined by the routine chemical characterization on TLC and by IR spectra analysis. Moreover, there was no protein detected by lowery total protein assay. Finally, the optimal temperature, pH and NaCl concentration to reach highest E24 values (85.7, 77.0, and 79.0 %) were found at respective 40 °C, pH = 9 and 0 % w/v. Our results revealed the practically potential of strain Dezful Isolate for BS large-scale production as environmentally friendly oil-eliminating agents.
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Keywords: Planococcus; Biosurfactant; Halotolerant; Biodegradation


This study highlights the role of marine microbial biosurfactants on solubilization/removal of crude-oil contamination from four different soils in an aqueous phase. Soil of four different types, viz., sandy, fine sand soil, clay, and clay loam, were collected and saturated with crude oil. Marine isolate MTCC 5514 (Bacillus licheniformis) was chosen for the study and comparisons were made with synthetic surfactants and commercially available biosurfactant. In-situ studies were carried out with different percentages of crude oil to assess the growth and the percentage removal of oil. For ex-situ studies, soils were pre-saturated with crude oil and then treated with the chosen biosurfactant at a 10% concentration level using flask and column methods. After time intervals of 30–120 min, samples were collected and then subjected to extraction with hexane and the percentage removal was calculated. Results revealed, at 2% concentration of crude oil, that complete solubilization was achieved. With regard to ex-situ studies, clay soil absorbed the maximum percentage of oil compared to other soil types, and with regard to the removal, all the synthetic surfactants showed <60% removal irrespective of soil type. In the case of biosurfactants even at 10% concentration, >85% removal was achieved.

Keywords: Crude oil; Bacillus licheniformis; Biosurfactant; Oil removal; Bioremediation; Contaminated soil

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Bioremediation of crude oil contaminated soil is an effective process to clean petroleum contaminant from the environment. In this study, we isolated 39 native crude oil degrading bacteria from different crude oil contaminated soils. From 16S rDNA sequences, we confirmed that the isolated bacteria belong to the genera Lysinibacillus, Brevibacillus, Bacillus, Paenibacillus, Stenotrophomonas, Alcaligenes, Delftia, Achromobacter and Pseudomonas. Four most effective strains (designated as AS03, N108, N002 and N78) were used for batch culture and microcosm evaluation. Gas chromatography analysis, further confirmed that the strain AS03, N108, N002 and N78 were able to degrade crude oil under both shake culture and microcosm study. Under microcosm, the soil quality was further improved significantly in the treatments of BF1-Mix (N108-AS03) and BF2-Mix (N002-N78). The improvement of soil quality was also confirmed by earthworm mortality bioassay and in plant test on rice (Oryza
sativa) and mung (Vigna radiata). These findings demonstrated that the combine use of crude oil degrading bacteria along with nutrient supplements could revive crude oil contaminated soil effectively in large scale.

**Keywords:** Environment; Soil; Crude oil; Pollutant; Bioremediation

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*Scripus triqueter* (ST), an oil tolerant plant in the wetland of Huangpu-Yangtze river estuary (HYRE) can be used for phytoremediation. As a dominant root exudate of the ST, palmitic acid was examined for its impact on the ST and the rhizosphere soil enzymes of simulated diesel-spiked wetland. Pot experiment showed that palmitic acid inhibited the growth (height, stem weight, root weight) of the ST and the activities of soil enzymes. The activities of superoxide dismutase (SOD) and catalase (CAT) in the root and stem of the ST decreased in diesel-spiked soil planted with ST and inoculated with 400 mg kg$^{-1}$ of palmitic acid (STDP) compared with those in the diesel-spiked soil planted with ST (STD). It was found that the activities of polyphenol oxidase (PPO) and dehydrogenase (DHG) were lower in the STDP than those in the STD throughout the experiment. Compared to the STD, the concentration of residual diesel in the STDP was significantly higher, indicating that inhibited degradation abilities of soil microbes and plants. The results suggested palmitic acid might act as an allelochemicals to the ST and the rhizosphere soil enzymes in the simulated diesel-spiked wetland.

**Keywords:** Diesel; *Scripus triqueter*; Palmitic acid; Growth parameters; Enzyme activity


Bioprecipitation of uranium (U) into uranyl phosphate (U-P) mediated by soluble orthophosphate is an attractive proposition for U bioremediation. As an alternative to the microbial phosphatase, we have investigated the dissolution of phosphate by the organic acids produced by bacteria to aid in U precipitation. The bacterium *Acinetobacter* sp. YU-SS-SB-29, isolated from monazite sand of natural background radiation site solubilized 952.0 ± 46.7 mg L$^{-1}$ phosphate from tri-calcium phosphate (TCP) in the Pikovskaya's medium and showed tolerance to 120 ppm U(VI). U(VI) bioprecipitation was investigated by adding different concentrations of U(VI) to a cell-free culture supernatant containing ortho-phosphate released from TCP by the bacterium. A yellow precipitate was immediately formed following which there was a reduction in U(VI) concentration. A strong positive correlation ($R^2 = 0.98$) was observed between % decrease in phosphate and U(VI) concentration (up to 750 ppm U) added. FTIR and EDX spectra of the yellow precipitate demonstrated the involvement of phosphate groups in U(VI) binding. Furthermore, the XRD pattern of the precipitate agrees well with that of chernikovite, a uranyl phosphate mineral. The results from this study demonstrate the potential of the U tolerant,
phosphate solubilizing bacterium *Acinetobacter* sp. YU-SS-SB-29 for non-reductive *in situ* bioprecipitation of uranium.

**Keywords:** Uranium; Bioremediation; Phosphate solubilizing bacteria; Uranyl phosphate; Bioprecipitation; *Acinetobacter*

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In this study, the biosorption of lead (II) ions on *Sargassum ilicifolium*, brown seaweed, was investigated in a batch system. The effects of operating parameters such as initial pH, initial metal ion concentration, and biosorbent concentration on the lead (II) biosorption were studied using response surface methodology (RSM). The optimum biosorption conditions were determined as initial pH 3.7, biosorbent concentration 0.2 g l\(^{-1}\), and initial lead (II) ion concentration 200 mg l\(^{-1}\). The maximum uptake capacity of *S. ilicifolium* for lead (II) ions was found to be 195 ± 3.3 mg g\(^{-1}\) at optimum conditions. The nature of biomass–metal ion interactions was evaluated by FTIR analysis. The equilibrium biosorption data fit very well with both Langmuir and Freundlich isotherm models. The biosorption performance of *S. ilicifolium* for lead (II) ions (\(q_{\text{max}} = 195\) mg g\(^{-1}\)) was significantly higher than the Pb(II) biosorption performance reported for other biosorbents. The abundant and economic biomass *S. ilicifolium* could be used for removal of Pb(II) from wastewater.

**Keywords:** Biosorption; Lead (II); *Sargassum ilicifolium*; Response surface methodology (RSM); Isotherm


Research and development of an effective color removal system is needed to reduce the severity of water pollution caused by effluent that contains dyes. In this study, the integrated biosorption and biodegradation system of chitosan coated *Lentinus polychrous* Lév. was developed and evaluated for its decolorization efficiency with regard to anionic reactive dye mixtures of Reactive Blue 19, 160, and 198. The fungi were coated with 0.1, 0.5, and 1.0% w/v of low molecular weight chitosan. The scanning electron micrographs confirmed that chitosan was successfully coated on the surface of the fungi. Studies of changes in UV–visible absorption spectra, dye desorption, ligninolytic enzyme activity, and Fourier transform infrared spectroscopy showed that within 6 h, the biosorption was the control mechanism and the dyes were reduced to 91.50, 77.66, 37.39, and 26.93% by the fungi coated with 0, 0.1, 0.5, and 1.0% w/v chitosan, respectively. From the 36th hour to the end of colorization at the 72nd hour, the fungal biodegradation by laccase and manganese peroxidase was dominant and all treatments had 5–8% of the dye remaining. Therefore, the chitosan coat acted as an efficient biosorbent for
the anionic reactive dyes, thereby effectively improving the decolorization efficiency of the white rot fungus.

**Keywords:** Chitosan; *Lentinus polychrous* Lév.; Biosorption; Biodegradation; Decolorization; Reactive dyes

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The aim of this research was to determine the role poultry droppings (PD) play in the process of reducing the concentrations of total petroleum hydrocarbon (TPH) and poly-aromatic hydrocarbon (PAH) in crude-oil-contaminated soils, and to check the effects of PD application rate on the bioremediation of such soils. This bioremediation trial was done ex-situ and the concentrations of TPH and PAH were used as an indicator of soil productivity restoration. The PD serving as amendment was applied to the crude-oil-contaminated soils collected from three sites in southern Nigeria at the rate of 0% (control), 5%, 10%, and 25% per dry mass of contaminated soil. Soil samples were evaluated for pH, TPH, and PAH after 6 wk. The results showed that the application rate of PD had significant reductive effects on the concentrations of TPH and PAH in the contaminated soils (\(P \leq 0.05\) and \(\leq 0.01\), respectively). There was a general increase in the mean percentage reduction in the concentrations of both TPH and PAH with increasing rate of PD application for all the three soils, with \(R^2\) values of 0.91 for TPH and 0.82 for PAH. The range of \(R^2\) for the regression between TPH/PAH concentration and PD rate across the three soils was narrower for TPH (0.89–0.94) than for PAH (0.69–0.94), suggesting a greater influence of the age of the crude oil contaminant in the soils on the bioavailability of the PAH. There were also linear correlations between the final soil pH and percentage reduction in the concentrations of both TPH (\(r = 0.53\)) and PAH (\(r = 0.48\)). Based on these relationships, a further increase of the PD above 25% may likely increase the soil pH to above tolerable limits for survival of plants and microorganisms.

**Keywords:** Bioremediation; Total petroleum hydrocarbon; Poly-aromatic hydrocarbons; Poultry droppings; Application rate; Crude-oil-contaminated soil; Southern Nigeria


This study looked at incorporation of iron(III) hexacyanoferrate onto chemically treated pine cone via iron(III) surface loading and its application for cesium (Cs) adsorption in the presence of alkali/alkali earth metals. The optimum iron(III) loading concentration was 2.50 mol\(\text{L}^{-1}\) at pH 7, while optimum hexacyanoferrate (HCF) loading was achieved at a hexacyanoferrate concentration of 0.26 mol\(\text{L}^{-1}\). The best-fitting kinetic model was confirmed using the closeness of the predicted equilibrium capacities to the experimentally determined capacity, three error
determination methods, and the comparative plots of predictive and experimental uptake of Cs with time. The adsorption rate constants were reduced by alkali/alkali metal addition and the reduction was higher in the HCF-modified pine. The mechanism of Cs adsorption onto raw pine followed the pseudo-second-order model and involved stripping of the hydration water from the metal ion. The presence of Na⁺ did not alter the adsorption mechanism but Ca²⁺ addition changed the best-fitting model to pseudo-first-order. The diffusion-chemisorption model best fitted Cs adsorption onto HCF-modified pine and involved adsorption of Cs in its hydrated form, which migrated easily through the zeolite-like lattice of hexacyanoferrate. Addition of Na⁺ and Ca²⁺ changed the best-fitting model to a pseudo-first-order model.

**Keywords:** Cesium; Alkali/alkali earth metals; Cation competition; Pine cone; Iron(III) hexacyanoferrate

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Presumed non-viable high resistant *Pseudomonas aeruginosa* CA207Ni, *Burkholderia cepacia* AL96Co, *Corynebacterium kutscheri* FL108 Hg, and *Rhodococcus* sp AL03Ni were studied for Cd³⁺ adsorption potentials. Moderate temperature, acidic pH, and high ionic strength were required for bacterial-sorption of cadmium, attaining isothermic equilibrium within 20 min. Experimental cadmium-biosorption data fitted well into biosorption isotherms. The adsorption capacities of the bacterial cell masses spanned 0.003–0.009 l mg⁻¹ (Langmuir model) and 0.43–0.68 l mg⁻¹ (Freundlich model), while binding capacity ranged from 1.14 to 56.16 mg gdw⁻¹, with maximum achievable cadmium uptake of 62.07–109.37 mg gdw⁻¹. The bacteria selectively removed the metal at low concentration (100.0 mg l⁻¹) with an efficiency ranging from 50.0% to 80.0%, while approximately 80.0–92.0% removal efficiency was obtained at higher ionic concentrations (450.0 mg l⁻¹). About 92.66% of the adsorbed metal was recovered from strain CA207Ni upon desorption, and approximately 91.7% of Cd³⁺ in solution was re-adsorbed onto the biomasses. In this work, effective feasible biosorption of Cd³⁺ in simulated wastewater system at harsh physico-chemistry, using non-viable resistant bacterial strains was demonstrated. The results indicate that the bacterial strains are sustainable tools for the detoxification of cadmium ions in industrial effluents via wastewater treatment, and cadmium demobilisation in contaminated ecosystem.

**Keywords:** Biosorption; Industrial wastewater; Heavy metal; Cadmium; Resistant bacteria

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**Abstract Vol. No. 25, December 2014**
Plants of the black nightshade (Solanum nigrum L.) Korean ecotype were exposed to a gradient of cadmium (Cd) concentrations (0, 10, 30, 50, and 80 mg kg$^{-1}$ of dry sand). The results showed a significant ($p < 0.05$) reduction in biomass, root-shoot length, and chlorophyll contents in the plants exposed to Cd compared to the control. Cd concentrations significantly increased in different parts of the plants as indicated by inductively coupled plasma mass spectrometry (ICP-MS) analysis. The amount of Cd accumulated by the plants in the leaves, stems, and roots was 307, 1536, and 3163 mg kg$^{-1}$ of dry matter, respectively, when treated with Cd 80 mg kg$^{-1}$. The translocation factor (TF) declined with higher Cd concentrations, whereas the bioconcentration factor (BCF) increased with elevated Cd levels. The response to oxidative stress induced by Cd was modulated by the enzymatic activity of peroxidase and polyphenol peroxidase. In terms of non-enzymatic antioxidant biochemicals such as reduced glutathione and polyphenols, its contents in the leaves significantly increased in a dose-dependent manner. The overall increased antioxidant defense response in leaves might have contributed to the higher accumulation and tolerance of plants against Cd-induced oxidative stress. The Korean ecotype of S. nigrum has potential phytoremediation utility for phytostabilization of Cd-contaminated marginal land. However, further genomic insights could contribute to the identification of potential Cd translocation genes.

Keywords: Cadmium contamination; Bio-concentration; Phytoremediation; Phytostabilization; Solanum nigrum

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The adsorption capacities of six biosorbents (Pseudomonas aeruginosa, Pseudomonas fluorescens, Escherichia coli, Chlorella vulgaris, and Spirulina platensis) for Zn(II) ions under batch condition have been studied. The optimum pH range was found to be 5.0–6.0. The amount of adsorbed Zn(II) ions were between 18 and 128 mg/g. Characterization of biosorption equilibrium was evaluated with Langmuir and Dubinin-Radushkevich model using non-linear regression. The adsorption capacities of Ca-alginate, chitosan, and immobilized Spirulina platensis-maxima cells were also determined in packed-bed column in continuous system. The results show, free Spirulina cells have the highest adsorption capacity for Zn(II) ions (128 mg/g). The chitosan-Spirulina system has slightly decreased adsorption capacity 98 mg/g per dry weight content. Thomas and Yoon-Nelson models were fitted for the evaluation of experimental data.

Keywords: Biosorbents; Zn(II); Ca-alginate; Chitosan; Modeling
Adsorption is one of the best methods for arsenic removal from water which is established in the last few decades. Biosorption by natural biosorbents and agricultural by-product is an environmental friendly approach and has proved to be a cost-effective and non-hazardous technology for the removal of heavy metals from water. This paper describes batch test findings conducted to evaluate the feasibility of using sugarcane bagasse (SCB) as an industrial by-product of sugar industry to remove arsenic (As) from water and compare the results with the efficiency of activated carbon (AC) for arsenic (As) removal. The effects of three parameters, such as pH, adsorbent dosage ($C_a$), and initial metal concentration ($C_0$) on the adsorption of arsenic were evaluated by using response surface methodology (RSM). It is discovered that AC and SCB removed up to ~89 and ~98% of arsenic, respectively. The uptake capacities yielded from the batch experiment were about 31.25 mg/g for AC at pH ~7.4 and 11.9 mg/g for SCB at pH ~9. The equilibrium times achieved were 120 and 150 min for SCB and AC, respectively. This study shows that SCB is an efficient low-cost biosorption for arsenic removal from water.

**Keywords:** Arsenic removal; Activated carbon; Sugarcane bagasse; Adsorption; Response surface methodology (RSM)

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The outstanding biological performance and non-food utilization of bioenergy grass possibly make it to be the best candidate for phytoremediation of heavy metal-contaminated soil, but evidence is limited. In this study, we conducted pot experiments to quantify the performance of two promising energy grasses, Arundo donax and Miscanthus sacchariflorus, in the phytoremediation of Zn- and Cr-contaminated soil. The results showed that (1) the biomass and root length of the two grasses were firstly increased and then kept stable or slightly decreased with increasing soil Zn/Cr concentration, implying that the two grasses had strong tolerance to Zn/Cr contamination; (2) the Zn/Cr concentration in the grass roots was two to seven times of that in the shoots, while both of them were positively correlated with the Zn/Cr concentration in soil; (3) the total accumulation of Zn/Cr in the grass (shoots + roots) was firstly determined by their concentration in the shoots and secondly determined by the shoots’ biomass, indicating that most of the Zn/Cr could be removed from contaminated soil by harvesting the aboveground parts; (4) the accumulating amount of the two grasses for Zn were 17.5 and 12.1 mg plant⁻¹, respectively; while the accumulating amount for Cr were 3.9 and 2.9 mg plant⁻¹, respectively. Taken together, the two energy grasses had strong tolerance and high accumulating ability for Zn/Cr, and therefore, they are promising candidates for the phytoremediation of Zn-/Cr-contaminated soil.
Keywords: Arundo donax; Miscanthus sacchariflorus; Heavy metal contamination; Soil contamination; Soil remediation; Hyperaccumulator


Textile industry is responsible for a large amount of wastewater inappropriate for both human consumption and aquatic species. Aquatic ecosystems are way more sensitive to the release of textile wastewater, and the usage of Winogradsky columns is interesting, once they are a simulated aquatic ecosystem in which the growth of algae and other microorganisms can be observed. In this research, simulated textile effluents with the dye Acid Blue 40 were treated with an electrolytic reactor, for a later ecotoxicological evaluation using Winogradsky columns. The algal and microbial population and primary production were measured. The results have shown that the electrolytic treatment was satisfactory when it comes to color removal, but the presence of the treated effluent in the Winogradsky columns changed the microecosystem. The number of algae identified decreased when exposed to certain effluents, and some algae groups even disappeared, while others such as Cyanophyceae were benefited.

Keywords: Winogradsky; Microalgae; Textile; Electrolysis

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The objective of this study was to develop a remediation strategy for soil co-contaminated with decabromodiphenyl ether (BDE-209) and heavy metals (Cd, Pb and Zn) using co-plantation of the hyperaccumulator plant (Sedum alfredii) with tall fescue (Festuca arundinaceae) associated with a BDE degrader (Bacillus cereus strain JP12).

A 120-day remediation experiment was conducted under greenhouse conditions. S. alfredii and tall fescue were grown in monoculture and intercropped in artificially contaminated soil. Plant biomass, concentration of polybrominated diphenyl ethers, density of soil bacteria, soil enzyme activity, and the physiological profile of the soil microbial community were determined.

Inoculation with JP12 significantly increased BDE-209 dissipation in soil. Phytoextraction of metals was also enhanced by JP12 inoculation due to the improved plant growth. Planting of tall fescue significantly enhanced BDE-209 dissipation as compared to that in the bare soil because of the increased soil microbial activity. Tall fescue showed higher Pb phytoextraction efficiency than S. alfredii, but Pb was principally retained in the roots of tall fescue. BDE-209 dissipation and metal phytoextraction were highest when co-planting S. alfredii with tall fescue inoculated with strain JP12. Pyrosequencing analysis revealed that the inoculated JP12 could functionally adapt to the introduced soil, against competition with indigenous microorganisms in soil.
Co-planting of S. alfredii with tall fescue combined with BDE-degrading bacterial strain JP12 is promising for remediation of soil co-contaminated with BDE-209 and metals.

Keywords: BDE-209; Co-contamination; Co-planting; Mineralization; Pyrosequencing;

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The remediation of copper-contaminated soils by aided phytostabilisation in 16 field plots at a wood preservation site was investigated. The mobility and bioavailability of four potentially toxic trace elements (PTTE), i.e., Cu, Zn, Cr, and As, were investigated in these soils 4 years after the incorporation of compost (OM, 5 % w/w) and dolomite limestone (DL, 0.2 % w/w), singly and in combination (OMDL), and the transplantation of mycorrhizal poplar and willows. Topsoil samples were collected in all field plots and potted in the laboratory. Total PTTE concentrations were determined in soil pore water (SPW) collected by Rhizon soil moisture samplers. Soil exposure intensity was assessed by Chelex100-DGT (diffusive gradient in thin films) probes. The PTTE phytoavailability was characterized by growing dwarf beans on potted soils and analyzing their foliar PTTE concentrations. OM and DL, singly and in combination (OMDL), were effective to decrease foliar Cu, Cr, Zn, and As concentrations of beans, the lowest values being numerically for the OM plants. The soil treatments did not reduce the Cu and Zn mineral masses of the bean primary leaves, but those of Cr and As decreased for the OM and DL plants. The Cu concentration in SPW was increased in the OM soil and remained unchanged in the DL and OMDL soils. The available Cu measured by DGT used to assess the soil exposure intensity correlated with the foliar Cu concentration. The Zn concentrations in SPW were reduced in the DL soil. All amendments increased As in the SPW. Based on DGT data, Cu availability was reduced in both OM and OMDL soils, while DL was the most effective to decrease soil Zn availability.

Keywords: Soil contamination; Copper; Amendment; Phytoavailability; Phytostabilisation; Trace elements

Zea mays (L.) is a crop widely cultivated throughout the world and can be considered suitable for phytomanagement due to its metal resistance and energetic value. In this study, the effect of two plant growth-promoting rhizobacteria, Ralstonia eutropha and Chryseobacterium humi, on growth and metal uptake of Z. mays plants in soils contaminated with up to 30 mg Cd kg$^{-1}$ was evaluated. Bacterial inoculation increased plant biomass up to 63% and led to a decrease of up to 81% in Cd shoot levels (4–88 mg Cd kg$^{-1}$) and to an increase of up to 186% in accumulation in the roots (52–134 mg Cd kg$^{-1}$). The rhizosphere community structure changed throughout the experiment and varied with different levels of Cd soil contamination, as revealed by molecular biology techniques. Z. mays plants inoculated with either of the tested strains may have potential application in a strategy of soil remediation, in particular short-term phytostabilization, coupled with biomass production for energy purposes.

**Keywords:** Zea mays; Soil; PGPR; Phytomanagement; Cadmium; Biomass production; Remediation

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The main purpose of this study was to determine typical concentrations of heavy metals (HM) in wood from willows and poplars, in order to test the feasibility of phytoscreening and phytoextraction of HM. Samples were taken from one strongly, one moderately, and one slightly polluted site and from three reference sites. Wood from both tree species had similar background concentrations at 0.5 mg kg$^{-1}$ for cadmium (Cd), 1.6 mg kg$^{-1}$ for copper (Cu), 0.3 mg kg$^{-1}$ for nickel (Ni), and 25 mg kg$^{-1}$ for zinc (Zn). Concentrations of chromium (Cr) and lead (Pb) were below or close to detection limit. Concentrations in wood from the highly polluted site were significantly elevated, compared to references, in particular for willow. The conclusion from these results is that tree coring could be used successfully to identify strongly heavy metal-polluted soil for Cd, Cu, Ni, Zn, and that willow trees were superior to poplars, except when screening for Ni. Phytoextraction of HMs was quantified from measured concentration in wood at the most polluted site. Extraction efficiencies were best for willows and Cd, but below 0.5% over 10 years, and below 1‰ in 10 years for all other HMs.

**Keywords:** Extraction efficiencies; Phytoremediation; Phytotechnologies; Plant uptake; Soil contamination; Toxic elements; Tree core sampling; Wood


In real environmental applications, such as heavy metal bioremediation, microorganisms are generally not kept at their optimum growth conditions; therefore, it is imperative to investigate their heavy metal removal performance under diverse environmental conditions. The present study aims to investigate the effects of pH, temperature and growth phases on the removal of
Cu$^{2+}$ and Cr$^{6+}$ by two environmental isolates identified as Ochrobactrum intermedium LBr and Cupriavidus metallidurans CH34. Results showed that cells in logarithmic phase presented better biosorption capacity than cells in stationary phase for both isolates. The Cr$^{6+}$ metal was removed more efficiently by live O. intermedium LBr than dead cells; while dead C. metallidurans CH34 biosorbed better than live ones. It was also found that the pH and temperature affected the biosorption capacity. The optimum temperatures were determined to be 37 °C and 27 °C, and the optimum pH values were 6 and 7 for O. intermedium LBr and C. metallidurans CH34, respectively. Additionally, both microorganisms preferentially adsorbed Cu$^{2+}$ in Cu$^{2+}$/Cr$^{6+}$ mixtures. The main mechanism of adsorption was determined to be through carboxylic, hydroxyl, and amino functional groups.

Keywords: Copper; Chromium; Cupriavidus metallidurans CH34; Ochrobactrum intermedium LBr; Biosorption

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This review summarizes the bioremediation and phytoremediation technologies proposed so far to detoxify PCB-contaminated sites. A critical analysis about the potential and limits of the PCB pollution treatment strategies by means of plants, fungi and bacteria are elucidated, including the new insights emerged from recent studies on the rhizosphere potential and on the implementation of simultaneous aerobic and anaerobic biodegradation processes.

The review describes the biodegradation and phytoremediation processes and elaborates on the environmental variables affecting contaminant degradation rates, summarizing the amendments recommended to enhance PCB degradation. Additionally, issues connected with PCB toxicology, actual field remediation strategies and economical evaluation are discussed.

Keywords: Polychlorinated biphenyls; Phytoremediation; Bioremediation; PCB; Biodegradation

Ting Wang$^{a,b}$, Hongwen Sun$^{a,*}$, Hongjun Mao$^b$, Yanfeng Zhang$^a$, Cuiping Wang$^a$, Zhiyuan Zhang$^a$, Baolin Wang$^a$, Lei Sun$^a$. (* MOE Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China, $^b$Urban Transport Emission Control Research Centre, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China). The immobilization of heavy metals in soil by bioaugmentation of a UV-mutant Bacillus subtilis 38 assisted by NovoGro biostimulation and changes of soil microbial community. Journal of Hazardous Materials, Volume 278(2014): 483–490

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Bacillus subtilis 38 (B38) is a mutant species of Bacillus subtilis acquired by UV irradiation with high cadmium tolerance. This study revealed that B38 was a good biosorbent for the adsorption of multiple heavy metals (cadmium, chromium, mercury, and lead). Simultaneous application of B38 and NovoGro (SNB) exhibited a synergetic effect on the immobilization of heavy metals in soil. The heavy metal concentrations in the edible part of the tested plants (lettuce, radish, and soybean) under SNB treatment decreased by 55.4–97.9% compared to the control. Three single extraction methods, diethylenetriaminepentaacetic acid (DTPA), Mehlich 3 (M3), and the first step of the Community Bureau of Reference method (BCR1), showed good predictive capacities for metal bioavailability to leafy, rhizome, and leguminous plant, respectively. The polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) profiles revealed that NovoGro could enhance the proliferation of both exotic B38 and native microbes. Finally, the technology was checked in the field, the reduction in heavy metal concentrations in the edible part of radish was in the range between 30.8% and 96.0% after bioremediation by SNB treatment. This study provides a practical strategy for the remediation of farmland contaminated by multiple heavy metals.

Keywords: Heavy metals; Immobilization; Microbial remediation; PCR–DGGE; Bioavailability prediction

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Algae grown in outdoor reactors (volume: 10 L and depth: 20 cm) were fed directly with filtered and sterilised municipal wastewater. The nutrient removal efficiencies were 86%, 90%, 89%, 70% and 76% for TOC, TN, NH\textsubscript{4}\textsubscript{-N}, TP and OP, respectively, and lipid content varied from 18% to 28.5% of dry algal biomass. Biomass productivity of $\sim$ 122 mg/l/d (surface productivity 24.4 g/m\textsuperscript{2}/d) and lipid productivity of $\sim$ 32 mg/l/d were recorded. Gas chromatography and mass spectrometry (GC–MS) analyses of the fatty acid methyl esters (FAME) showed a higher content of desirable fatty acids (bearing biofuel properties) with major contributions from saturates such as palmitic acid [C16:0; $\sim$ 40%] and stearic acid [C18:0; $\sim$ 34%], followed by unsaturates such as oleic acid [C18:1(9); $\sim$ 10%] and linoleic acid [C18:2(9,12); $\sim$ 5%]. The decomposition of algal biomass and reactor residues with an exothermic heat content of 123.4 J/g provides the scope for further energy derivation.

Keywords: Mixotrophic algal consortia; Lipid; Wastewater treatment; GC–MS; SEM-EDXA

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Contribution rates of factors controlling sludge dewaterability during bioleaching, such as sludge pH, microbial quantity, extracellular polymeric substances (EPS), etc., were investigated in this study. Results showed that the dewaterability of bioleached sludge was jointly enhanced by the growth of *Acidithiobacillus* sp., the increase of Fe³⁺ concentration, the decreases of sludge pH, heterotrophic microorganism quantity change, and the decreases of EPS and bound water contents. Ridge regression analysis further revealed that the contribution rates of microbial quantity change, bound water content and slime EPS content on sludge dewaterability enhancement were 32.50%, 24.24%, and 22.37%, respectively, all of which are dominant factors. Therefore, the enhancement of sludge dewaterability was mainly controlled by microbial quantity change and the decrease of bound water and slime EPS contents during bioleaching.

**Keywords:** Municipal sewage sludge; Bioleaching; Microbial quantity; Dewaterability; Extracellular polymeric substances


This study determined the optimal conditions required to attain maximum metal recovery in the bioleaching process of dewatered metal-plating sludge using *Acidithiobacillus ferrooxidans* (A. ferrooxidans). Adaptation of this strain was carried up to 1% (w/v) of the sample. Three factors including initial pH, initial Fe³⁺ concentration and pulp density were selected as the effective factors and were optimized using a central composite design of response surface methodology. An initial pH of 1, pulp density of 9 g/l and initial Fe³⁺ concentration of 1 g/l were determined to be optimum values by the statistical models. The highest extractions for Cr and Ni under optimal conditions were 55.6% and 58.2%, respectively. Bioleaching kinetics was investigated using a modified shrinking core model to better understand the mechanism of the leaching reaction. The model predictions indicate that the diffusion step controlled the overall dissolution kinetics and is the rate controlling step.

**Keywords:** Bioleaching; Dewatered metal-plating sludge; *Acidithiobacillus ferrooxidans*; Kinetics; Optimization

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In this study, the cometabolic degradation of carbazole (CA), dibenzofuran (DBF), and dibenzothiophene (DBT) by immobilized Arthrobacter sp. W1 cells pregrown with naphthalene was investigated. Four kinds of polymers were evaluated as immobilization supports for strain W1. After comparison with agar, alginate, and κ-carrageenan, gellan gum was selected as the optimal immobilization support. Furthermore, magnetic Fe₃O₄ nanoparticle was selected as most suitable nanoparticle for immobilization and the optimal concentration was 80 mg/L. The relationship between specific degradation rate and the initial concentration of CA, DBF and DBT was described well by Michaelis–Menten kinetics. The recycling experiments demonstrated that the magnetically immobilized cells coupling with activation zeolite showed highly bioremediation activity on the coking wastewater containing high concentration of phenol, naphthalene, CA, DBF and DBT during seven recycles. Toxicity assessment indicated the treatment of the coking wastewater by magnetically immobilized cells with activation zeolite led to less toxicity than untreated wastewater.

**Keywords:** Cometabolic degradation; Heterocyclic compounds; Immobilization; Nanoparticles; Coking wastewater

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A naphthalene-utilizing bacterium, Arthrobacter sp. W1, was used to investigate the cometabolic degradation of carbazole (CA), dibenzofuran (DBF) and dibenzothiophene (DBT) using naphthalene as the primary substrate. Both the growing and washed cells of strain W1 could degrade CA, DBF, DBT, and naphthalene simultaneously and quickly. Inhibition kinetics confirmed that the presence of CA, DBF and DBT in the growing system would inhibit the cells growth and biodegradability of strain W1. The relationship between ln(C/C₀) and time, and specific degradation rate and CA, DBF and DBT concentration could be described well by First-order and Michaelis–Menten kinetics. The treatment of real coking wastewater containing high concentration of phenol, naphthalene, CA, DBF, DBT and NH₃-N was shown to be highly efficient by naphthalene-grown W1 coupling with activation zeolite. Toxicity assessment indicated the treatment of the coking wastewater by strain W1 coupling with activation led to less toxicity than untreated wastewater.

**Keywords:** Cometabolic degradation; Arthrobacter sp. W1; Naphthalene; Heterocyclic compound; Coking wastewater

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**Biotransformation**

Igor Lehnherr. (Department of Earth and Environmental Sciences, University of Waterloo, 200 University Ave. W., Waterloo, ON N2L 3G1, Canada. E-mail : ilehnher@uwaterloo.ca.). Methylmercury biogeochemistry: a review with special reference to Arctic aquatic ecosystems. Environmental Reviews, 22(3)(2014): 229-243
There has been increasing concern about mercury (Hg) levels in marine and freshwater organisms in the Arctic, due to the importance of traditional country foods such as fish and marine mammals to the diet of Northern Peoples. Due to its toxicity and ability to bioaccumulate and biomagnify in food webs, methylmercury (MeHg) is the form of Hg that is of greatest concern. The main sources of MeHg to Arctic aquatic ecosystems, the processes responsible for MeHg formation and degradation in the environment, MeHg bioaccumulation in Arctic biota and the human health implications for Northern Peoples are reviewed here. In Arctic marine ecosystems, Hg(II) methylation in the water column, rather than bottom sediments, is the primary source of MeHg, although a more quantitative understanding of the role of dimethylmercury (DMHg) as a MeHg source is needed. Because MeHg production in marine waters is limited by the availability of Hg(II), predicted increases in Hg(II) concentrations in oceans are likely to result in higher MeHg concentrations and increased exposure to Hg in humans and wildlife. In Arctic freshwaters, MeHg concentrations are a function of two antagonistic processes, net Hg(II) methylation in bottom sediments of ponds and lakes and MeHg photodemethylation in the water column. Hg(II) methylation is controlled by microbial activity and Hg(II) bioavailability, which in turn depend on interacting environmental factors (temperature, redox conditions, organic carbon, and sulfate) that induce nonlinear responses in MeHg production. Methylmercury bioaccumulation—biomagnification in Arctic aquatic food webs is a function of the MeHg reservoir in abiotic compartments, as well as ecological considerations such as food-chain length, growth rates, life-history characteristics, feeding behavior, and trophic interactions. Methylmercury concentrations in Arctic biota have increased significantly since the onset of the industrial age, and in some populations of fish, seabirds, and marine mammals toxicological thresholds are being exceeded. Due to the complex connection between Hg exposure and human health in Northern Peoples—arising from the dual role of country foods as both a potential Hg source and a nutritious, affordable food source with many physical and social health benefits—reductions in anthropogenic Hg emissions are seen as the only viable long-term solution.

**Keywords:** mercury, methylation, Arctic, bioaccumulation, freshwater, marine


Microbial fuel cells (MFCs) are a device using microorganisms as biocatalysts for transforming chemical energy into bioelectricity. As soil is an environment with the highest number of microorganisms and diversity, we hypothesized that it should have the potential for energy generation. The soil used for the study was Mollic Gleysol collected from the surface layer (0–20 cm). Four combinations of soil MFC differing from each other in humidity (full water holding capacity [WHC] and flooding) and the carbon source (glucose and straw) were constructed. Voltage (mV) and current intensity (µA) produced by the MFCs were recorded every day or at 2-day intervals. The fastest and the most effective MFCs in voltage generation (372.2 ± 5 mV) were those constructed on the basis of glucose (MFC-G). The efficiency of straw MFCs (MFC-S) was noticeable after 2 weeks (319.3 ± 4 mV). Maximal power density.
Abstract Vol. No. 25, December 2014

The biotransformation of lignin components during co-composting of sewage sludge-activated palm tree waste was studied for six months using Py-GC-MS. Two main groups of compounds were distinguished. The first includes 7 compounds which occurred during the co-composting process listed here in decreasing order of abundance: toluene, 2,4-dimethylbenzene, ethylbenzene, styrene, 1-ethyl-2-methylbenzene, 4-methylphenol and 2-methylnaphthalene. The reduction of their concentrations during the process is due to metabolization and biotransformation into other compounds. A second group of 4 components showed concentrations that increased with co-composting time: phenol, benzofuran, ethylmethoxyphenol and dimethoxyphenol. These lignin constituents are probably released parallel with lignin degradation to become incorporated into humic substances.

Keywords: Biotransformation of lignin; Sewage sludge activated-palm tree waste; Py-GC-MS; Humic substances

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Thermally-enhanced bioremediation is a promising treatment approach for petroleum contamination; however, studies examining temperature effects on anaerobic biodegradation in zones containing light non-aqueous phase liquids (LNAPLs) are lacking. Herein, laboratory microcosm studies were conducted for a former refinery to evaluate LNAPL transformation, sulfate reduction, and methane generation over a one-year period for temperatures ranging from 4 to 40 °C, and microbial community shifts were characterized. Temperatures of 22 and 30 °C significantly increased total biogas generation compared to lower (4 and 9 °C) and higher temperatures (35 and 40 °C; p < 0.1). Additionally, at 22 and 30 °C methane generation commenced ~6 months earlier than for 35 and 40 °C. Statistically significant biodegradation of benzene, toluene and xylenes was observed at elevated temperatures but not at lower temperatures (p < 0.1). Additionally, a novel differential chromatogram approach was developed to overcome challenges associated with resolving losses in complex mixtures of hydrocarbons, and application of this method revealed greater losses of hydrocarbons at 22 and 30 °C as compared to lower and higher temperatures. Finally, molecular biology assays revealed that the

(P_max = 32 mW m⁻²) was achieved by the MFC-G at current density (CD) of 100 mA m⁻². Much lower values of P_max (10.6–10.8 mW m⁻²) were noted in the MFC-S at CD of ca. 60–80 mA m⁻². Consequently, soil has potential for production of renewable energy.

Keywords: Microbial fuel cell; Soil; Electricity generation; Soil microorganisms

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composition and activity of microbial communities shifted in a temperature-dependent manner. Collectively, results demonstrated that anaerobic biodegradation processes can be enhanced by increasing the temperature of LNAPL-containing soils, but biodegradation does not simply increase as temperature increases likely due to a lack of microorganisms that thrive at temperatures well above the historical high temperatures for a site. Rather, optimal degradation is achieved by holding soils at the high end of, or slightly higher than, their natural range.

**Keywords:** Biodegradation; LNAPL; Petroleum hydrocarbons; Pyrosequencing; Thermal enhancement

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The white-rot fungi *Irpex lacteus* KB-1.1 and *Lentinus tigrinus* LP-7 have been shown in previous studies to have high biobleaching activity in vivo. The aim of this study was to investigate the activities and stabilities of extracellular enzymes, prepared from *I. lacteus* and *L. tigrinus* culture grown in three types of economical media of agricultural and forestry wastes, for biobleaching of *Acacia* oxygen-delignified kraft pulp using kappa number reduction as an indicator of delignification. After 3 days of incubation, the extracellular enzymes preparations from *I. lacteus* and *L. tigrinus* cultures in media of *Acacia mangium* wood powder supplemented with rice bran and addition 1% glucose (WRBG), resulted in significant decrease of 4.4 and 6.7 %, respectively. A slightly higher kappa number reduction (7.4 %) was achieved with the combine extracellular enzymes from *I. lacteus* and *L. tigrinus*. One of the strategies for reducing the cost of enzyme production for treatment processes in the pulp and paper industry is the utilization of agricultural and forestry waste. Thus, WRBG has potential as a culture medium for producing stable lignolytic enzymes simply and economically.

**Keywords:** *Acacia* kraft pulp; Biobleaching; Economical medium; Extracellular enzymes


This study examines the potential for use of biogenic sulphuric acid for leaching of a low-grade Brazilian ore containing 0.64% copper occurring as brochantite, Cu₄SO₄(OH)₆, and as malachite, Cu₂CO₃(OH)₂. Ore was agglomerated with elemental sulphur and sulphur-oxidizing microorganisms. Copper extraction was more efficient from inoculated ore amended with 6.67 kg S°/t of ore, compared to uninoculated ore with no added S° and leached with an acid solution maintained at pH 2.0 with sulphuric acid. The rate and extent of copper bioleaching were proportional to the amount of added S°. A maximum of 88% copper was extracted after
9 weeks of bioleaching with 13.3 kg S added/t of ore. Cu extraction was more efficient if sulphur was uniformly mixed with the ore than if the sulphur was added only to the upper zone of the ore column. There was no evidence that acid leaching of Cu from this ore was far more efficient in ore crushed by a high pressure grinding rolls (HPGR) compared to a conventional jaw crusher.

**Keywords:** Bioleaching; Sulphuric acid leaching; Biooxidation of sulphur; Copper bioleaching

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Ferric iron (Fe$^{3+}$) is an important low-cost oxidant in hydrometallurgical processing. High-rate iron oxidation and removal of iron and sulphate from solution at low pH and ambient temperature and pressure were successfully demonstrated at laboratory scale. A two-stage continuous stirred tank reactor (CSTR) system operated with a mixed culture of mesophilic iron oxidisers was fed with a range of influent solutions (pH 1.0–2.2) containing (g L$^{-1}$): 15 Fe$^{2+}$, 1.5 Cu, 1.5 Ni, nutrients and trace elements. An overall iron oxidation efficiency of 96–99% was achieved with rates of 1.0–1.1 g L$^{-1}$ h$^{-1}$. The two-stage sequential reactor design permitted optimisation of overall oxidation kinetics by facilitating the growth of low (460–480 mV vs Ag/AgCl) and high (530–700 mV) redox potential iron-oxidisers in the respective reactors. Molecular methods confirmed that Acidithiobacillus ferrooxidans was able to thrive in the two-stage system along with Leptospirillum ferriphilum despite the high redox potential in the final effluent. Iron and sulphate precipitation rates and efficiencies increased with increasing influent pH. The effluent pH of the CSTR system, where acid consuming iron oxidation and acid producing iron precipitation reactions were balanced, was found to be at 2.05–2.06. The percentages of influent Fe, S, Cu and Ni removed as precipitates were 8.2–54%, 3.7–33%, 0.25–2.5% and 0.01–0.26%, respectively, with the amount depending on the influent pH. The two-stage CSTR system for bio-catalysed iron oxidation and precipitation is promising as a unit process for a variety of hydrometallurgical processes, as it allows iron removal from ferrous solutions without the requirement for additional neutralising chemicals and has low losses of base metals due to co-precipitation. This work demonstrates that a high rate of iron oxidation and removal can be achieved without the use of a physical growth support matrix, thereby facilitating continuous removal of iron and sulphate which, in turn, enables the process to be readily scaled up.

**Keywords:** Continuous stirred tank reactor; Ferric iron regeneration; Biological iron oxidation; Iron precipitation; Microbial community

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Colchicinoids into Their Corresponding 3-O-Glucosyl Derivatives by Selected Strains of *Bacillus megaterium*. Molecular Biotechnology, Volume 56(7) (2014): 653-659

Natural colchicinoids and their semisynthetic derivatives are important active ingredients for pharmaceutical applications. Thiocolchicoside (3-demethoxy-3-glucosyloxythiocolchicine) is used in several countries as standard therapy for the treatment of diseases of the muscle–skeletal system, due to its potent antiinflammatory and myorelaxant properties. Manufacturing of thiocolchicoside requires a key step, the regioselective demethylation and glucosylation of chemically derivative thiocolchicine. High selectivity and efficiency of this transformation cannot be achieved in a satisfactory way with a chemical approach. In particular, the chemical demethylation, a part requiring toxic and aggressive reagents, generates a complex mixture of products with no industrial usefulness. We report herein an efficient, direct and green biotransformation of thiocolchicine into thiocolchicoside, performed by a specific strain of *Bacillus megaterium*. The same process, with minor modifications, can be used to convert the by-product 3-O-demethyl-thiocolchicine into thiocolchicoside. In addition, we describe the *B. megaterium* strain selection process and the best conditions for this effective double biotransformation. The final product has a pharmaceutical quality, and the process has been industrialised.

**Keywords:** Colchicine; Thiocolchicoside; Demethylation; Glucosylation; Biotransformation; Fermentation; Bacillus megaterium

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Dihydroxylation of dehydroepiandrosterone (DHEA) is an essential step in the synthesis of many important pharmaceutical intermediates. However, the solution to the problem of low biohydroxylation conversion in the biotransformation of DHEA has yet to be found. The effects of natural oils on the course of dihydroxylation of DHEA to 3β,7α,15α-trihydroxy-5-androsten-17-one (7α,15α-diOH-DHEA) were studied. With rapeseed oil (2%, v/v) addition, the bioconversion efficiency was improved, and the 7α,15α-diOH-DHEA yield was increased by 40.8% compared with that of the control at DHEA concentration of 8.0 g/L. Meantime, the ratio of 7α,15α-diOH-DHEA to 7α-OH-DHEA was also increased by 4.5 times in the rapeseed oil-containing system. To explain the mechanism underlying the increase of 7α,15α-diOH-DHEA yield, the effects of rapeseed oil on the pH of the bioconversion system, the cell growth and integrity of *Gibberella intermedia* CA3-1, as well as the membrane composition were systematically studied. The addition of rapeseed oil enhanced the substrate dispersion and maintained the pH of the system during bioconversion. Cells grew better with favorable integrity. The fatty acid profile of *G. intermedia* cells revealed that rapeseed oil changed the cell membrane composition and improved cell membrane permeability for lipophilic substrates.
Keywords: Biotransformation; Gibberella intermedia CA3-1; Hydroxylation; Dehydroepiandrosterone; Natural oils

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The alginate extraction products from Brazilian brown seaweed \textit{Sargassum filipendula} were studied for chromium biosorption. Batch experiments were conducted at pH 2 and 3 and 20 °C to determine the sorption capacity of this biosorbents for chromium (VI) and (III). The biomass was characterized before and after metal binding by X-ray photoelectron spectroscopy (XPS) in order to determine the mechanisms of chromium biosorption. The residue has a high adsorption capacity, close the value obtained with seaweed and higher than that of alginate for both Cr(III) and Cr(VI). XPS analysis of the biosorbents revealed that carboxyl, amino and sulfonate groups are responsible for the binding of the metal ions. The analysis also indicated that the Cr(VI) bound to the biomass was reduced to Cr(III).

Keywords: Biosorption; Seaweed; Alginate-extraction; Chromium; XPS

**Biomarker**

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Studies characterizing the sources of organic matter (OM) to the west coast of India (WCI) and its continental shelf are limited. This study examined sedimentary OM in 35 estuaries along the WCI using molecular biomarkers (lignin phenol), elemental ratio (C/N), and stable carbon isotope (δ\textsuperscript{13}C) values. Multivariate statistical techniques, such as cluster analysis, identified similar sedimentary chemical properties among the estuaries and their distribution patterns highlight the strong control of geographical provenance on sedimentary OM composition from south to north along the WCI. Results of an end-member mixing model reveal that terrigenous sources (C\textsubscript{3} plants, C\textsubscript{4} plants, and soil) contribute ~80% of estuarine OM, with the remaining 20% derived from marine sources (marine plankton and estuarine macrophytes). In the estuaries of large rivers, such as the Narmada and Sabarmati rivers, C\textsubscript{4} plants and soil OM were found to be the dominant contributors of OM, which is likely the result of an abundance of C\textsubscript{4} vegetation and agriculture in their catchment areas. High OC (organic carbon content) of sediments (0.5-5%) from the WCI estuaries indicates that large amounts of OM are present in the sediments.
The sources of OM (plant and soil) shift substantially throughout the study area, corresponding to changes in land use patterns along the Western Ghats. Sediments with low nitrogen contents (C/N>15-20) and degraded lignin ((Ad/Al)=0.4-0.6 and DHBA/V=0.16-0.34) were observed in all estuaries, indicating humification and/or degradation of OM originating from terrestrial plants (bio-degradation) and soil (de-mineralization). The collective results of this study illustrate the benefits of using biomarkers (lignin phenols) along with C/N and δ\(^{13}\)C values for evaluating land use changes and the impacts of land use changes on aquatic ecosystems.

**Keywords:** Biomarkers; Carbon isotopes; India; Organic matter; Sediments; West coast

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The present study was undertaken to investigate the degradation and removal of direct yellow 9 (DY9) by the electro-Fenton (EF) process in batch reactor using iron and stainless steel electrodes. DY9 removal decreased with the increase in pH (3 to 8) and increased with the increase in current intensity (0.05 to 0.2A) and [H\(_2\)O\(_2\)] (0 to 0.5gL\(^{-1}\), but not with high doses which led to low rates of DY9 removal and OH\(^{-}\) uptake). The regression quadratic models describing DY9 degradation yield "R (percent)" and electrical energy consumption "EEC (kWhkg\(^{-1}\))" were validated by the analysis of variance (ANOVA) and were both noted to fit well with the experimental data. The R\(^2\) correlation coefficients (0.995, 0.978), those adjusted coefficients (0.986, 0.939), and F values (110.7, 24.9) obtained for the responses validated the efficiency of model. The results revealed that among several other parameters, EEC depended essentially on the degradation yield. The eco-toxicity tests showed a positive correlation between catalase activity and DY9 concentration, and catalase could be qualitatively identified to assess the effect of dye and its by-products generated during the EF process.

**Keywords:** Biomarker; Catalase; DY9; Electro-Fenton; RSM

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Carboniferous source rocks have been gaining increasing attention after the discovery of the Kelameili gasfield in the Eastern Junggar Basin in 2005. Two sets of source rocks within the Lower Carboniferous Dishuiquan Formation (C\(_{1d}\)) and Upper Carboniferous Batamayineishan Formation (C\(_{2h}\)), respectively, have been identified in this area. In this paper, clear differences between these two source rocks are demonstrated in molecular and carbon isotopic
compositions. The C_{1d} source rocks, in comparison with the C_{2b} source rocks, have remarkably lower Pr/
\text{-}C_{17} ratio, higher Ts/(Ts+Tm) and C_{30} dihophane/(C_{30} dihophane+C_{30} hopane) ratios, lower concentration of C_{24} tetracyclic terpane relative to C_{23} and C_{26} tricyclic terpanes and higher
gammacerane/C_{31} hopane ratio. $\delta^{13}$C values of individual n-alkanes decrease with carbon number for C_{1d} source rocks while the opposite is true for C_{2b} source rocks. Among the 10 oils collected from the studied area, four were generated exclusively from C_{1d} source rocks while the others were mainly derived from C_{1d} source rocks but contaminated by a small or trace amount of oil components which were derived from the C_{2b}, Middle Permian Pingdiquan Formation (P_{2p}) and Lower Jurassic source rocks. The free, adsorbed and inclusion oils from three oil-containing volcanic reservoir rocks within the C_{2b} formation generally correlate with C_{1d} source rocks based on molecular and carbon isotopic data. However, the three types of oil from the same reservoir rock vary significantly in molecular and carbon isotopic compositions, reflecting facies and maturity changes of charging oil during the reservoir filling.

**Keywords:** Biomarker; Carbon isotope; Carboniferous source rocks; Eastern Junggar Basin

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The blue mussel Mytilus galloprovincialis has been used as monitoring organism in many biomonitoring programs because of its broad distribution in South European sea waters and its physiological characteristics. Different pollution-stress biomarkers, including gene expression biomarkers, have been developed to determine its physiological response to the presence of different pollutants. However, the existing information about basal expression profiles is very limited, as very few biomarker-based studies were designed to reflect the natural seasonal variations. In the present study, we analyzed the natural expression patterns of several genes commonly used in biomonitoring, namely ferritin, metallothionein, cytochrome P450, glutathione S-transferase, heat shock protein and the kinase responsive to stress KRS, during an annual life cycle. Analysis of mantle-gonad samples of cultured populations of M. galloprovincialis from the Delta del Ebro (North East Spain) showed natural seasonal variability of these biomarkers, pointing to temperature and oxidative stress as major abiotic modulators. In turn, the reproductive cycle, a process that can be tracked by VCLM7 expression, and known to be influenced by temperature, seems to be the major biotic factor involved in seasonality. Our results illustrate the influence of environmental factors in the physiology of mussels through their annual cycle, a crucial information for the correct interpretation of responses under stress conditions.

**Keywords:** Biomonitoring; Gene expression; Mytilus galloprovincialis; qRT-PCR; Seasonal variations

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The present study documents individual and combined sub-lethal effect of one redox active (copper) and one non-redox active (cadmium) metal on green mussel (*Perna viridis*). The mussels were exposed to 60 µg L\(^{-1}\) of Cu and 150 µg L\(^{-1}\) of Cd (individually and in combination) for 21 days. Histopathological and ultrastructural studies revealed significant metal induced alterations such as vacuolization, fusion of gill lamellae, enhance mucous deposition, hyperplasia and necrosis in gills. Antioxidant enzyme assays revealed significant increase in superoxide dismutase (SOD), glutathione S-transferase (GST) and glutathione peroxidase (GPx) activity. Similarly, single exposure to Cd and Cu caused significant induction in Malate dehydrogenase (MDH) activity. However, combined Cu+Cd exposure modulated suppression in MDH activity. Unlike MDH, Cu and Cd individual exposure resulted in a decrease in esterase (EST) activity, but their combined exposure caused an induction. Non-enzymatic biomarkers such as lipid peroxidation (LPO) and metallothionein (MT) levels showed no significant change in response to Cu exposure, whereas, individual Cd exposure or Cd exposure in combination with Cu caused significant changes in their levels. Comet assay revealed a significant increase in DNA damage upon metal exposure. These results indicate that Cu (redox active) and Cd (non-redox active) can induce measurable physiological, biochemical as well as genotoxic perturbations in mussels even at sub-lethal concentrations. A monitoring programme based on the biomarkers discussed here would be useful to study the effect of metal pollutants reaching the coastal waters.

**Keywords:** Antioxidant enzymes; biomarker; DNA damage; genotoxicity; histology; metal-metal interaction; *Perna viridis*; sub-lethal toxicity; ultrastructure


Facies, biomarker and stable isotopic records from the Miocene lacustrine sediments in the northwestern Qaidam basin were investigated to reconstruct the Miocene sedimentary environment and climatic history. Three distinct facies can be recognized. These include the following: (1) gray-black laminated mudstone and marlstone, which represent a semi-deep fresh to semi-brackish lake environment; (2) gray, yellowish massive mudstone, marlstone and siltstone; and (3) yellowish massive sandstone, which imply a shallow brackish lake environment. The decreasing C\(_{27}/\)C\(_{31}\) and (C\(_{27}+\)C\(_{29}\))/(C\(_{31}+\)C\(_{33}\)) values, the increasing ACL (mean
chain length) values of n-alkanes and the vertical evolution of sedimentary environments indicate the overall intensified aridity, which is considered to be an integrated result of high elevation of the Himalaya-Tibetan system, retreat of the Paratethys and global cooling. High fluctuations of the δ18O values and primary dolomite contents reveal the hydrologically closed paleidam basin with intermittently open conditions in the study area during middle-late Miocene. The Qaidam basin is suggested to be hydrologically segmented, based on the stable isotopic data comparison between the study area and the northeastern area. The most negative end of the oscillations of the δ18O values (indicating the minimal evaporation), which likely represents the isotopic ratio of the meteoric water, surprisingly conveys stability in the Shang Youshashan and Shizigou Formations and displays a positive ~2.5‰ shift. This significant shift was probably due to the climatic aridification and air mass changes around 10-8Ma rather than the global cooling.

**Keywords:** Aridity; Lacustrine carbonate; Organic matter; Oxygen isotope; Qaidam basin; Tibetan plateau

Liu, J.-L. , Xu, X.-R. , Yu, S. , Cheng, H. , Peng, J.-X. , Hong, Y.-G. , Feng, X.-B. . ( Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of SciencesGuangzhou, China, Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of SciencesXiamen, China, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of SciencesGuangzhou, China, State Key Laboratory of Tropical Oceanography, South China Sea Institute of Oceanology, Chinese Academy of SciencesGuangzhou, China, State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of SciencesGuiyang, China, University of Chinese Academy of SciencesBeijing, China). Mercury contamination in fish and human hair from Hainan Island, South China Sea: Implication for human exposure. Environmental Research, Volume 135(2014): 42-47

Hair has long been recognized as a good biomarker for human exposure to Hg. The mercury concentrations in 14 species of marine fish and hair samples from 177 coastal residents in Hainan, South China Sea were investigated to assess the status of mercury exposure associated with marine fish consumption. Concentrations of total Hg (THg) and methylmercury (MeHg) in the fish muscles were 0.094±0.008 and 0.066±0.006. µg/g. ww, respectively, which were far below the limit considered safe for consumption (0.5. µg/g). The average THg concentrations in hair of adults (1.02±0.92. µg/g) were lower than the provisional tolerable weekly intake (PTWI) level of 2.2. µg/g. However, 23.7% of children had a hair THg level exceeding the RfD level of 1. µg/g, indicating a great risk of Hg exposure to children via fish consumption. The concentration of THg in hair was significantly correlated with fish consumption but not with gender-specific fish intake. With higher fish consumption frequency, the fishermen had significantly elevated hair Hg levels compared to the students and the other general public, who had similar hair THg levels but different fish consumption patterns, indicating the existence of other sources of Hg exposure to the residents of Hainan Island.

**Keywords:** Dietary exposure; Fish consumption; Human hair; Mercury pollution; South China Sea

Marasinghe Wadige, C.P.M. , Taylor, A.M., Maher, W.A., Krikowa, F. ( Ecochemistry Laboratory, Institute for Applied Ecology, University of CanberraCanberra, ACT, Australia). Bioavailability and toxicity of zinc from contaminated freshwater sediments:

To evaluate the use of the freshwater bivalve Hyridella australis as a potential biomonitor for zinc contamination in freshwater sediments, the bioavailability and toxicity of zinc contaminated sediments (low 44. ± 5, medium 526. ± 41, high 961. ± 38. µg/g dry mass) were investigated in laboratory microcosms for 28 days by examining H. australis exposure-dose-response relationships. Zinc concentrations in sediments and surface waters were measured as zinc exposure. Zinc in whole organism soft body tissues and five individual tissues were measured as organism zinc dose. Sub-cellular localisation of zinc in hepatopancreas tissues was investigated to further understand the zinc handling strategies and tolerance of H. australis. Total antioxidant capacity, lipid peroxidation and lysosomal membrane stability were measured in hepatopancreas tissues as zinc induced biomarker responses. Accumulated zinc concentrations in whole body tissues of H. australis reflected the zinc exposure and exhibited exposure dependent zinc accumulation at day 28. Gills accumulated significantly higher zinc concentrations than other tissues, however, no significant differences in zinc accumulation between treatments were detected for any of the individual tissues analysed. Analysis of individual tissue zinc concentrations, therefore, may not offer any advantages for monitoring bioavailable zinc in freshwater environments with this organism. Relationships between tissue zinc and calcium concentration suggest accumulation of zinc by H. australis may have occurred as an analogue of calcium which is a major constituent in shell and granules of unionid bivalves. A high percentage of accumulated zinc in the hepatopancreas tissues was detoxified and stored in metallothionein like proteins and metal rich granules. Of the zinc accumulated in the biologically active metal pool, 59-70% was stored in the lysosome. +. microsome fraction. At the concentrations tested, increasing zinc exposure resulted in decreasing total antioxidant capacity and measurable increases in the sublethal effects, lipid peroxidation and lysosomal membrane destabilisation, were observed. Based on exposure-dose analysis, H. australis partially regulates zinc uptake and weakly exhibits bioavailability of zinc in freshwater environments, however, exposure-response analysis shows zinc induced toxicological effects, suggesting the potential of this organism as a biomonitor for zinc in heavily contaminated freshwater environments.

**Keywords:** Biologically active zinc; Biologically detoxified zinc; Biomarkers; Freshwater bivalve; Oxidative stress; Sub-cellular partitioning

**Biofertilizer**

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The extensive research, production and use of microorganisms to improve plant nutrition have resulted in an inconsistent definition of the term “biofertiliser” which, in some cases, is due to the different microbial mechanisms involved. The rationale for adopting the term biofertiliser is that it derives from “biological fertiliser”, that, in turn, implies the use of living microorganisms.
Here, we propose a definition for this kind of products which is distinguishing them from biostimulants or other inorganic and organic fertilisers. Special emphasis is given to microorganism(s) with multifunctional properties and biofertilisers containing more than one microorganism. This definition could be included in legal provisions regulating registration and marketing requirements. A set of rules is also proposed which could guarantee the quality of biofertilisers present on the market and thus foster their use by farmers.

**Keywords:** PGPR; Mycorrhizal fungi; Rhizosphere; Regulation; Production standards

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The development of communities of three important composting players including actinobacteria, fungi and clostridia was explored during the composting of wheat straw for mushroom production. The results revealed the presence of highly diversified actinobacteria and fungal communities during the composting process. The diversity of the fungal community, however, sharply decreased in the mature compost. Furthermore, an apparent succession of both actinobacteria and fungi with intensive changes in the composition of communities was demonstrated during composting. Notably, cellulolytic actinomycetal and fungal genera represented by *Thermopolyspora*, *Microbispora* and *Humicola* were highly enriched in the mature compost. Analysis of the key cellulolytic genes revealed their prevalence at different composting stages including several novel glycoside hydrolase family 48 exocellulase lineages. The community of cellulolytic microbiota also changed substantially over time. The prevalence of the diversified cellulolytic microorganisms holds the great potential of mining novel lignocellulose decomposing enzymes from this specific ecosystem.

**Keywords:** Mushroom compost; Actinobacteria; Fungi; Cellulase

### Biocomposting

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Vermicomposting of organic waste has an important part to play in an integrated waste management strategy. The aim of the present study was to investigate the ability of an epigeic earthworm *Eisenia fetida* to transform anaerobically digested sewage sludge (SS) amended with hazelnut husk (HH) and cow manure (CM) in different proportions under laboratory conditions (in darkness at 25°C ± 0.5°C). Three approaches investigated in the study were: (1) to find the
best medium for growth and reproduction of *E. foetida* in different feed mixtures, (2) to analyze the heavy metal concentrations in different feed mixtures of SS&HH;CM before and after vermicomposting, and (3) to explore heavy metals accumulation of earthworms in sewage sludge with different feed mixtures. Number and biomass of earthworms and heavy metal contents in feed mixtures and earthworms were periodically monitored. The results indicated that maximum earthworm biomass was attained in a feed mixture of 20% SS + 40% CM + 40% HH, while the earthworm number was highest in a feed mixture of 30% SS + 35% CM + 35% HH during the vermicomposting period. Heavy metals concentration (Zn, Cu, Cd, Pb, Ni, and Cr) in all feed mixtures decreased associated with the increasing vermicomposting time. The heavy metals’ content in the feed mixtures was lower than that of initial mixtures. Metal analysis of earthworms revealed considerable bioaccumulation of heavy metals in their bodies’ tissue. Heavy metal analysis of earthworm body showed that increasing proportion of SS in the feed mixtures promoted the heavy metal content of earthworm body.


Earthworm digested wastes (vermicompost) are being produced in increasing quantities to make farming sustainable. A study was carried out for two consecutive years (2007–09) at the Agricultural Experimental Farm of Indian Statistical Institute, Giridih, India on sandy loam soil in factorial randomized block design with three replications. Baby corn (cv. Early Composite) was grown without vermicompost (V₀) or with vermicompost (V₁: @ 10 Mg ha⁻¹) in combination with three recommended doses of fertilizers [F₁: 50%, F₂: 100% (N:P₂O₅:K₂O = 150:60:60 kg ha⁻¹) and F₃: 150% RDF] besides an absolute control (F₀: no-NPK) to assess their effect on baby corn productivity and soil health. Vermicompost applied plots recorded considerably higher cob (0.717 Mg ha⁻¹) and green fodder (17.58 Mg ha⁻¹) yield. Among the fertilizers, baby corn grown with F₃ yielded maximum cob (0.759 Mg ha⁻¹) and green fodder (18.46 Mg ha⁻¹). Vermicompost application built-up soil nutrient like nitrogen (145 kg ha⁻¹), phosphorus (16 kg ha⁻¹), potassium (190 kg ha⁻¹), organic carbon (0.78%), and enhanced cation exchange capacity (12.19 Cmol·kg⁻¹), microbial [basal soil respiration, microbial biomass carbon, microbial quotient, and metabolic quotient] and enzyme activities (urease and acid phosphatase). However, microbial and enzyme activities were minimum with F₃. Vermicompost and F₂ treatments were most remunerative. Use of vermicompost not only reduces the requirement of chemical fertilizers but also supplements important all essential nutrients to increase crop yield besides improving the soil properties and processes.


*Lantana camara* is an evergreen, which is the most notorious toxic weed of the terrestrial ecosystem. It is native to subtropical and tropical America, but a few taxa are indigenous to tropical Asia and Africa. An enormous quantity of green foliage is produced by this weed, which cannot be used as livestock feed due to its toxic properties. Management through utilization
seems the only sustainable option for this problem. In this study, the composting of Lantana biomass was done and changes in chemical characteristics of waste biomass were measured. The composting caused decreases in pH, organic carbon, C:N ratio, K and C by 2.0-, 1.25-, 1.66-, and 19-fold, respectively, but increases in electrical conductivity (EC), ash content, P, Zn, and Mg of 2.0-, 1.11-, 3.36-, 1.76-, 1.28-, and 1.70-fold, respectively. The C/N ratio (20.1) and soil respiration rate (47.12–66.20 mg CO$_2$-C/100 g) suggested the compost maturity at 52 days. The high bacterial (38.67 CFU × 10$^{-7}$ g$^{-1}$), fungal (30.0 CFU × 10$^{-3}$ g$^{-1}$), and actinomyces (32.0 CFU × 10$^{-5}$ g$^{-1}$) population in composted material suggested the suitability of compost for agronomic purposes. Phytotoxicity measured through compost:water extract and compost pot trial suggested the germination index (GI) in the ranges of 52.3%–122.3% and 74.5%–166.9%, respectively. The high ranges of chlorophyll, protein, and carotenoids in seedling than control suggested the non-toxicity of ready materials. Results suggested that composting can be a potential technology to manage Lantana biomass for sustainable land fertility management programs.


This work investigates the impact of municipal solid waste compost (MSW-compost) application (0, 50, and 100 t/ha) on the growth, and on nutrient and trace elements content in lettuce and tomato plants grown in large, 40-L pots. Our findings showed inhibition of plants’ growth with increasing dose of MSW-compost, compared to plants receiving conventional fertilization. Growth inhibition was associated with a sharp decrease in soil NO$_3$–N content. On the other hand, a slower decrease in soil NO$_3$–N content occurred in non-planted pots amended with MSW-compost. These findings provide evidence that N immobilization and/or decreased N mineralization were responsible for inhibited growth by constraining N availability. With regard to the other macro-nutrients, K, P, Mg, Ca, and Fe, their contents in leaves of both crops were maintained at optimum levels. Higher zinc and copper content was measured in leaves of both crops but they did not exceed the optimum range for growth. No accumulation of trace elements was found in the fruits. The content of heavy metals in the tissues of plants grown in MSW-compost amended soil, remained at levels similar to those of the non-amended soil, suggesting that they do not pose a significant risk either for plant growth or public health. The findings of our study suggest that further emphasis should be given on the investigation of the factors regulating N mineralization and availability in order to avoid reductions in crop yield.

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The aerial parts of invasive crofton weed (Eupatorium adenophorum) in Southwest China were used as the main material and fermented by microbial inoculant Bacillus subtilis to produce bio-organic fertilizer. Results showed that the allelochemicals of crofton weed had no significant negative effect on the activity of B. subtilis. No seeds of crofton weed germinated during the composting period and thereafter. Compared with uncomposted crofton weed, the nitrogen and
phosphorus contents in composted crofton weed increased. In addition, the main allelochemical of composted crofton weed, i.e., 9-β-hydroxy-ageraphorone, was significantly reduced. The potassium content remained stable. Furthermore, tomatoes cultivated with bio-organic fertilizer grew better in terms of plant height than those cultivated with uncomposted crofton weed and ordinary red soil. Therefore, crofton weed can be used to produce bio-organic fertilizers, thereby controlling crofton weed infestation.

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Growth responses of potted ornamental crops to municipal biosolids in the semiarid southwestern USA are not adequately known. In 10- to 11-wk greenhouse pot studies, we evaluated the effects of dried biosolids-amended growing media on four ornamental crop species: Garden chrysanthemum (Dendranthe Xgrandiflorum ‘Megan’), butterfly bush (Buddleia davidii ‘Nanho Blue’), Japanese honeysuckle (Lonicera japonica ‘Purpurea’), and blanket flower (Gaillardia Xgrandiflora ‘Goblin’). The biosolids were composted without bulking agents (100% sewage sludge) and incorporated into growing media at rates ranging from 0 to 593 kg m⁻³, or 0 to 72% by volume. Biosolids increased substrate pH from 5.8 to 7.2 and electrical conductivity (EC) from 2.6 to 47.3 dS m⁻¹. Any addition of biosolids (≥30 kg m⁻³) reduced total plant dry matter (DM) of chrysanthemum. Conversely, shoot DM of blanket flower and butterfly bush increased by four- to five-fold at biosolids rates of 59 to 148 kg m⁻³ (7 to 18% by volume) with corresponding increases in shoot N and P concentrations. Biosolids rates higher than 148 kg m⁻³ reduced top growth of the latter two species and of Japanese honeysuckle. For all species, growth reductions with excessive biosolids rates likely resulted from osmotic stress and specific NH₄ toxicity. However, based on the substantial growth stimulations at moderate biosolids rates, xeric and salt-adapted species, such as blanket flower and butterfly bush, may be ideally suited for expanding the use of highly saline biosolids at semiarid nursery production sites.

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The physical and chemical parameters were monitored for seven weeks during Trichoderma/Aspergillisinoculated rice straw composting at various pH levels. Three treatments (A, B, and C) were inoculated with lignocellulolytic microbial consortium (Aspergillus niger, F44 and Trichoderma viride, F26) and three were un-inoculated (D, E, and
pH of the starting materials was amended to 5.75 (A and D), 6.75 (B and E), and 7.75 (C and F) with either acetic acid or sodium hydroxide. Three typical phases of temperature were observed both in inoculated and un-inoculated treatments during composting: mesophilic phase, thermophilic phase, and followed by cooling and maturation phase. The bioconversion were maximum in *Trichoderma/Aspergillus* inoculated treatments within 14–21 days as indicated by the profiles of electrical conductivity, bulk density, total carbon and nitrogen, and germination index. After day 21, the germination index of *Trichoderma/Aspergillus* inoculated treatment (B) without any pH amendment was increased to 74.5 indicating the maturity of compost and suitability for field application.

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To ensure the safety of compost products, the Canadian Council of Ministers of the Environment's compost guidelines specify upper limits for certain pathogenic and indicator microbes, which are presumably achieved by exposing every particle of compost to temperatures ≥55°C for at least three consecutive days. A rugged temperature probe that behaves like a random compost particle was used to investigate whether every compost particle meets the time temperature criterion and to measure sanitation efficacy. An inoculum consisting of *Salmonella enterica* var. Meleagridis, *Escherichia coli* K12, and phi-S1 bacteriophage (all at levels of ∼1 × 10⁶ CFU/PFU mL⁻¹) was added into 17 probes. The probes were randomly introduced into a covered, aerated static pile along with 17 probes that only monitored the temperature. After 56 days of composting, with one pile turn the probes were recovered. Organism levels were determined via culture-based methods. Before turning, 80% of the randomly introduced probes satisfied the time-temperature criterion. After turning, this number increased to 87%, demonstrating that turning is somewhat useful for sanitation. The cool zones largely remained mesophilic with the pile turning having minimal impact, which could potentially be an indication that the pile was not turned thoroughly. One of the 17 probes with cryovials reached only 40.2°C, and survival of *S. meleagridis* (2.5 × 10⁶ CFU ml⁻¹) was observed. The remaining probes with cryovials exceeded 55°C and were pathogen free. It appears that the specified time-temperature conditions are likely adequate. However, more observations are needed before a firm conclusion can be made.


The potential of keratin wastes originating from poultry farms in practical application as a valuable organic fertilizer, gives rise to the need for intensive study on their effect on plants. In this study, for the first time there has been examined the influence of hen feather keratin bio-hydrolysate (FKH) and hen feather keratin compost (FKC) on plant growth, and the following features that indicate the plant condition: the leaf chlorophyll content, the activity of phenylalanine ammonia lyase (PAL) and guaiacol peroxidase (GPX) enzymes. The results of pot experiments showed a potential plant growth promoting effect of FKH, applied as a leaf
Fertilization of tested plants with FKC resulted in significant increase of their fresh weight. There was no effect on plant biomass after FKH treatment. The stimulating effect on plant physiology was expressed by decreased PAL activity after FKC treatment, and enhanced GPX activity, after FKH and FKC treatment. The application of FKC as a manure gave better effects for the plant condition expressed by the activity of PAL and GPX in comparison with FKH spraying. The chlorophyll content did not prove to be an efficient parameter to evaluate the impact of FKH or FKC on white cabbage, tomato, and maize plant. However, a significant increase in the leaf chlorophyll a, b, and a+b concentration was observed in cucumber plant after FKC treatment. Among the tested plants, the cucumber has shown the most profitable effect of feather compost on plant growth.


Infra-red spectroscopy (FTIR) and thermogravimetric and differential thermal analysis (TGA/DTA) were used to characterize the chemical structure of the humic acids (HA) extracted from two composts of date palm waste: in one the date palm waste was alone (DPW) and in the other, it was mixed with couch-grass clippings (DPCG). FTIR showed that the HA of both origins were rich in aromatic and phenolic structures indicating the presence of lignin degradation products. Both aliphatic and aromatic components were found in the HA. The persistence of certain aliphatic structures resistant to degradation was equally observed. For the DPCG mixture, the HA formed had a structure richer in peptides and carboxylic compounds than DPW compost, which was richer in aliphatic compounds. We can deduce that the DPCG mixture reached greater maturity than the DPW compost. The IR absorption ratios did not show the same patterns of change during the gradual degradation of substrate occurring on composting. Underlying the variations specific to each compost was a different degree of humification. It was higher for the DPCG mixture. FTIR results confirmed those of TDA/TGA thermal analysis, again showing the aliphatic and aromatic character of HA. The R_{TDA/TGA} ratio stresses that functionalization of organic matter was more marked than its aromatization.

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Influence of fungal consortium and different turning frequency on composting of organic fraction of municipal solid waste (OFMSW) was investigated to produce compost with higher agronomic value. Four piles of OFMSW were prepared: three piles were inoculated with fungal consortium containing 51 each spore suspensions of *Trichoderma viride*, *Aspergillus niger* and *Aspergillus flavus* and with a turning frequency of weekly (Pile 1), twice a week (Pile 2) and daily (Pile 3), while Pile 4 with weekly turning and without fungal inoculation served as control. The fungal consortium with weekly (Pile 1) turning frequency significantly affected temperature, pH, TOC, TKN, C/N ratio and germination index. High degradation of organic matter and early maturity was observed in Pile 1. Results indicate that fungal consortium with weekly turning frequency of open windrows were more cost-effective in comparison with other technologies for efficient composting and yield safe end products.

**Keywords:** Composting; Organic fraction of municipal solid waste; Fungal consortium; Inoculation; Turning frequency

Ying Zhou, Ammaiyappan Selvam, Jonathan W.C. Wong. (Sino-Forest Applied Research Centre for Pearl River Delta Environment and Department of Biology, Hong Kong Baptist University, Hong Kong Special Administrative Region, PR China). Evaluation of humic substances during co-composting of food waste, sawdust and Chinese medicinal herbal residues. Bioresource Technology, Volume 168(2014): 229–234

Humification during co-composting of food waste, sawdust and Chinese medicinal herbal residues (CMHRs) was investigated to reveal its correlation with compost maturity. Food waste, sawdust and CMHRs were mixed at 5:5:1 and 1:1:1 (dry weight basis) while food waste:sawdust at 1:1 (dry wt. basis) served as control. Lime at 2.25% was added to all the treatments to alleviate low pH, and composted for 56 days. Humic acid/fulvic acid (HA/FA) ratio increased to 0.5, 2.0 and 3.6 in the control and treatment at 5:5:1, and 1:1:1 mixing ratio, respectively at the end of composting. The decrease in aliphatic organics in HA demonstrated the degradation of the readily available organics, while an increase in aromatic functional groups indicated the maturity of compost. Disappearance of hemicellulose and weak intensity of lignin in the CMHRs treatments indicated that the lignin provided the nucleus for HA formation; and the CMHRs accelerated the compost maturity.

**Keywords:** Composting; Food waste; Chinese medicinal herbal residues; Humic acids; Fulvic acid

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To investigate morphine degradation and optimize turning frequency in opium poppy processing waste composting, a pilot scale windrow composting trial was run for 55 days. Four treatments were designed as without turning (A1), every 5 days turning (A2), every 10 days turning (A3) and every 15 days turning (A4). During composting, a range of physicochemical parameters
including the residual morphine degradation, temperature, pH, and the contents of total C, total N, total P and total K were investigated. For all treatments, the residual morphine content decreased below the detection limit and reached the safety standards after day 30 of composting, the longest duration of high temperature (≥50 °C) was observed in A3, pH increased 16.9–17.54%, total carbon content decreased 15.5–22.5%, C/N ratio reduced from 46 to 26, and the content of total phosphorus and total potassium increased slightly. The final compost obtained by a mixture of all four piles was up to 55.3% of organic matter, 3.3% of total nutrient (N, P₂O₅ and K₂O) and 7.6 of pH. A turning frequency of every ten days for a windrow composting of opium poppy processing waste is recommended to produce homogenous compost.

**Keywords:** Composting; Opium poppy processing waste; Turning frequency; Morphine degradation


The present work was focused on vermicomposting apple pomace waste and its mixtures with straw in volume proportions of 25%, 50%, and 75%. The feasibility was evaluated on the basis of agrochemical properties and earthworm biomass. Vermicomposting was able to reduce the weight and volume of the feedstock by 65% and 85%, respectively. The resulting vermicomposts were characterized by slightly acidic to neutral pH (5.9–6.9), and optimal EC (1.6–4.4 mS/cm) and C:N ratios (13–14). The total content of nutrients increased during vermicomposting for all of the treatments with the following average final values: N = 2.8%, P = 0.85%, K = 2.3%, and Mg = 0.38%. The addition of straw to apple pomace did not enhance earthworm biomass, but did increase the available content of nutrients during vermicomposting. The data reveals that vermicomposting is a suitable technology for the decomposition of apple pomace waste into a value added product.

**Keywords:** Apple pomace; Earthworms; Straw; Vermicomposting

Jining Zhang, Fan Lü, Liming Shao, Pinjing He. (a State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, Shanghai 200092, China, b Institute of Waste Treatment and Reclamation, Tongji University, Shanghai 200092, China, c Centre for the Technology Research and Training on Household Waste in Small Towns & Rural Area, Ministry of Housing and Urban-Rural Development of PR China (MOHURD), China). The use of biochar-amended composting to improve the humification and degradation of sewage sludge. Bioresource Technology, Volume 168(2014): 252–258

Wood biochar (6%, 12% and 18% of fresh sludge weight) adding to a sludge-and-straw composting system was investigated to assess the potential of biochar as a composting amendment. Organic degradation efficiency, temporal humification profile of the water-extractable organic fraction and solid organic matter, through spectroscopic, microscopic and elementary analysis were monitored. Fluorescent excitation and emission matrix indicated that concentrations of aqueous fulvic-acid-like and humic-acid-like compounds were, respectively, 13–26% and 15–30% higher in the biochar-amended treatments, than those in the control.
without biochar-amended. On the first day of sludge aerobic incubation, the presence of biochar resulted in increased oxygen uptake rates of 21–37% due to its higher nano-porosity and surface area. SEM indicated that, in the biochar-amended sludge, the dense microstructure on the sludge surface disintegrated into fragments with organic fraction degraded and water lost. Results indicated that 12–18% w/w addition of wood biochar to sludge composting was recommended.

**Keywords:** Water-extractable organic matter; Fluorescence excitation and emission matrix; FT-IR; SEM; Humus substances

L. Goswami, S. Sarkar, S. Mukherjee, S. Das, S. Barman, P. Raul, P. Bhattacharyya, N.C. Mandal, S. Bhattacharya, S.S. Bhattacharya. ("Department of Environmental Science, Tezpur University, Assam 784028, India, b Department of Zoology, Visva-Bharati, Santiniketan, West Bengal, India, c Department of Botany, Visva-Bharati, Santiniketan, West Bengal, India, d Defence Research Laboratory, DRDO, Tezpur, Assam, India, e Indian Statistical Institute, North East Centre, Tezpur, Assam 784028, India). Vermicomposting of Tea Factory Coal Ash: Metal accumulation and metallothionein response in *Eisenia fetida* (Savigny) and *Lampito mauritii* (Kinberg). Bioresource Technology, Volume 166, August 2014, Pages 96–102

Earthworms can accumulate heavy metals in their intestines to a great extent. Impact of feed materials and duration of metal exposure on natural activity of earthworms are rather unclear; this investigation therefore addresses the impact of metal rich Tea Factory Coal Ash (TFCA) on reproduction, composting and metal accumulation ability of *Eisenia fetida* and *Lampito mauritii*. Earthworm count and cocoon production increased significantly during vermicomposting. pH of the vermicomposted mixtures shifted toward neutrality, total organic C decreased substantially and total N enhanced significantly compared to composting. High heavy metal (Mn, Zn, Cu, As) accumulation was recorded in the intestine of both the earthworm species. Moreover, gradual increase in the metal-inducible metallothionein concentration indicated the causal mechanism of metal accumulation in these species. TFCA + cow dung (CD) (1:1) were most favorable feed mixture for *E. fetida* and TFCA + CD (1:2) were good for *L. mauritii* in regard to metal accumulation and compost quality.

**Keywords:** Heavy metal; Metallothionein; Tea Factory Coal Ash; *Eisenia fetida*; *Lampito mauritii*

**Biopesticides**

Mee Kyung Sang and Ki Deok Kim. ("Laboratory of Plant Disease and Biocontrol, Division of Biotechnology and Institute of Life Science and Natural Resources, Korea University, Anam-dong Sungbuk-ku, Seoul, 136-713, Republic of Korea. Email: kidkim@korea.ac.kr"). Biocontrol activity and root colonization by *Pseudomonas corrugata* strains CCR04 and CCR80 against Phytophthora blight of pepper. BioControl, Volume 59(4) (2014): 437-448

Previously, we selected *Pseudomonas corrugata* strains CCR04 and CCR80 as rhizobacteria suppressive to Phytophthora blight of pepper caused by *Phytophthora capsici*. In this study, we investigated soil microbial activity in pepper plants root-drenched with strains CCR04 and CCR80 in relation to their biocontrol activity, root colonization by using bacterial population counts and scanning electron microscopy, biofilm formation and cell motility as well as cell
sensitivity to hydrogen peroxide (H$_2$O$_2$). As a result, strains CCR04 and CCR80 more effectively suppressed disease expression in pepper plants through root colonization than did *Paenibacillus polymyxa* AC-1 (positive control), *Escherichia coli* DH5α (negative control) or MgSO$_4$ solution (untreated control). Strains CCR04 and CCR80 had efficient biofilm formation and cell motility (swimming and swarming activities) abilities and responded to certain tested compounds (amino acids, organic acids and sugars), which can be found in root exudates. Strains CCR04 and CCR80 and the positive control strain AC-1 were relatively insensitive to H$_2$O$_2$, a reactive oxidative species at concentration up to 20 mM, unlike the negative control strain DH5α. Taken together, these results suggest that *P. corrugata* CCR04 and CCR80 can effectively inhibit *P. capsici* infection of pepper plants through successful colonization of plant roots. This bacterial colonization may be facilitated by the biofilm formation ability and cell motility in addition to reduced sensitivity to H$_2$O$_2$ and probably the production of antimicrobial compounds. These findings highlight the potential of strains CCR04 and CCR80 as biocontrol agents for the management of Phytophthora blight of pepper.

**Keywords:** Bacterial motility Biofilm formation Root colonization Hydrogen peroxide *Phytophthora capsici* *Pseudomonas corrugata*

Martin Stadler$^1$ and Andreas von Tiedemann$^1$. ($^1$Division of Plant Pathology and Crop Protection, Department of Crop Sciences, Georg-August-University Göttingen, Grisebachstrasse 6, 37077 Göttingen, Germany. Email: atiedem@gwdg.de). Biocontrol potential of *Microsphaeropsis ochracea* on microsclerotia of *Verticillium longisporum* in environments differing in microbial complexity. BioControl, Volume 59(4) (2014): 449-460

The potential of the fungal antagonist *Microsphaeropsis ochracea* to control the soilborne pathogen *Verticillium longisporum* was investigated in environments with varying microbial complexity (in vitro vs. in vivo, sterile vs. unsterile, controlled conditions vs. field). A semi-quantitative PCR assay was developed for the detection of *M. ochracea* on unsterile plant debris. In vitro, *M. ochracea* caused high levels of mortality to *V. longisporum* microsclerotia (51–100 %) from 4 to 24 °C, with a broad optimum between 16 and 24 °C. In controlled conditions, *M. ochracea* significantly reduced the viability of *V. longisporum* microsclerotia grown on dead rapeseed stems in autoclaved sand, but not in unsterile soil. Likewise, in two experimental years, no significant reduction of *V. longisporum* inoculum was detectable on rapeseed straw buried in small plots in the field in any of the treatments (soil depths, exposure duration, doses of *M. ochracea*). Germination of *M. ochracea* pycnidiospores was inhibited by general soil fungistasis in unsterile soil from a field, botanical garden and grassland. Accordingly, *V. longisporum* infection of rapeseed plants in the greenhouse was reduced only at artificially high doses of *M. ochracea* inoculum and no biocontrol efficacy in disease control was recorded in field experiments conducted with winter oilseed rape during two subsequent seasons in an experimental field near Göttingen, with a soil homogenously infested with *V. longisporum*. The results demonstrate that *M. ochracea*, although having shown promising potential in controlling pathogens with melanised resting structures on leaf litter, evidently lacks microbial competitiveness to effectively control pathogens in the soil such as *V. longisporum*, even though the latter is effectively inhibited in vitro.
**Keywords:** Soilborne diseases Microsclerotia Mycoparasitism Biological control Oilseed rape Soil fungistasis

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*Candida sake* CPA-1 is an antagonistic yeast that has previously been shown to effectively control *Botrytis* bunch rot in grapes. The efficacy of biological control agents is dependent on their survival, which may also depend on climatic conditions. However, few studies have evaluated the effect of abiotic factors affecting the survival of biological control agents, such as temperature (T) or relative humidity (RH). In this study, efficacy of *C. sake* (5 × 10\(^7\) CFU mL\(^{-1}\)), which was applied with the additive Fungicover (FC; 50 g L\(^{-1}\)), was tested against BBR in the laboratory and in field trials under the Atlantic climate conditions of the Bordeaux region (France). The study also evaluated the survival of *C. sake* under T and RH regimes simulated in climatic chambers. Two or five applications of *C. sake* plus FC during the growing season significantly reduced BBR severity at harvest by 48% and 82%, respectively, when compared to the control. Similar reductions were achieved after inoculation with selected virulent *Botrytis cinerea* strains (75% compared to control) in laboratory experiments. *C. sake* populations showed minimal decreases between field applications and were favored by simulated Atlantic climate conditions. The survival pattern of *C. sake* exposed to 40 and 45 °C combined with 30% and 100% of RH was described, demonstrating a sharp decrease during the first 24 h. Allowing 48 h for *C. sake* to incubate and become established on fruits prior to the exposure to 40 °C and 30% RH increased survival (*P* < 0.05). These results confirm the efficacy of treatment with *C. sake* plus FC under favorable climatic conditions for BBR development, while survival studies may help to improve the survival and efficacy of yeast BCAs, such as *C. sake* CPA-1.

**Keywords:** Abiotic factors; Extreme conditions; Fatty acids; Fungal disease; Grapevine; *Vitis vinifera*


Banker plants with *Aphidius colemani* were tested in greenhouse for control of *Myzus persicae* on arugula and sweet pepper crops and compared to inoculative releases of parasitoids. Banker plants system consisted of pots of oat (non-crop plant) infested with *Rhopalosiphum padi* (non-pest herbivore). The non-pest herbivore serves as an alternative host for *A. colemani* (parasitoid of the target crop pest). In the arugula crop significant differences in the pest population between the two strategies of biological control showed the lowest densities of

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the pest when introducing the banker plant system. In the sweet pepper crop, there was no difference in the pest population between the two strategies of biological control.

**Keywords:** *Aphidius colemani*; Aphids; Alternative host; Vegetable crop; Greenhouse

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The production of maize, a major staple food crop in sub-Saharan Africa is being constrained by the parasitic weed *Striga hermonthica*. The fungus *Fusarium oxysporum* f. sp. *strigae* (Foxy 2) that causes fusarium wilt of *Striga* in Ghana, West Africa, is being considered for biological control of the weed in Western Kenya. The present study investigated the efficacy of *F. oxysporum* f. sp. *strigae* (Foxy 2) for *S. hermonthica* management in Western Kenya. Research was conducted in post-entry quarantine (PEQ) facilities at Alupe, Busia, Homabay, Kibos and Siaya field stations for two seasons. Each PEQ was a split-plot, with 4 main blocks each having 6 treatment subplots. The treatments included seeds of two *S. hermonthica*-susceptible maize varieties, either coated with Foxy 2 using gum Arabic, gum Arabic alone, or left untreated. Data was collected over seven sampling periods on *S. hermonthica* population per plant, percentage of those that were wilting, and the severity of wilting. Maize plant growth parameters assessed included duration to 50% anthesis and 50% silking, plant height, number of leaves, stover and cob weights, and maize yield per hectare. Statistical analysis was done using SAS 9.1 software. Data on *S. hermonthica* population were analyzed by $\chi^2$-test using Proc Genmod (Poisson); while the other parameters were analyzed by Proc Mixed using study location, season and blocks as random effects, and the sampling periods as repeated effects. All the assessed parameters were similar between plants grown from seeds inoculated with *F. oxysporum* f. sp. *strigae* (Foxy 2), those coated with gum Arabic, and the ones without any coating. These parameters were also not different between the maize varieties. There are varying reasons for the disparities between results on *F. oxysporum* f. sp. *strigae* (Foxy 2) obtained in this Kenyan study, and those from researches outside this country. In conclusion, *F. oxysporum* f. sp. *strigae* strain Foxy 2 is predominantly safe on maize growth, but its efficacy in controlling *S. hermonthica* was not evident on the tested Kenyan soils.
Sustainable agriculture must provide for growing human demands for crops while minimizing impacts on ecosystems. This is a daunting challenge as agroecosystems have trended towards monocultures with intensive synthetic inputs. Moreover, agricultural landscapes often lack natural habitats that are necessary to support biodiversity. Furthermore, problems associated with agricultural intensification and land-use change may be exacerbated by climate change, which increases the frequency of disturbances, modifies the suitability of habitats, and changes the way species interact. To meet this challenge, farmers must increasingly rely on integrated pest management strategies, including biological control. Biological control of arthropods, weeds, and diseases can promote the stability and diversity of agricultural communities and aid in reducing synthetic inputs. Promoting biological control may thus help farming systems adapt to a rapidly changing world. This special issue considers how multiple global change drivers such as agricultural intensification, land-use change, and climate change affect biological control.

Here, we discuss these papers and highlight concepts that remain relatively unexplored in the context of global change and biological control. Future research addressing these issues will promote biological control and enhance agricultural sustainability in a rapidly changing world.

Keywords: Agricultural intensification; Climate change; Conservation; Ecosystem functioning; Habitat modification

Agricultural systems around the world are faced with the challenge of providing for the demands of a growing human population. To meet this demand, agricultural systems have intensified to produce more crops per unit area at the expense of greater inputs. Agricultural intensification, while yielding more crops, generally has detrimental impacts on biodiversity. However, intensified agricultural systems often have fewer pests than more "environmentally-friendly" systems, which is believed to be primarily due to extensive pesticide use on intensive farms. In turn, to be competitive, less-intensive agricultural systems must rely on biological control of pests. Biological pest control is a complex ecosystem service that is generally positively associated with biodiversity of natural enemy guilds. Yet, we still have a limited understanding of the relationships between biodiversity and biological control in agroecosystems, and the mechanisms underlying these relationships. Here, we review the effects of agricultural intensification on the diversity of natural enemy communities attacking arthropod pests and weeds. We next discuss how biodiversity of these communities impacts pest control, and the mechanisms underlying these effects. We focus in particular on novel conceptual issues such as relationships between richness, evenness, abundance, and pest control. Moreover, we discuss novel experimental approaches that can be used to explore the relationships between biodiversity...
and biological control in agroecosystems. In particular, we highlight new experimental frontiers regarding evenness, realistic manipulations of biodiversity, and functional and genetic diversity. Management shifts that aim to conserve diversity while suppressing both insect and weed pests will help growers to face future challenges. Moreover, a greater understanding of the interactions between diversity components, and the mechanisms underlying biodiversity effects, would improve efforts to strengthen biological control in agroecosystems.

**Keywords:** Agricultural intensification; Conservation; Ecosystem functioning; Evenness; Richness


Agroecosystems contain complex networks of interacting organisms and these interaction webs are structured by the relative timing of key biological and ecological events. Recent intensification of land management and global changes in climate threaten to desynchronize the temporal structure of interaction webs and disrupt the provisioning of ecosystem services, such as biological control by natural enemies. It is therefore critical to recognize the central role of temporal dynamics in driving predator–prey interactions in agroecosystems. Specifically, ecological dynamics in crop fields routinely behave as periodic oscillations, or cycles. Familiar examples include phenological cycles, diel activity rhythms, and crop-management cycles. The relative timing and the degree of overlap among ecological cycles determine the nature and magnitude of the ecological interactions among organisms, and ultimately determine whether ecosystem services, such as biological control, can be provided. Additionally, the ecological dynamics in many cropping systems are characterized by a pattern of frequent disturbances due to management actions such as harvest, sowing and pesticide applications. These disturbance cycles cause agroecosystems to be dominated by dispersal and repopulation dynamics. However, they also serve as selective filters that regulate which animals can persist in agroecosystems over larger temporal scales. Here, we review key concepts and examples from the literature on temporal dynamics in ecological systems, and provide a framework to guide biological control strategies for sustainable pest management in a changing world.

**Keywords:** Population dynamics; Diel activity cycle; Predator–prey dynamics; Ecological disturbance; Climate change; Early-season predation

**Paul J. Chisholm**, Mary M. Gardiner, Elliott G. Moon, David W. Crowder. (a Department of Entomology, Washington State University, PO Box 646382, Pullman, WA, USA, b Department of Entomology, Ohio State University, Columbia, OH, USA). Tools and techniques for investigating impacts of habitat complexity on biological control. Biological Control, Volume 75(2014): 48–57

Across the globe, landscapes are becoming altered as natural habitats are converted to agriculture or development. Consequently, a critical question is how changes in habitat complexity and composition might influence ecosystem services such as biological control.
Although the development of new statistical, molecular, and digital technologies offers exciting opportunities to explore this issue, the appropriate usage of these tools is crucial to any successful study. This review examines the tools and techniques employed to investigate relationships between habitat complexity and biological control, and their appropriateness in different contexts. We examine various definitions of the explanatory variable, habitat complexity, and methods to experimentally measure the response variable, biological control. We conclude with a summary of the different statistical techniques available to assess linkages between habitat complexity and biological control. This review will facilitate future research on habitat complexity and biological control and will thus aid in the conservation of this valuable ecosystem service.

**Keywords:** Agricultural landscapes; Geographical information systems; Landscape ecology; Landscape simplification; Predator–prey interactions


Terrestrial landscapes, including those with embedded agroecosystems, are a mosaic of cover types varying in size. Creating or maintaining habitats that support natural enemy populations to combat agricultural pests is the primary method of conservation biological control. Non-crop habitats can be managed in an attempt to maximize the exchange of natural enemies with adjacent agroecosystems with the expectation that they will suppress damaging pest outbreaks. Despite this goal, current habitat management relying on natural enemy spillover into crops has been unreliably effective at reducing pest abundance or increasing crop yield. Furthermore, the expansion and intensification of agriculture and changes in global climate patterns threaten the foundations of conservation biological control in future agroecosystems. However, the aquatic–terrestrial interface offers a natural boundary similar to the one between agroecosystems and their neighboring non-crop habitats that can provide useful insights to the challenges facing growers. Research of the exchanges between water and land suggests general biological and physical processes that govern the movement of organisms between disparate habitats. We propose that like aquatic insects moving from water to land, natural enemy dispersal from non-crop donor habitats into recipient crop patches on the landscape is a function of (1) the production of natural enemies in the source habitat which establishes the abundance of organisms that can disperse, (2) how and why mobile natural enemies disperse themselves into neighboring recipient habitats, and (3) the configuration of donor and recipient habitats on the landscape. We suggest that conservation biological control practitioners can focus on these main components of natural enemy production and dispersal to predict the effectiveness of conservation biological control measures and guide their adaptation to future global change.

**Keywords:** Mobile organisms; Aquatic–terrestrial linkage; Conservation biological control

Due to the considerable losses caused by slugs in terms of agricultural production and revenue, there is an urgent need for a cost effective biological control agent. The malacophagous nature of the sciomyzid fly, *Tetanocera elata* (Fab.) makes it a possible contender to meet this demand. This study examined the effect of constant temperatures (14, 17, 20, 23, and 26 °C), in addition to ambient outdoor and laboratory temperatures on *T. elata* larval duration and predation. In general, the mean and median larval stage duration decreased as temperature increased with percentage survival for the overall larval stage (62%) greatest at 20 °C with a median duration of 44 days. There was no significant difference between temperatures with regard to the number of slugs killed per larva and while predation rate increased with increasing constant temperature, there was also no significant difference between the constant temperatures. Our results show that puparial weight can be used to predict the sex of adult flies prior to their emergence. The results are discussed in the context of the suitability of *T. elata* as a biological control agent of pestiferous slugs.

**Keywords:** Larval duration; Predation rate; Malacophagy; Slug predator

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The worldwide important crop tomato is attacked by various pathogens, for which management is still primarily reliant on fungicides despite increasing concerns and constraints on their use. Other approaches are investigated, including the use of biocontrol organisms to manage tomato diseases. In this review we discuss and compare the interaction of major biocontrol fungi (BCF) with tomato, including the endophytic arbuscular mycorrhizal fungi and *Piriformospora indica*, the free-living opportunistic symbionts*Trichoderma* spp. and non-pathogenic *Fusarium oxysporum*, as well as the oomycete *Pythium oligandrum*. We cover recent advances that have been made in unraveling biocontrol modes of action against the most important tomato pathogens, encompassing direct effects of the BCF on pathogens and their indirect effects through the plant, with a main focus on induced systemic resistance. It is an exciting era for the study of biocontrol tripartite interactions, with the emergence of next-generation sequencing tools and the higher pace at which new genomes are being sequenced nowadays, as was recently also achieved for tomato. In addition, plant pathology and biocontrol research domains are increasingly reaching out to each other, because of the parallels that we are only beginning to discover between the interactions of beneficial and detrimental micro-organisms with a plant. Considering the enormous technological possibilities at hand today, this seems a timely opportunity to review the most recent advances in this field and to anticipate to what is ahead of us, discussing breakthroughs expected in our understanding of biocontrol interactions and remaining hurdles on the way to reach them.

**Keywords:** Mycorrhizal fungi; *Piriformospora indica*; *Trichoderma*; *Fusarium oxysporum*; *Pythium oligandrum*; ISR

Bancy Waweru, Losenge Turoop, Esther Kahangi, Daniel Coyne, Thomas Dubois. (a) Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200,
Endophytic colonization by the fungus *Fusarium oxysporum* can result in increased host resistance to pests and diseases, and greater biomass production. However, few studies have assessed the field performance of this fungus for biological control of pests and diseases in banana. Further to greenhouse assessment, studies were carried out to evaluate the performance of *F. oxysporum* strains against plant-parasitic nematodes on banana (*Musa* sp., cv. Giant Cavendish and cv. Grand Nain) in the field using tissue-cultured plants. Plants were inoculated separately with one of three strains (V5W2, Eny 7.11o and Emb 2.4o) before being inoculated with Pratylenchus goodeyi and Helicotylenchus multicinctus in an on-station trial and in an on-farm trial planted in a field naturally infested with the same nematodes. All three endophytic strains significantly suppressed *P. goodeyi* and *H. multicinctus* densities and damage in the field. On-station, nematode population densities were reduced by >45% in endophyte-inoculated plants compared to non-inoculated plants, while percentage root necrosis was reduced by >20%. Similarly, on-farm, nematode damage to roots and densities were also significantly lower in endophyte-inoculated plants compared with control plants. Significantly improved yields were observed for plants inoculated with endophytes when compared to the control plants, with inoculation with strains Emb 2.4o and V5W2 resulting in up to 35% and 36% increased banana yields, respectively, for the on-station trial. For the on-farm trial, up to 20% increase in yields were observed for strain Eny 7.11o compared to control plants. This study provides the first report from the field in Africa on the reduction of nematode populations and damage, and the increase in banana production by fungal endophytes. The study shows that endophytes have potential to enhance yields of tissue-cultured banana plants and protect them against pests.

**Keywords:** Africa; Biological control; Endophyte inoculation; Microbial antagonist; Plant-parasitic nematode; Tissue culture


*Glycine max* (soybean) production can be dramatically affected by frogeye leaf spot (FLS) caused by *Cercospora sojina* Hara. The inoculation of biocontrol agents may be an alternative strategy for *C. sojina* control. The native biocontrol bacterium *Bacillus* sp. CHEP5 reduced the severity of FLS in soybean by inducing systemic resistance. We suggest that the defense response was primed since the expression of the defense related gene *GmAOS* was enhanced in induced plants treated with both methyl jasmonate and *C. sojina*. Furthermore, as *GmAOS* is related to jasmonic acid biosynthesis, we assume that this phytohormone is involved in induced systemic resistance signaling defense pathway in soybean against *C. sojina*.

**Keywords:** Soybean; Bacillus sp.; Cercospora sojina; Induced systemic resistance; Priming; Jasmonic acid
Biodegradation


In Acatzingo, Puebla, Mexico (east-central), oil spills have mainly affected agricultural fields. *Pleurotus ostreatus* is a white rot basidiomycete and produces extracellular enzymes (laccases, manganese peroxidases, versatile peroxidases and veratryl alcohol oxidases). The production of edible mushrooms generates spent mushroom substrate that may have a biotechnological application. The aim of this study was to evaluate the mushroom substrate of *P. ostreatus* in a microcosm for the bioremediation of an agricultural soil contaminated with diesel. We evaluated the participation of microbial populations and specific enzymatic laccases, manganese peroxidases, versatile peroxidases, veratryl alcohol oxidases activities of mushroom substrate in the biodegradation of a soil contaminated with 11030 ppm of diesel in four treatments: E1, E2, E3 and E4. All the experiments were performed in triplicate at 25 and 37°C for 28 days, with a soil:substrate ratio of 4:1. The treatments incubated at 37°C were quantified for diesel-tolerant bacteria, and treatments incubated at 25°C were quantified for diesel-tolerant fungi. Mushroom substrate participated in the biostimulation (91% organic material, 0.56% total nitrogen and 0.3% phosphorus) and bioaugmentation of the microorganisms of the microcosm. Bacteria-tolerant populations increased significantly (*p = 0.000*) in all the treatments. Laccases (8.62 U g⁻¹) activity was stimulated at 25°C and was the only one related to biodegradation; however, the highest biodegradation rate (72%) was at 37°C (bacterial biodegradation) being promising for future research.

**Keywords:** Bioremediation, diesel, laccase, veratryl alcohol oxidase, *Pleurotus ostreatus*.

Nwadinigwe Alfreda Ogochukwu*, Obi-Amadi Achuna. (Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria, Centre for Environmental Management and Control (CEMAC) University of Nigeria, Nsukka, Enugu State, Nigeria. Corresponding Author Email: fredanwad@yahoo.com). Crude oil
Pollution by crude oil and its products is one of the most prevalent environmental problems that cause greenhouse effects and global warming. The crude oil-degrading potentials of *Pennisetum glaucum* was investigated using 0.2, 0.9, 5.0 and 6.0% w/v concentrations of crude oil, which were employed to pollute soil planted with the seeds of the plant. These treatments were repeated in soil without seeds and the control had no crude oil pollution. Total petroleum hydrocarbons (TPH) were determined for all soil samples using gas-liquid chromatography. Microbial count was carried out on soil rhizosphere using standard methods. The results show that percentage TPH degraded in soil planted with *P. glaucum* was 100, 99.53, 99.44 and 99.47 for 0.2, 0.9, 5.0 and 6.0% w/v concentrations, respectively. *P. glaucum* alone degraded 0.56, -0.29, 0.39 and 0.31% for the same treatments. The total viable count of microorganisms from the polluted, vegetated soil samples was significantly (*P < 0.05*) higher than that of the unvegetated ones. *P. glaucum* might have enhanced the biodegradation of crude oil by stimulating the proliferation of microorganisms in the soil and hence may be used for phytoremediation of crude oil polluted soils.

**Keywords:** *Pennisetum glaucum*, crude oil, total petroleum hydrocarbons (TPH), microorganisms.

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Bacteria capable of degrading the sulphonated azo dye Red HE7B were isolated from textile mill effluent contaminated soil. The most efficient isolate was identified as *Bacillus* sp. Azo1 and the isolate could successfully decolorize up to 89% of the dye. The decolorized cultural extract analyzed by HPLC confirmed degradation. Enzymatic analysis showed twofold and fourfold increase in the activity of azoreductase and laccase enzymes, respectively, indicating involvement of both reductive and oxidative enzymes in biodegradation of Red HE7B. Degraded products which were identified by GC/MS analysis included various metabolites like 8-nitroso-1-naphthol, 2-diazonium naphthalene. Mono azo dye intermediate was initially generated from the parent molecule. This mono azo dye was further degraded by the organism, into additional products, depending on the site of cleavage of R–N=N–R molecule. Based on the degradation products identified, three different pathways have been proposed. The mechanism of degradation in two of these pathways is different from that of the previously reported pathway for azo dye degradation. This is the first report of a microbial isolate following multiple pathways for azo dye degradation. Azo dye Red HE7B was observed to be phytotoxic, leading to decrease in root development, shoot length and seedling fresh weight. However, after biotreatment the resulting degradation products were non-phytotoxic.

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Polyphosphate kinases 2 (PPK2) are key enzymes for polyphosphate utilisation in bacteria. The genome of Ruegeria pomeroyi, a marine α-proteobacterium, includes three Pseudomonas aeruginosa PPK2 homologs. We expressed these homologs in Escherichia coli as soluble proteins, purified the protein products and compared their metal, pH and nucleotide preferences. The optimal pH was 8.0 for SPO1727 and 9.0 for SPO1256. The SPO0224 gene product had two pH optima at eight and ten. The SPO0224 protein showed little dependence on metal presence, while SPO1256 required Mg$^{2+}$. SPO1727 required Mg$^{2+}$ but accepted other ions as well.

Keywords: Calgon; Calcium salts; Polyphosphate degradation; Polyphosphate kinase; PPK2 homolog Ruegeria pomeroyi

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Gene therapy has provided great potential to revolutionize the treatment of many diseases. This therapy is strongly relied on whether a delivery vector efficiently and safely directs the therapeutic genes into the target tissue/cells. Nonviral gene delivery vectors have been emerging as a realistic alternative to the use of viral analogs with the potential of a clinically relevant output. Dendritic polymers were employed as nonviral vectors due to their branched and layered architectures, globular shape and multivalent groups on their surface, showing promise in gene delivery. In the present review, we try to bring out the recent trend of studies on functional and biodegradable dendritic polymers as nontoxic and efficient gene delivery vectors. By regulating dendritic polymer design and preparation, together with recent progress in the design of biodegradable polymers, it is possible to precisely manipulate their architectures, molecular weight and chemical composition, resulting in predictable tuning of their biocompatibility as well as gene transfection activities. The multifunctional and biodegradable dendritic polymers possessing the desirable characteristics are expected to overcome extra- and intracellular obstacles, and as efficient and nontoxic gene delivery vectors to move into the clinical arena.

Keywords: Gene therapy; Gene vectors; Gene transfection; Functionalization; Dendritic polymers; Dendrimer; Biodegradable; Biocompatibility

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Marine bacteria, *Vibrio alginolyticus* and *Vibrio parahemolyticus* isolated from sediments were evaluated for their ability as a consortia, to degrade polyvinyl alcohol-low linear density polyethylene (PVA-LLDPE)-blended plastic films in shake flask conditions at 120 rpm at 37 °C over 15 weeks. Results indicated that relatively 20% decrease in tensile strength of the film could be achieved with 25 and 30% blend of PVA in the PVA-LLDPE plastic film compared to other ratios. Micrographs obtained with scanning electron microscope showed visible cracks and grooves on the surface of the PVA-LLDPE blend film after 15 weeks of incubation with bacterial consortium. The decrease in tensile strength of the PVA-blended plastic films after treatment and the results of the scanning electron microscopic analysis evidence that the consortium could cause degradation of PVA-LLDPE plastic blends compared to suitable controls. This is the first report on polyvinyl alcohol degrading *Vibrio* sp. from marine sediments and its application in microbial degradation of polyvinyl alcohol-low linear density polyethylene plastic blends. The study indicated potential of marine benthic vibrios that have novel enzymes and unique characteristics for application in bioremediation and solid waste management particularly in handling synthetic polymers such as PVA-blended plastic films.


The efficiency of denitrification and enhanced biological phosphorus removal in biological nutrient removal activated sludge systems is strongly dependent on the availability of appropriate carbon sources. Due to high costs of commercial compounds (such as methanol, ethanol, acetic acid, etc.) and acclimation periods (usually) required, the effective use of internal substrates is preferred. The aim of this study was to determine the effects of slowly biodegradable compounds (particulate and colloidal), as internal carbon sources, on denitrification, phosphate release/uptake and oxygen utilization for a full-scale process mixed liquor from two large wastewater treatment plants located in northern Poland. Since it is difficult to distinguish the effect of slowly biodegradable substrate in a direct way, a novel procedure was developed and implemented. Four types of one- and two-phase laboratory batch experiments were carried out in two parallel reactors with the settled wastewater without pre-treatment (reactor 1) and pre-treated with coagulation–floculation (reactor 2). The removal of colloidal and particulate fractions resulted in the reduced process rates (except for phosphate release). The average reductions ranged from 13% for the oxygen utilization rate during the second phase of a two-phase experiment (anaerobic/aerobic), up to 35% for the nitrate utilization rate (NUR) during the second phase of a conventional NUR measurement.

An investigation was carried out to compare the ability of two bacteria *Pseudomonas aeruginosa* PSA5 and *Rhodococcus* sp. NJ2 isolated from petroleum sludge for degradation of benzo(a)pyrene [B(a)P], a HMW PAH compound in MSM. During 25 days of incubation, 50 ppm B(a)P was degraded by 88 and 47 % by *P. aeruginosa* PSA5 and *Rhodococcus* sp. NJ2, respectively. Besides, involvement of different catabolic enzymes, that is, salicylate hydroxylase, 2-carboxybenzaldehyde dehydrogenase, catechol 1,2-dioxygenase and catechol 2,3-dioxygenase, was also examined to identify their differential role in B(a)P degradation. Among these enzymes, the highest induction of 2-carboxybenzaldehyde dehydrogenase (773.5 nmol mg$^{-1}$ protein) was recorded in *P. aeruginosa* PSA5, while salicylate hydroxylase was highly expressed (839.6 nmol mg$^{-1}$ protein) in *Rhodococcus* sp. NJ2. Both the bacteria were found biosurfactant (glycolipid) producing, and role of biosurfactant in PAH degradation was also ascertained by reduced surface tension, higher emulsification index and increased cell surface hydrophobicity.

Keywords: Biosurfactant; Cell surface hydrophobicity; Degradative enzymes; Surface tension

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Acrylamide finds diverse industrial applications but is considered an environmental threat because of its neurotoxic, carcinogenic, and teratogenic effects. Certain bacteria enzymatically degrade acrylamide to acrylic acid and ammonia. The present investigation was carried out to isolate and identify an acrylamide-degrading bacterium from industrial effluent. Bacterial growth and extent of acrylamide degradation in the presence of different acrylamide concentrations, nutrients, varied range of pH, and temperature were analyzed. Among the eight acrylamide-degrading isolates, isolate BAC-6 demonstrated the highest degradation, and based upon the partial 16S rDNA sequencing, it was identified as *Pseudomonas aeruginosa*. *P. aeruginosa* BAC-6 grew over a wide range of acrylamide concentrations, but the highest degradation was recorded at 500 mg/L concentration with concomitant cell growth. Among the carbon supplements, mannitol supported the highest growth and degradation. Maximum degradation was reported at neutral pH. A mesophilic temperature range (25–40 °C) facilitated conducive bacterial growth followed by degradation. The highest degradation and bacterial growth were observed at 30 and 35 °C, respectively. Thus, it could be inferred from the present investigation that cultural conditions strongly affected the degradation potential of *P. aeruginosa* BAC-6 and advocated the utilization of the isolate in bioremediation of sites polluted with acrylamide.
Chemical synthesis of 1,3-propanediol (1,3-PD) is environmentally unfriendly and hence its microbial production is preferred, especially for biomedical, cosmetic and textile applications. In this work, production of 1,3-PD by co-fermentation of glucose and glycerol by *Lactobacillus reuteri* was investigated under different cultivation conditions such as aeration, acetate concentration and different molar ratios of glucose/glycerol. The final concentration of 1,3-PD and yield attained under unaerated conditions was close to that obtained under anaerobic conditions. Addition of acetate in the initial medium at 5 g/l increased the productivity of 1,3-PD but above this concentration it was found to be inhibitory. Batch reactor experiments showed that the molar ratio of glucose and glycerol in the medium affected the fermentation pattern. The effect of molar ratios was further investigated in fed-batch fermentation and the optimum ratio was found to be 1.5. In repeated fed-batch fermentation with co-feeding of glucose and glycerol in the molar ratio of 1.5, 1,3-PD concentration reached up to 65.3 g/l, which is the highest 1,3-PD concentration reported so far for this strain. The yield (0.97 mol/mol) based on glycerol utilized also approached the theoretical value (1 mol/mol).

**Keywords:** Glycerol; 1,3-Propanediol; *Lactobacillus reuteri*; Unaerated; Fed-batch; Co-fermentation

Cheese whey is a by-product of cheese production and has high concentrations of lactose (about 5%) and other nutrients. *Pseudozyma antarctica* produces a unique cutinase-like enzyme, named PaE, that efficiently degrades biodegradable plastics. A previous study showed that a combination of 1% oil and 0.5% lactose increased cutinase-like enzyme production by another species of yeast. In this study, to produce PaE from cheese whey, we investigated the effects of soybean oil on PaE production (expressed as biodegradable plastic-degrading activity) by *P. antarctica* growing on lactose or cheese whey. In flask cultures, the final PaE activity was only 0.03 U/ml when soybean oil was used as the sole carbon source, but increased to 1.79 U/ml when a limited amount of soybean oil (under 0.5%) was combined with a relatively high concentration of lactose (6%). Using a 5-L jar fermentor with lactose fed-batch cultivation and periodic soybean oil addition, about 14.6 U/ml of PaE was obtained after 5 days of cultivation.

**Keywords:** Acrylamide; Pseudomonas aeruginosa; Degradation; High-performance liquid chromatography

**Chemical synthesis of 1,3-propanediol (1,3-PD)**
When the lactose was replaced with cheese whey, PaE production was 10.8 U/ml after 3 days of cultivation.

**Keywords:** Cheese whey; Cutinase-like enzyme; Phyllosphere yeast; *Pseudozyma antarctica*; Biodegradable plastics; Jar fermentor

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Polycyclic aromatic hydrocarbons (PAHs) and arsenic often co-occur at polluted sites, but remediation strategies for this are scarce. In this study, the effect of bacterial inoculation on plant growth and arsenic uptake by *Pteris vittata* and phenanthrene dissipation was investigated hydroponically using an arsenate-reducing and PAH-degrading *Pseudomonas* isolate. In a 12-d experiment, despite reduced dry weight in some cases, the isolate generally promoted the growth of *P. vittata*. The aboveground and belowground biomass increased by 21.0–38.7% and 3.5–66.3%, respectively. In addition, bacterial inoculation greatly enhanced arsenic uptake by *P. vittata* compared to un-inoculated treatments (from 246.7–438.9 and 102.6–231.4 to 754.1–1425.7 and 121.5–351.4 mg kg\(^{-1}\) As in aboveground and belowground biomass, respectively). Accordingly, arsenic transfer factor increased by 116–315%. The enhancement was attributed to the bacteria-mediated As(V) reduction in growth media. A dissipation of phenanthrene from growth media was observed and attributed to the degradation of the chemical by the isolate, as the contribution of *P. vittata* in phenanthrene removal was negligible. The present results demonstrated the versatile arsenate-reducing and PAH-degrading bacteria can effectively enhance arsenic uptake and translocation by *P. vittata* and remove phenanthrene.

**Keywords:** Co-contamination; Phenanthrene; Arsenic; *Pteris vittata*; Bioremediation

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The effect of aflatoxin B1 (AFB1) on an anaerobic digestion process (AD) was studied. Batch anaerobic digestion trials were performed with both non-contaminated AFB1 corn grain (Control A) and contaminated-AFB1 corn grain at different doses (AFB1 contents of 0.54, 66.2, and
110 µg kg⁻¹ wet weight). Both cumulative biogas production and the degradation rate of AFB1 were studied. Results indicated that no adverse effects on AD were detected during the processes which could be attributed to the presence of AFB1. AFB1 degradation ranged from 69% to 87% of the total initial AFB1 content.

Anaerobic digestion trials using Completely Stirred Tank Reactors (CSTR) were also carried out, comparing the biogas production of a mix of contaminated corn grain plus pig slurry (AFB1 content of 7.2 µg kg⁻¹ wet weight) with a mix of non-contaminated corn grain plus pig slurry (Control B). No adverse effect of AFB1 on biogas production was detected. The CSTR trial resulted in an average degradation of AFB1 of 42%. The further storage of the digestate for 30 days resulted in an overall degradation (CSTR plus storage) of AFB1 of 61% of the starting content.

**Keywords:** Aflatoxin B1; Anaerobic digestion; Biodegradation; Batch trials; CSTR trials


Three bacterial isolates enriched from historically contaminated soil samples were investigated for their ability to use 100 mg L⁻¹ of technical grade endosulfan as sole source of carbon and energy under aerobic conditions in liquid phase. Among the three isolates, maximum biodegradation ability was obtained by PT-3 which degraded >99% of 100 mg L⁻¹ of endosulfan after 90 h of incubation and also used it as the source of sulfur. The isolate, PT-3 was identified as *Agrobacterium tumefaciens* and was able to use both α and β isomers of endosulfan with equal efficiencies without the accumulation of known toxic intermediates or end products. Endosulfan is known to be highly toxic to nitrogen fixing bacteria such as *Rhizobium* sp thereby reducing the fertility of soil. *A. tumefaciens* is a well known inhabitant of agricultural fields and therefore may be a valuable bioaugmenting agent for remediation of endosulfan contaminated sites.

**Keywords:** Endosulfan; Bacteria; Biodegradation; *Agrobacterium tumefaciens*

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A moderately halophilic bacterial enrichment was able to degrade 120 mg/L of phenol in the presence of 1–2 M of NaCl within 3 d or 2.5–3 M of NaCl within 6 d. The optimal degradation was achieved at 1.5 M of NaCl and 350 mg/L of phenol. PCR-DGGE profile of the enrichment showed that the *Acidobacterium* sp. and *Chloroflexus* sp. dominated the community. The phenol-
Degradation of rhamnolipid-solubilized hexadecane and mass hexadecane as a separate phase by Pseudomonas aeruginosa CCTCC AB93066 treated with rhamnolipid biosurfactant was studied for better understanding on the roles of rhamnolipid in hydrocarbon biodegradation. The results of hexadecane solubilization experiment showed that solubility of hexadecane was linearly related to the concentration of rhamnolipid below or above its critical micelle concentration (CMC), and the ability of monorhamnolipid (monoRL) to solubilize hexadecane was stronger at concentration below CMC than above CMC. MonoRL was then used for treating cells in degradation experiment. Results showed that 75 µM (1 CMC) monoRL treatment had a small inhibitory effect on cell growth on glucose or mass hexadecane, however 750 µM (10 CMC) monoRL treatment accelerated degradation of mass hexadecane by reducing the lag phase of cell growth for 36 h; this effect was not caused by initial cell surface hydrophobicity enhancement. No degradation of hexadecane solubilized by 750 µM monoRL was observed for the cells treated with or without monoRL, indicating that the pseudo-solubilized hexadecane is not available to cells. It is inferred from the data that the effectiveness of rhamnolipid to accelerate degradation of hydrocarbons by enhancing solubilization of the hydrocarbons may not always be guaranteed, which is of importance for evaluation of rhamnolipid biosurfactant application to hydrocarbon-contaminated sites during remediation.

**Keywords:** Pseudomonas aeruginosa; Rhamnolipid; Pseudo-solubilized hexadecane; Cell surface hydrophobicity; Degradation

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Abstract Vol. No. 25, December 2014

The study of wood decay fungi that naturally biodegrade lignocellulosic polymers has been steadily increasing during the past two decades due to their industrial and innovative applications. In this work, we compare ten species of lignicolous macrofungi which develop fruiting bodies on poplar in relation to their capacity for growing on poplar wood chips and sawdust and of secreting cell wall degrading enzymes. All the fungi studied appeared to be able to grow well in these conditions and to secrete cellulase and hemicellulase, Mn-peroxidase and celllobiose dehydrogenase, while Li-peroxidase and laccase were produced by seven and six out of the ten species, respectively. Variability in the levels of all these enzymatic activities was assessed. Two species, never investigated before, showed the best performances as regards production of cellulytic and hemicellulytic activities (Lenzites warnieri) and Mn-peroxidase (Perenniporia meridionalis). The highest laccase level was detected in the well known plant pathogen Fomes fomentarius, and the brown-rot Daedalea quercina proved to be the best producer of lignin peroxidase and celllobiose dehydrogenase.

Keywords: Lignicolous fungi; Cell wall degrading enzymes; Poplar wood


The textile and dye industries are considered as one of the foremost sectors that pollutes environment. Technologies employing biological methods showed promising approach to remediate sites polluted with dye and dye intermediates. Bacterial consortium AR1 developed through culture enrichment method was comprised of four distinct bacterial strains. The synergistic metabolic activities of AR1 led to complete decolourization of monoazo dye Reactive Red 195 within 14 h under microaerophilic environment. The co-metabolic decolourization of Reactive Red 195 by consortium, in presence of maltose and proteose peptone (0.1%, w/v, each) in minimal medium, easily reduced Reactive Red 195 (100 mg/L) for five consecutive cycles without any replenishment of nutrient. The maximum decolourization was observed at pH 8.0 and 40 °C. The consortium was acclimatized to decolourize Reactive Red 195 at higher concentration (2000 mg/L) even under high salt concentration (1 M NaCl). Consortium exhibited broad substrate specificity where it decolourized 15 structurally different dyes and more than 50% decolourization was observed in a medium containing mixture of dyes. The degradation products analyzed using FTIR, HPTLC and 1H NMR revealed the formation of 2-amino-naphthalene, 1-amino-benzene and 1-nitro-benzene. Thus the ability of bacterial consortium for simultaneous decolourization and degradation of azo compounds signifies its potential application in dye remediation.

Keywords: Bioremediation; Azo dyes; Textile effluents; Co-substrates; Microaerophilic; Aromatic amines

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A phenanthrene (PHE) degrading bacterium strain BZ-3 was isolated from the crude oil contaminated soil in Binzhou, China. The isolate was identified as *Pseudomonas* sp. BZ-3 on the basis of 16S rRNA gene sequence. Various experiments were conducted to investigate the effect of pH, salinity and PHE concentration on the degradation efficiency of PHE. The degradation efficiency and degradation metabolites of PHE were detected by using GC–MS and HPLC-MS analyses. The strain BZ-3 could degrade 75% of PHE at an initial concentration of 50 mg/L under 20 g/L salinity in 7 days. PHE degradation kinetics was estimated in a first-order degradation rate model and the rate coefficient was calculated as 0.108 d\(^{-1}\). On the basis of the identified degradation metabolites, the strain BZ-3 could degrade PHE in the salicylate metabolic pathway. In a mixture system consisting of PHE and other PAHs including naphthalene (NA), anthracene (ANTH), and pyrene (PYR), the strain BZ-3 showed an efficiently degradation capability. Further study showed that the strain BZ-3 could also use NA, ANTH, PYR, xylene, 1-hydroxy-2-naphthoic acid, and hexane as the sole carbon and energy source, but did not grow on nitrobenzene-containing medium.

**Keywords:** Biodegradation; Phenanthrene; Halophilic microorganism; Metabolic pathway; *Pseudomonas*

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Most Trichloroethylene (TCE) biodegradation reports refer to methanogenic conditions, however, in this work, enhanced sulfidogenesis and TCE biodegradation were achieved in an upflow anaerobic sludge blanket (UASB) reactor in which a completely sulfidogenic sludge, from hydrothermal vents sediments, was developed. The work was divided in three stages, (i) sludge development and sulfate reducing activity (SRA) evaluation, (ii) TCE biodegradation and (iii) SRA evaluation after TCE biodegradation. For (i) SR was 98 ± 0.1%, 84% as sulfide (H\(_2\)S, 1200 ± 28 mg/L), sulfate reducing activity (SRA) was 188 ± 50 mg COD H\(_2\)S/g VSS*d. For (ii) The reactor reached 74% of TCE removal, concentrations of vinyl chloride of 16 ± 0.3 µM (5% of the TCE added) and ethene 202 ± 81 µM (67% of the TCE added), SRA of 161 ± 7 mg COD H\(_2\)S/g VSS*d, 68% of sulfide (H\(_2\)S) production and 93% of COD removal. For (iii) SRA was of 248 ± 22 mg COD H\(_2\)S/g VSS*d demonstrating no adverse effects due to TCE.
Among the genera of the microorganisms identified in the sludge during TCE biodegradation were: *Dehalobacter*, *Desulfofotomaculum*, *Sulfospirillum*, *Desulfotibacterium*, *Desulfovibrio* and *Clostridium*. To the best of our knowledge, this is the first report using a sulfidogenic UASB reactor to biodegrade TCE. The overall conclusions of this work are that the reactor is efficient on both, sulfate and TCE biodegradation and it could be used to decontaminate wastewater containing organic solvents and relatively high concentrations of sulfate.

**Keywords:** Sulfidogenic UASB reactors; Trichloroethylene biodegradation; Sulfate reducing bacteria; Dehalorespiring bacteria; *Desulfovibrio* sp.; *Clostridium* sp.

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The present study examines an improved detoxification and rapid biological degradation of toxic pollutant acrylamide using a bacterium. The acrylamide degrading bacterium was isolated from the soil followed by its screening to know the acrylamide degrading capability. The minimal medium containing acrylamide (30 mM) served as a sole source of carbon and nitrogen for their active growth. The optimization of three different factors was analyzed by using Response Surface Methodology (RSM). The bacteria actively degraded the acrylamide at a temperature of 32 °C, with a maximum growth at 30 mM substrate (acrylamide) concentration at a pH of 7.2. The acrylamidase activity and degradation of acrylamide was determined by High Performance Liquid Chromatography (HPLC) and Matrix Assisted Laser Desorption and Ionization Time of Flight mass spectrometer (MALDI-TOF). Based on 16S rRNA analysis the selected strain was identified as Gram negative bacilli *Stenotrophomonas acidaminiphila* MSU12. The acrylamidase was isolated from bacterial extract and was purified by HPLC, whose mass spectrum showed a molecular mass of 38 kDa.

**Keywords:** Acrylamidase; Acrylamide; Biodegradation; MALDI-TOF; *Stenotrophomonas acidaminiphila* MSU12

Esmail AL-Saleh, Christian Obuekwe. (Department of Biological Sciences, College of Science, Kuwait University, Kuwait). Crude oil biodegradation activity in potable water. International Biodeterioration & Biodegradation, Volume 93 (2014): 18–24

A wide range of aliphatic and aromatic hydrocarbon concentrations were detected in potable water samples obtained from various locations in Kuwait city. Detected aliphatic compounds included hexadecane (2.19 µg l\(^{-1}\)), heptadecane (2.49 µg l\(^{-1}\)), nonadecane (2.24 µg l\(^{-1}\)), eicosane (1.79 µg l\(^{-1}\)), docasane (1.4 µg l\(^{-1}\)) and pentacosane (1.48 µg l\(^{-1}\)), while the aromatic hydrocarbons contaminants included benzene (1.62 µg l\(^{-1}\)), phenanthrene (3.19 ng l\(^{-1}\)) and several aromatic-degradation intermediates. Culturable microbial loads in the water samples were low, ranging from 3 CFU ml\(^{-1}\) to 41 CFU ml\(^{-1}\), but included a high proportion of
hydrocarbon degrading bacteria. Hydrocarbon-utilizing bacteria isolated included *Cupriavidus gilardii*, *Pseudomonas* sp., *Bacillus cereus* and *Paenibacillus ehimensis* that constituted >30% of the total number of isolates. Under current experimental conditions, the hydrocarbon-utilizing capacities of individual isolates from the water samples were wide representing self-cleaning activity of hydrocarbons present in water samples. Interestingly, the hydrocarbon-utilizing microbial contaminants also exhibited a wide-range of antibiotic resistance characteristics.

**Keywords:** Biodegradation; Hydrocarbon utilization; Pollution; Bacterial contamination; Phylogenetic


To better understand the process of fuel biodeterioration, Jet-A and F-76 diesel fuel were exposed to *Pseudomonas aeruginosa*, a common fuel contaminant, and *Marinobacter hydrocarbonoclasticus*, a marine hydrocarbon degrader, and the extent of hydrocarbon decomposition produced by these bacteria determined. Degradation assays containing fuel-minimal media mixtures and bacteria were analyzed by gas chromatography (GC) to discern the consumption of fuel hydrocarbons. Experiments were conducted in closed systems to prevent evaporation of hydrocarbons and allow accurate quantitation. Results indicated that *P. aeruginosa* preferred to consume mid-range normal alkanes (C₁₂–C₁₈) followed by higher chain n-alkanes (C₁₉–C₂₃). Cycloparaffins were consumed at much lower rates, while aromatic and isoparaffins were not consumed. However, *M. hydrocarbonoclasticus* showed a different profile with preferential degradation of shorter n-alkanes (C₈–C₁₁) and specific aromatic compounds. Both types of bacteria were incapable of degrading branched alkanes. During larger scale bioreactor tests, bacteria were able to degrade similar hydrocarbons. This study clearly demonstrated that the effects of fuel biodeterioration can go well beyond corrosion and filter fouling, with different bacteria metabolizing different fuel hydrocarbons and presenting the possibility for microbes to directly change fuel composition and properties. Results are discussed in light of the use of newer alternative fuels which can have dramatically different hydrocarbon profiles compared to conventional petroleum fuels.

**Keywords:** Fuel; Biofuels; Alternative fuels; Hydrocarbons; Biodegradation; Biodeterioration; Jet-A; F-76; Jet fuel; Diesel; Biorremediation; Bacteria; Gas chromatography

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N-Methylpyrrolidone (NMP), a kind of nitrogen-containing heterocyclic pollutant, is widely used in chemical industry. Microbial degradation is an important environmental fate process in soil and water, however, the microbial metabolic mechanism is still unknown. Strain NMD-4, capable of utilizing NMP as the sole source of carbon and nitrogen, was isolated from the activated sludge of a pesticide plant in Jiangsu, China, and identified as Paracoccus sp. based on its physiological–biochemical properties, as well as 16S rRNA gene sequence analysis. The degradation characteristic of NMP by strain NMD-4 was studied in a liquid culture, and the metabolic pathway of NMP by the strain was investigated. Two metabolites, 1-methyl-2,5-pyrrolidinedione and succinic acid, were detected and identified by liquid chromatography-mass spectrometry analysis, and a plausible microbial degradation pathway of NMP was proposed by the first time.

Keywords: N-Methylpyrrolidone; Paracoccus sp.; Biodegradation; Metabolic pathway

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The aim of this study was to screen microorganisms belonging to Rhodococcus and Cupriavidus genera, for their DHT degrading capability and to select microbes with the best biodetoxification potential. Bioluminescent bioreporters (Saccharomyces cerevisiae BLYAS and BLYR) were successfully used to monitor DHT degradation and the results were confirmed by chemical analysis (GC–MS). Several strains with high potential for DHT biotransformation were selected on the base of analytical method, but bioreporters were able to monitor the samples for bioavailable toxicants and androgenic potential even after substantial degradation. Based on this integrated chemical and biological analysis DHT-degrading microbes, ones that produced minimal toxic products and products with lower androgenic potential could be selected. Out of the selected microbes Rhodococcus pyridinivorans strain AK37 was further investigated by using extracellular extracts of the microbe, which demonstrated a DHT-degradation with the total loss of androgenicity after 9 h. Results indicated that the degradation is enzymatic and that the enzymes responsible for the degradation of DHT are extracellular and constitutively produced.

Keywords: Dihydrotestosterone; Saccharomyces cerevisiae; Androgen; Biomonitoring; Biodegradation

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This study aimed to evaluate changes in the chemical composition and mechanical properties of static bending of wood from two fast-growing eucalypt species (blue gum and lemon-scented gum) after exposure for one year in a field test. To achieve this, decayed untreated wood (after 1 yr of exposure in the field test) was characterised by chemical analysis, thermogravimetric analysis, and infrared spectroscopy, and was compared with control samples. Mass loss and modulus of elasticity (MOE) and modulus of rupture (MOR) as a function of the exposure time were evaluated. The main findings showed that lignin and carbohydrate content of the two decayed woods decreased after exposure in the field test. Mass loss increased with increasing time of exposure, while MOE and MOR decreased for both woods. Nevertheless, blue gum wood was more susceptible to decay.

**Keywords:** Chemical composition; Decay fungi; Wood degradation; Mechanical strength; Wood species

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Bacterial strains having high BPA tolerance were isolated from seawater and sediment samples collected from coastal regions of Chennai, Tamil Nadu, India. Three different bacteria, designated as K-6, K-8, and KU-3, showed the best degradation efficiency of 78 ± 4%, 81 ± 3%, and 74 ± 2%, respectively, at 150 rpm (12 days at 37 °C) in mineral medium supplemented with 1000 ppm BPA. The best degradation was obtained at pH 7.0 and it subsequently decreased with alteration of pH. Sodium glutamate as co-substrate accelerated the degradation efficiency 85 ± 3% (K-8), 83 ± 2% (K6), and 81 ± 3% (KU-3). The bacterial strains K-6, K-8, and KU-3 exhibited degradation rates of 78%, 70%, and 61%, respectively, for 150 rpm (15 days at 37 °C) in the seawater containing BPA (1000 ppm) as a sole carbon and energy source. Residual BPA was analyzed by gas chromatography. These strains were identified as *Pseudomonas* sp. strain KU1, *Pseudomonas* sp. strain KU2, and *Bacillus* sp. strain KU3 by partial 16S rDNA analysis.

**Keywords:** Bisphenol A; Biodegradation; BPA tolerance; Bacteria; Seawater

L. Wagner\(^a\), T.K. Bader\(^a\), J. Eberhardsteiner\(^a\), K. de Borst\(^b\). (\(^a\) Institute for Mechanics of Materials and Structures, Vienna University of Technology, Karlsplatz 13/202, 1040 Vienna, Austria, \(^b\) School of Engineering, University of Glasgow, Glasgow G12 8LT, Scotland, UK). Fungal degradation of softwood cell walls: Enhanced insight through
Fungal degradation is among the greatest hazards for standing trees as well as timber constructions. Herein we aim at gaining more detailed insight into the degradation strategies of wood destroying fungi and the consequences on the mechanical performance of wood. At the macroscale, the occurring losses of mass and of mass density mask effects of altered chemical composition and microstructure. Thus, it is necessary to step down the hierarchical organization of wood to the cell wall scale in order to resolve these changes and their mechanical impact. We present a multiscale micromechanical model which is used to estimate the stiffnesses of the S2 cell wall layer and the compound middle lamella of fungal degraded wood. Data from a detailed chemical, microstructural and micromechanical characterization of white rot and brown rot degraded Scots pine sapwood is analyzed. Comparing predicted cell wall stiffnesses with measured ones confirms the suitability of the approach. The model enables to establish structure–stiffness relationships for fungal degraded wood cell walls and to test hypotheses on yet unknown effects of fungal decay. The latter include the evolution of porosity, modifications of the cell wall polymers resulting in changes of their stiffnesses, as well as increasing cell wall crystallinity. The model predictions in general showed good agreement with the predictions not considering pores in the cell wall. However, this finding does not rule out the formation of porosity. Other degradation related effects like modifications of the cell wall polymers as well as increased crystallinity have the potential to account for stiffness decreases upon the formation of pores.

**Keywords:** Fungal decay; Softwood; Brown rot; White rot; Micromechanical modeling

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Nonylphenol (NP) has been a contaminant of great environmental concern due to its ubiquity, toxicity and endocrine activity. Biodegradation is an ideal way to clean up NP pollution. In this study, two NP degraders were isolated from river sediment. Their ability to degrade NP was tested in both liquid culture and sediment microcosm. Phylogenetic analysis indicated that one isolate belonged to genus *Rhizobium*, while another was a *Sphingobium* species. The *Rhizobium* strain contained ALK gene, while the *Sphingobium* strain harbored ALK and C23O genes. Both of the two strains showed strong NP degradation ability in liquid culture. However, only the *Rhizobium* strain demonstrated a potential of bioremediating NP-contaminated sediment. This study can provide some new insights towards NP biodegradation and bioremediation.

**Keywords:** Alkylphenol; Bioremediation; Bioaugmentation; *Rhizobium*; *Sphingobium*; Sediment

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This research describes indigenous *Raoultella planticola* bacterial cells which were isolated from the wastewater treatment plant of a herbicide factory. The optimum conditions for degrading atrazine were at pH = 7 and 28 °C, with a degradation rate of 10 mg L\(^{-1}\) h\(^{-1}\). Biodegradation was observed at temperatures of 45 and 4 °C and partial degradation was also observed at extreme pH values (3 and 10). The degradation rates to reach 50% depletion of atrazine were 9.42, 7.42 and 5.42 mg L\(^{-1}\) h\(^{-1}\) in the presence of acetonitrile, phenol or toluene, respectively. Successful inoculation of *R. planticola* into the original sludge from the herbicide factory led to atrazine degradation within 3 h, instead of 3 days without the inoculation. *R. planticola* developed a massive biofilm when exposed to atrazine. The results indicate that the isolated *R. planticola* strain can be added to the arsenal of atrazine-degrading bacterial cells that have the ability to degrade this substance under unfavorable conditions, such as those existing in the sludge of herbicide factories. In addition, the isolated strain showed an ability to form a biofilm, which can be utilized for improving the wastewater treatment.

**Keywords:** Atrazine; Degradation; *Raoultella planticola*; Toxic solvents; Sludge; Biofilm

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This study highlights the application potential of Microbial Desalination Cell in effective bioremediation of dye house effluent by biodecolourisation of industrial dyes. The study showed, for the first time, that the dye house effluent could be used as an organic substrate in MDC, achieving biodecolourisation of effluent along with considerable desalination and power production. Utilization of these wastes in MDC can protect the environment from dye-containing wastewater contamination and the treated water can be reused for other purposes. Two bacterial strains, a novel isolate *Bacillus subtilis moh3* and a MTCC strain *Aeromonas hydrophila* subsp. *hydrophila* 8049 were used for decolourisation of two model dyes-Malachite Green (C.I.42,000) and Sunset Yellow (C.I.15,985). Decolourisation of dyes by these cultures was studied in batch cultures and optimized the growth conditions. The same cultures when used in Microbial Desalination Cell, achieved complete biodecolourisation along with considerable desalination (62.2 ± 0.4% and 57.6 ± 0.2%) and power production.

**Keywords:** Microbial desalination cell; Biodecolourisation; Dye house effluent; *Bacillus subtilis moh3*; *Aeromonas hydrophila* subsp. *hydrophila* 8049

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University, P.O. Box 46414-356, Noor, Iran, bWaste Management Organization of Mashhad Municipality, Mashhad, Iran). Microbial biodegradation of waste materials for nutrients enrichment and heavy metals removal: An integrated composting-vermicomposting process. International Biodeterioration & Biodegradation, Volume 92 (2014): 41–48

The aim of present study was to improve the quality of vermicompost through different substrates and adding active sewage sludge as a source of N-fixing and P-solublizing bacteria in a shorter time than conventional composting process. The experiment setup included 15-L reactors used for pre-composting, a vermicomposting mixture of activated sewage sludge (control, 2000, 4000 and 6000 mg L$^{-1}$) and corn stalk residue (40, 60 and 80%). The physicochemical changes in vermicompost caused by the microbial biodegradation and their combinations were measured over a period of 70 days. The results showed that the values of total organic carbon (TOC), total volatile solid (TVS), total Kjeldahl nitrogen (TKN) and carbon to nitrogen ratio (C/N) decreased in all treatments, while those of electrical conductivity (EC), total phosphorous (TP), nitrate and heavy metals increased. A minimum C/N ratio of 13.16% obtained in the 40% corn stalk waste substrate with 4000 mg L$^{-1}$ activated sludge treatment while it was 23.44% in the 80% corn stalk waste substrate without activated sludge treatment. Results indicated that with the increase in 6000 mg L$^{-1}$ sewage sludge and with the decrease of 40% corn waste substrate lead a decrease in TKN and an increase in nitrate, viz. 1.36–2.06% and n.d.–1889 mg kg$^{-1}$, respectively. However, in comparison to decrease in TKN nitrogen, decrease in TOC (39.94–27.32%), TVS (63.48–43.48%), C/N ratio (63.48–13.43) and pH (7.33–3.15) and increase in EC (1.55–3.15 mS cm$^{-1}$) and TP (2.395–3.31 g kg$^{-1}$) was obtained. The decrease of heavy metals in the final vermicompost materials was detected by noting a low heavy metals concentration in the corn residue.

Keywords: Vermicompost; Corn stalk residue; Activated sludge; Heavy metals; N-fixing and P-solubilizing bacteria


Two sequencing batch reactors (SBR) were constructed and filled with different inocula of activated sludge (AS) and mature fine tailings (MFT) to treat oil sands process-affected water (OSPW). The COD was reduced by 82% in the AS-SBR and 43% in the MFT-SBR during phase I using 10% OSPW and 90% synthetic wastewater as reactor feed. However, COD removal reached 12% and 20% in the AS-SBR and the MFT-SBR, respectively, when 100% raw OSPW was fed into the reactors. Maximum removal of acid-extractable organics (AEO) was 8.7% and 16.6% in the AS-SBR and the MFT-SBR, respectively with a hydraulic retention time of one day. Pyrosequencing analysis revealed that Proteobacteria was the dominant phylum and beta- and gamma-Proteobacteria were dominant classes in both reactors. Evidence of a microbial community change was observed when influent raw OSPW was switched from 50 to 100%. More significant changes in the AS-SBR community were detected.

Keywords: Sequencing batch reactors; Activated sludge; Mature fine tailings; Oil sands process affected water; Pyrosequencing
Halotolerant strains of *Bacillus amyloliquefaciens* were isolated from salt spring in Ovca spa located in Republic of Serbia. Strains exhibit robust spore laccase with high temperature optimum of 65 °C while pH optimum is wide and substrate dependant. Ability to oxidize azo dyes was demonstrated. Under optimized conditions more than 85% removal of Congo red dye was achieved at pH 5.7. Substantial resistance to inhibition by high concentration of chloride ions was observed and tolerance of some commonly used cosolvents shows that applicability of these laccases goes beyond decolorization of textile effluents.

**Keywords:** *Bacillus amyloliquefaciens*; Laccase; Decolorization; Reactive dye; Congo red; Azo dye

The biodegradation of lignin, cellulose and hemicellulose from oil palm empty fruit bunches by white rot fungi *Phanerochaete chrysosporium* and *Pleurotus ostreatus* was investigated. The results showed higher mass loss and polysaccharide degradation when the biological treatment was carried out with *P. chrysosporium*. Biodegradation curves were modelled by a Weibull kinetic model and the kinetic parameters were obtained for each one of the components. Even though the lignin degradation rates were similar for both fungi, the biodegradation of this component reached 50% with *P. ostreatus*, a higher value than the 41% reached with *P. chrysosporium* after harvesting for four weeks. Higher polysaccharides biodegradation rates were observed for *P. chrysosporium* compared to *P. ostreatus*. Consequently, the *P. ostreatus* pretreatment can be considered adequate for the delignification of palm residues without considerably affecting the cellulose fraction, which is important for the production of fermentable sugars.

**Keywords:** Biodegradation kinetics; Oil palm empty fruit bunch; Biological pretreatment; White rot fungi; Lignocelullolosic biodegradation

Degradation of polychlorinated biphenyls (PCBs) by four bacterial isolates

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**Abstract Vol. No. 25, December 2014**
obtained from the PCB-contaminated soil and PCB-contaminated sediment. International Biodeterioration & Biodegradation, Volume 91 (2014): 52–59

We investigated the PCB-degrading abilities of four bacterial strains isolated from long-term PCB-contaminated soil (Alcaligenes xylosoxidans and Pseudomonas stutzeri) and sediments (Ochrobactrum anthropi and Pseudomonas veronii) that were co-metabolically grown on glucose plus biphenyl which is an inducer of the PCB catabolic pathway. The aim of study was to determine the respective contribution of biomass increase and expression of degrading enzymes on the PCB degrading abilities of each isolate. Growth on 5 g l⁻¹ glucose alone resulted in the highest stimulation of the growth of bacterial strains, whereas grown on 10 mg l⁻¹, 100 mg l⁻¹, 1 g l⁻¹, or 5 g l⁻¹ biphenyl did not effect the bacterial growth. None of the strains used in this study was able to grow on PCBs as the sole carbon source. Cells grown on glucose exhibited enhanced degradation ability due to an increased biomass. Addition of biphenyl at concentrations of 1 or 5 g l⁻¹ did not increase total PCB degradation, but stimulated the degradation of highly chlorinated congeners for some of the strains. The degradation of di- and tri-chlorobiphenyls was significantly lower for cells grown on 5 g l⁻¹ biphenyl independently on glucose addition. The highest degradation of the PCBs was obtained for A. xylosoxidans grown in the presence of glucose. Thus A. xylosoxidans appears to be the most promising among the four bacterial isolates for the purpose of bioremediation.

Keywords: Bacteria; Biodegradation; Biphenyl; Polychlorinated biphenyls

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In the present study, cultivation of aerobic granular biomass capable of biodegradation of dibutyl phosphate, an organophosphite, and isolation of dibutyl phosphate degrading bacterial strains, are reported for the first time. The strain AMGD5, identified as Sphingobium sp., based on 16S rRNA sequencing, degraded dibutyl phosphate efficiently and utilised it as the sole source of carbon and phosphorus. Microbial degradation of dibutyl phosphate caused a significant decrease in medium pH, leading to cessation of growth and further degradation of dibutyl phosphate. Under buffered conditions, complete degradation of up to 3 mM of dibutyl phosphate was achieved within 60 h. The strain showed almost similar growth pattern when either phosphite or dibutyl phosphite was used as the phosphorous source. A 4-fold enhancement in phosphatase activity was evident in dibutyl phosphate fed cells, implying their role in dibutyl phosphate degradation. Sphingobium sp. AMGD5 can be a potential candidate for bioremediation of dibutyl phosphate contaminated waters or sites.

Keywords: Aerobic granular sludge; Biodegradation; Dibutyl hydrogen phosphite; Dibutyl phosphite; Sphingobium sp.; Organophosphorous compounds

Zeeshanur Rahman, Ved Pal Singh. (Applied Microbiology and Biotechnology Laboratory, Department of Botany, University of Delhi, Delhi 110 007, India). Cr(VI) reduction by Enterobacter sp. DU17 isolated from the tannery waste dump site and characterization of the bacterium and the Cr(VI) reductase. International Biodeterioration & Biodegradation, Volume 91(2014): 97–103
Out of nineteen bacteria screened from the tannery waste dump site, the most effective isolate, strain DU17 was selected for Cr(VI) reduction process among the non-pathogenic once. Based on 16S rRNA gene sequence analysis, the bacterium was identified as Enterobacter sp. DU17. Its amplified Cr(VI) reductase gene showed maximum homology with flavoprotein of Enterobacter cloacae. Enterobacter sp. DU17 reduced Cr(VI) maximally at 37 °C and pH 7.0. Various co-metals, electron (e-) donors and inhibitors were tested to study their effect on Cr(VI) reduction. In presence (0.2% each) of glucose and fructose, Enterobacter sp. DU17 reduced Cr(VI) completely after 16 and 20 h, respectively. Since the concentration of total Cr was invariable after remediation as detected through AAS analysis, this experiment disclosed that responsible operation was associated with extracellular Cr(VI) reduction process rather than uptake mechanism. Multiple antibiotic resistance index of 0.08 for this bacterium was very low as compared to standard risk assessment value of 0.20. With high Cr(VI) reducing capability, non-pathogenicity and antibiotic sensitivity, Enterobacter sp. DU17 is found to be very efficient in removing Cr(VI) toxicity from the environment.

**Keywords:** Cr(VI) reductase; Cr(VI) reduction; Cr(VI) toxicity; Enterobacter; Tannery waste dump site


The aim of this study was to evaluate the impact of selected electron donors and electron acceptors on the anaerobic biodegradation of DDT and its major metabolites in a muck soil with a long history of exposure to the pesticide. Loss of DDT was measured in anaerobic microcosms supplemented with H2, lactate, and acetate. The greatest loss of DDT (approximately 87 %) was observed in microcosms amended with lactate and no additional electron acceptor compared to the no additional electron donor or acceptor sets. An increase in measureable concentrations of DDx was observed in un-amended microcosms. In larger scale mesocosms, significant increases in dissolved organic carbon (DOC) corresponded with low redox potentials. Increases in DOC corresponded with sharp increases in measured concentrations of DDx, followed by a decrease in measured DDT concentrations in lactate-amended mesocosms. Our studies indicate that sorbed DDx is released upon anaerobic incubation, and that indigenous microorganisms capable of DDx degradation respond to lactate additions. Both the potential for release of sorbed DDx and the potential for biodegradation of DDx should be considered during remediation of DDx-contaminated organic soils at low redox potentials.

**Keywords:** DDT; DDD; DDE; Muck soil; Anaerobic; Microcosm; Mesocosm; Degradation

Microbial degradation of dibenzothiophene (DBT) beyond 3-hydroxy-2-formylbenzothiophene (HFBT), a commonly detected metabolite of the Kodama pathway for DBT metabolism, and the catabolic intermediates leading to its mineralization are not fully understood. The enrichment cultures cultivated from crude oil contaminated soil led to isolation of ERI-11; a natural mixed culture, selected for its ability to deplete DBT in basal salt medium (BSM). A bacterial strain isolated from ERI-11, and tentatively named A11, degraded more than 90% of the initial DBT (270 µM), present as the sole carbon and sulfur source, in 72 h. Gas chromatography–mass spectrophotometry (GC–MS) analyses of the DBT degrading A11 culture medium extracts led to detection of HFBT. The metabolite HFBT, produced using A11, was used in degradation assays to evaluate its metabolism by the bacteria isolated in this study. Ultra violet–visible spectrophotometry and high-performance liquid chromatography analyses established the ability of the strain A11 to deplete HFBT, present as the sole sulfur and carbon source in BSM. GC–MS analyses showed the presence of 2-mercaptobenzoic acid in the HFBT degrading A11 culture extracts. The findings in this study establish that the environmental isolate A11 possesses the metabolic capacity to degrade DBT beyond the metabolite HFBT. The compound 2-mercaptobenzoic acid is an intermediate formed on HFBT degradation by A11.

Keywords: Biodegradation; Dibenzothiophene; 3-Hydroxy-2-formylbenzothiophene; 2-Mercaptobenzoic acid;

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Bromophenol is listed as priority pollutant by U.S. EPA, however, there is no report so far on its removal in mixed pollutants system by any biological reactor operated in continuous mode. Furthermore, bromophenol along with chlorophenol and nitrophenol are usually the major constituents of paper pulp and pesticide industrial effluent. The present study investigated simultaneous biodegradation of these three pollutants with specially emphasis on substrate competition and crossed inhibition by Arthrobacter chlorophenolicus A6 in an upflow packed bed reactor (UPBR). A 2³ full factorial design was employed with these pollutants at two different levels by varying their influent concentration in the range of 250–450 mg l⁻¹. Almost complete removal of all these pollutants and 97% effluent toxicity removal were achieved in the UPBR at a pollutant loading rate of 1707 mg l⁻¹ day⁻¹ or lesser. However, at higher loading rates, the reactor performance deteriorated due to transient accumulation of toxic intermediates. Statistical analysis of the results revealed a strong negative interaction of 4-CP on 4-NP biodegradation. On the other hand, interaction effect between 4-CP and 4-BP was found to be insignificant. Among these three pollutants 4-NP preferentially degraded, however, 4-CP exerted more inhibitory effect on 4-NP biodegradation. This study demonstrated the potential of A. chlorophenolicus A6 for biodegradation of 4-BP in mixed pollutants system by a flow through UPBR system.

Keywords: 4-Bromophenols; Arthrobacter chlorophenolicus A6; Substrate interaction; Biofilm reactor; Toxicity removal;
Uzochukwu C. Ugochukwu¹, Ian M. Head¹, David A. C. Manning¹, (¹School of Civil Engineering and Geosciences, University of Newcastle Upon Tyne, Drummond Building, Newcastle Upon Tyne, NE1 7RU, UK). Biodegradation and adsorption of C1- and C2-phenanthrenes and C1- and C2-dibenzo thiophenes in the presence of clay minerals: effect on forensic diagnostic ratios. Biodegradation, Volume 25 (4) (2014): 515-527

The impact of modified montmorillonites on adsorption and biodegradation of crude oil C1-phenanthrenes, C1-dibenzo thiophenes, C2-phenanthrenes and C2-dibenzo thiophenes was investigated in aqueous clay/oil microcosm experiments with a hydrocarbon degrading microorganism community. Consequently, the effect on C1-dibenzo thiophenes/C1-phenanthrenes, C2-dibenzo thiophenes/C2-phenanthrenes, 2+3-methyl dibenzo thiophene/4-methyl dibenzo thiophene and 1-methyl dibenzo thiophene/4-methyl dibenzo thiophene ratios commonly used as diagnostic ratios for oil forensic studies was evaluated. The clay mineral samples were treated to produce acid activated montmorillonite, organomontmorillonite and homoionic montmorillonite which were used in this study. The different clay minerals (modified and unmodified) showed varied degrees of biodegradation and adsorption of the C1-phenanthrenes, C1-dibenzo thiophenes, C2-phenanthrenes and C2-dibenzo thiophenes. The study indicated that as opposed to biodegradation, adsorption has no effect on the diagnostic ratios. Among the diagnostic ratios reviewed, only C2-dibenzo thiophenes/C2-phenanthrenes ratio was neither affected by adsorption nor biodegradation making this ratio very useful in forensic studies of oil spills and oil–oil correlation.

Keywords: Clay minerals; Biodegradation; Adsorption; Diagnostic ratio; Forensics

Sara Gallego¹, Joaquim Vila¹, Margalida Tauler¹, José María Nieto¹, Philip Breugelmans², Dirk Springael², Magdalena Grifoll¹. (¹Department of Microbiology, University of Barcelona, Diagonal 643, 08028, Barcelona, Spain, ²Division of Soil and Water Management, Catholic University of Leuven, Kasteelpark Arenberg 20, 3001, Leuven, Belgium). Community structure and PAH ring-hydroxylating dioxygenase genes of a marine pyrene-degrading microbial consortium. Biodegradation, Volume 25 (4) (2014): 543-556

Marine microbial consortium UBF, enriched from a beach polluted by the Prestige oil spill and highly efficient in degrading this heavy fuel, was subcultured in pyrene minimal medium. The pyrene-degrading subpopulation (UBF-Py) mineralized 31% of pyrene without accumulation of partially oxidized intermediates indicating the cooperation of different microbial components in substrate mineralization. The microbial community composition was characterized by culture dependent and PCR based methods (PCR-DGGE and clone libraries). Molecular analyses showed a highly stable community composed by Alphaproteobacteria (84%, Breog spina, Thalassospira, Paracoccus, and Martellella) and Actinobacteria (16%, Gordonia). The members of Thalassospira and Gordonia were not recovered as pure cultures, but five additional strains, not detected in the molecular analysis, that classified within the genera Novosphingibium, Sphingopyxis, Aurantimonas (Alphaproteobacteria), Alcanivorax (Gammaproteobacteria) and Micrococcus (Actinobacteria), were isolated. None of the isolates degraded pyrene or other PAHs in pure culture. PCR amplification of Gram-positive and Gram-negative dioxygenase genes did not produce results with any of the cultured strains. However, sequences related to the NidA3 pyrene dioxygenase present in mycobacterial strains were detected in UBF-
Py consortium, suggesting the representative of Gordonia as the key pyrene degrader, which is consistent with a preeminent role of actinobacteria in pyrene removal in coastal environments affected by marine oil spills.

**Keywords:** Biodegradation; Pyrene; PAHs; Marine microbial consortia; Gordonia; Dioxygenase; Community structure analysis;

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This study reports the biodegradation of carbon disulfide (CS₂) in air biofilters packed with a pelleted mixture of composted manure and sawdust. Experiments were carried out in two lab-scale (1.2 L) biofiltration units. Biofilter B was seeded with activated sludge enriched previously on CS₂-degrading biomass under batch conditions, while biofilter A was left as a negative inoculation control. This inoculum was characterized by an acidic pH and sulfate accumulation, and contained *Achromobacter xylosoxidans* as the main putative CS₂ biodegrading bacterium. Biofilter operation start-up was unsuccessfully attempted under xerophilic conditions and significant CS₂ elimination was only achieved in biofilter A upon the implementation of an intermittent irrigation regime. Sustained removal efficiencies of 90–100% at an inlet load of up to 12 g CS₂ m⁻³ h⁻¹ were reached. The CS₂ removal in this biofilter was linked to the presence of the chemolithoautotrophic bacterium *Thiobacillus thioparus*, known among the relatively small number of species with a reported capacity of growing on CS₂ as the sole energy source. DGGE molecular profiles confirmed that this microbe had become dominant in biofilter A while it was not detected in samples from biofilter B. Conventional biofilters packed with inexpensive organic materials are suited for the treatment of low-strength CS₂ polluted gases (IL <12 g CS₂ m⁻³ h⁻¹), provided that the development of the adequate microorganisms is favored, either upon enrichment or by inoculation. The importance of applying culture-independent techniques for microbial community analysis as a diagnostic tool in the biofiltration of recalcitrant compounds has been highlighted

**Keywords:** Gas biofiltration; Carbon disulfide; 16S rRNA; DGGE microbial profiling; *Thiobacillus thioparus*;

Ling Chang¹, Yongming Zhang¹, Lu Gan¹, Hua Xu¹, Ning Yan¹, Rui Liu², Bruce E. Rittmann³. (¹Department of Environmental Engineering, College of Life and Environmental Science, Shanghai Normal University, Shanghai, 200234, People’s Republic of China, ²Zhejiang Provincial Key Laboratory of Water Science and Technology, Department of Environmental Technology and Ecology, Yangtze Delta Region Institute of Tsinghua University, Zhejiang, Jiaxing, 314006, People’s Republic of China, ³Swette Center for Environmental Biotechnology, Biodesign Institute, Arizona State University, Tempe, 85287-5701, USA). Internal loop photo-biodegradation reactor used for accelerated quinoline degradation and mineralization. Biodegradation, Volume 25 (4) (2014):587-594
Biofilm biodegradation was coupled with ultra-violet photolysis using the internal loop photobiodegradation reactor for degradation of quinoline. Three protocols—photolysis alone (P), biodegradation alone (B), and intimately coupled photolysis and biodegradation (P&B)—were used for degradation of quinoline in batch and continuous-flow experiments. For a 1,000 mg/L initial quinoline concentration, the volumetric removal rate for quinoline was 38 % higher with P&B than with B in batch experiments, and the P&B kinetics were the sum of kinetics from the P and B experiments. Continuous-flow experiments with an influent quinoline concentration of 1,000 mg/L also gave significantly greater quinoline removal in P&B, and the quinoline-removal kinetics for P&B were approximately equal to the sum of the removal kinetics for P and B. P&B similarly increased the rate and extent of quinoline mineralization, for which the kinetics for P&B were nearly equal to the sum of kinetics for P and B. These findings support that the rate-limiting step for mineralization was transformation of quinoline, which was accelerated by the simultaneous action of photolysis and biodegradation.

**Keywords:** Quinoline; Biodegradation; Photolysis; Biofilm

Roy Geerts¹, Patrick Kuijer¹, Cornelis G. van Ginkel¹, Caroline M. Plugge². (¹ AkzoNobel N.V., P.O. Box 9300, 6800 SB, Arnhem, The Netherlands, ² Laboratory of Microbiology, Wageningen University, P.O. Box 8033, 6700 EJ, Wageningen, The Netherlands). Microorganisms hydrolyse amide bonds; knowledge enabling read-across of biodegradability of fatty acid amides. Biodegradation, Volume 25(4) (2014):605-614

To get insight in the biodegradation and potential read-across of fatty acid amides, N-[3-(dimethylamino)propyl] cocoamide and N-(1-ethylpiperazine) tall oil amide were used as model compounds. Two bacteria, *Pseudomonas aeruginosa* PK1 and *Pseudomonas putida* PK2 were isolated with N-[3-(dimethylamino)propyl] cocoamide and its hydrolysis product N,N-dimethyl-1,3-propanediamine, respectively. In mixed culture, both strains accomplished complete mineralization of N-[3-(dimethylamino)propyl] cocoamide. *Aeromonas hydrophila* PK3 was enriched with N-(1-ethylpiperazine) tall oil amide and subsequently isolated using agar plates containing dodecanoate. N-(2-Aminoethyl)piperazine, the hydrolysis product of N-(1-ethylpiperazine) tall oil amide, was not degraded. The aerobic biodegradation pathway for primary and secondary fatty acid amides of *P. aeruginosa* and *A. hydrophila* involved initial hydrolysis of the amide bond producing ammonium, or amines, where the fatty acids formed were immediately metabolized. Complete mineralization of secondary fatty acid amides depended on the biodegradability of the released amine. Tertiary fatty acid amides were not transformed by *P. aeruginosa* or *A. hydrophila*. These strains were able to utilize all tested primary and secondary fatty acid amides independent of the amine structure and fatty acid. Read-across of previous reported ready biodegradability results of primary and secondary fatty acid amides is justified based on the broad substrate specificity and the initial hydrolytic attack of the two isolates PK1 and PK3.

**Keywords:** Fatty acid amides; Hydrolysis; Biodegradation pathway; Substrate specificity; Read-across;

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Anaerobic/aerobic conditions affected bacterial community composition and the subsequent chlorophenols (CPs) degradation in biocathode microbial fuel cells (MFCs). Bacterial communities acclimated with either 4-chlorophenol (4-CP) or 2,4-dichlorophenol (2,4-DCP) under anaerobiosis can degrade the respective substrates more efficiently than the facultative aerobic bacterial communities. The anaerobic bacterial communities well developed with 2,4-DCP were then adapted to 2,4,6-trichlorophenol (2,4,6-TCP) and successfully stimulated for enhanced 2,4,6-TCP degradation and power generation. A 2,4,6-TCP degradation rate of 0.10 mol/m$^3$/d and a maximum power density of 2.6 W/m$^3$ (11.7 A/m$^3$) were achieved, 138 and 13% improvements, respectively compared to the controls with no stimulation. Bacterial communities developed with the specific CPs under anaerobic/aerobic conditions as well as the stimulated biofilm shared some dominant genera and also exhibited great differences. These results provide the most convincing evidence to date that anaerobic/aerobic conditions affected CPs degradation with power generation from the biocathode systems, and using deliberate substrates can stimulate the microbial consortia and be potentially feasible for the selection of an appropriate microbial community for the target substrate (e.g. 2,4,6-TCP) degradation in the biocathode MFCs.

Keywords: Microbial fuel cell; Biocathode; Biodegradation; Bacterial community; Stimulation; Chlorophenol

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Indigenous oil-degrading bacteria play an important role in efficient remediation of polluted marine environments. In this study, we investigated the diversity and abundance of indigenous oil-degrading bacteria and functional genes in crude oil-contaminated seawater of the Dalian coast. The gene copy number bacterial 16S rRNA in total were determined to be about $10^{10}$ copies L$^{-1}$ in contaminated seawater and $10^9$ copies L$^{-1}$ in uncontaminated seawater. Bacteria of Alcanivorax, Marinobacter, Novosphingobium, Rhodococcus, and Pseudoalteromonas were found to be predominant oil-degrading bacteria in the polluted seawater in situ. In addition, bacteria belonging to Algoriphagus, Aestuariibacter, Celeribacter, Fabibacter, Zobellia, Tenacibaculum, Citreicella, Roseivirga, Winogradskyella, Thioclava, Polaribacter, and Pelagibaca were confirmed to be the first time as an oil-degrading bacterium. The indigenous functional enzymes, including AlkB or polycyclic aromatic hydrocarbons ring-hydroxylating dioxygenases a (PAH-RHDα) coding genes from Gram-
positive (GP) and Gram-negative bacteria (GN), were revealed and quite diverse. About $10^{10}$ to $10^{11}$ copies L$^{-1}$ for the expression of $alkB$ genes were recovered and showed that the two-thirds of all the AlkB sequences were closely related to widely distributed *Alcanivorax* and *Marinobacter* isolates. About $10^9$ copies L$^{-1}$ seawater for the expression of $RHDaGN$ genes in contaminated seawater and showed that almost all $RHDaGN$ sequences were closely related to an uncultured bacterium; however, $RHDaGP$ genes represented only about $10^5$ copies L$^{-1}$ seawater for the expression of genes in contaminated seawater, and the naphthalene dioxygenase sequences from *Rhodococcus* and *Mycobacterium* species were most abundant. Together, their data provide evidence that there exists an active aerobic microbial community indigenous to the coastal area of the Yellow sea that is capable of degrading petroleum hydrocarbons.

**Keywords:** Oil spill, Biodegradation, Alkane hydroxylase, Ring-hydroxylating dioxygenase, Oil-degrading bacteria

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Municipal wastewaters contain a multitude of organic trace pollutants. Often, their biodegradability by activated sludge microorganisms is decisive for their elimination during wastewater treatment. Since the amounts of micropollutants seem too low to serve as growth substrate, cometabolism is supposed to be the dominating biodegradation process. Nevertheless, as many biodegradation studies were performed without the intention to discriminate between metabolic and cometabolic processes, the specific contribution of the latter to substance transformations is often not clarified. This minireview summarizes current knowledge about the cometabolic degradation of organic trace pollutants by activated sludge and sludge-inherent microorganisms. Due to their relevance for communal wastewater contamination, the focus is laid on pharmaceuticals, personal care products, antibiotics, estrogens, and nonylphenols. Wherever possible, reference is made to the molecular process level, i.e., cometabolic pathways, involved enzymes, and formed transformation products. Particular cometabolic capabilities of different activated sludge consortia and various microbial species are highlighted. Process conditions favoring cometabolic activities are emphasized. Finally, knowledge gaps are identified, and research perspectives are outlined.

**Keywords:** Wastewater treatment; Biodegradation; Activated sludge; Cometabolism; Enzymes; Emerging pollutants; Pharmaceuticals and personal care products; Antibiotics; Estrogens; Nonylphenol

Medhat Rehan$^{1,2}$, Martin Kluge$^1$, Stefan Fränzle$^1$, Harald Kellner$^1$, René Ullrich$^1$, Martin Hofrichter$^1$. ($^1$Department of Bio- and Environmental Sciences, International Institute Zittau, Markt 23, 02763, Zittau, Germany, $^2$Department of Genetics, Kafrelsheikh University, 33516, Kafr El-Sheikh, Egypt). Degradation of atrazine by *Frankia*
Atrazine is transformed to N-isopropylammelide through hydroxyatrazine as an intermediate as indicated by high-performance liquid chromatography/mass spectroscopy in culture filtrates of *Frankia alni* ACN14a and *Frankia* sp. EuI1c. Both *Frankia* strains have the ability to degrade atrazine via dechlorination and dealkylation and, subsequently, may be using it as a nitrogen and carbon source as detected here by increasing their growth patterns. Bioinformatic analysis of the *Frankiagenomes revealed that a potential gene cluster involved in atrazine decomposition contains three genes, namely, *trzN* (FRAAL1474 and FraEuI1c_5874), *atzB* (FRAAL1473 and FraEuI1c_5875), and *atzR* (FRAAL1471). The relative messenger RNA gene expression of the former genes was examined by qRT-PCR. The LysR-type transcriptional regulator *atzR* (FRAAL1471), which is expected to control the cluster expression, showed a 13-fold increase in the expression level under atrazine stress. Moreover, the putative adenosine aminohydrolase 3 *atzB* (FRAAL1473), which is expected to dealkylate the N-ethyl group of atrazine, showed also an increased expression by factor 16 with increased exposure. Eventually, the *trzN* (FRAAL1474) gene, which is predicted to encode a putative amidohydrolase catalyzing atrazine dechlorination, exhibited 31-fold increased expression. To our best knowledge, this is the first report about adenosine aminohydrolase 3 function in the dealkylation of the N-ethyl group from atrazine.

**Keywords:** *Frankia; Actinorhizal symbiosis; s-Triazine biodegradation; qRT-PCR; Huanlin Huang (1) (2)

**Dongsheng Shen**1, 2, **Na Li**1,2, **Dan Shan**3, **Jiali Shentu**1,2, **YuYang Zhou**1, 2. (1). School of Environmental Science and Engineering, Zhejiang Gongshang University, 310012, Hangzhou, China, 2. Zhejiang Provincial Key Laboratory of Solid Waste Treatment and Recycling, 310012, Hangzhou, China, 3. Hangzhou Yuhang Environmental Protection Bureau, 311100, Hangzhou, China. Biodegradation of 1,4-Dioxane by a Novel Strain and Its Biodegradation Pathway. *Water, Air, & Soil Pollution*, Volume 225(2014): 2135

A Gram-negative strain DD1, which could use 1,4-dioxane as the sole carbon and energy source, was isolated from the mixture of activated sludge obtained from Qige urban sewage treatment plant. According to the Biolog GNIII detection and the 16S ribosomal DNA (rDNA) sequence, DD1 was identified as *Acinetobacter baumannii*. Cells of *A. baumannii* DD1 precultured in 1,4-dioxane could completely degrade 100 mg/L 1,4-dioxane in 42 h with a cell yield of 0.414 mg protein (mg-1,4-dioxane)-1 and a generation time of 6.75 h, demonstrating that DD1 bears the highest 1,4-dioxane-degrading activity among the described strains. Moreover, DD1 tolerates higher 1,4-dioxane concentration almost up to 1,000 mg/L. The strain could also grow on several benzene homologues including benzene, toluene, ethylbenzene, o-xylene, m-xylene, and phenol. During the degradation process of 1,4-dioxane, the first oxidation was initiated by monooxygenase in DD1. However, the main second monooxygenation intermediate 2-hydroxyethoxyacetic acid was not detected. As replacer, 1,4-dioxene was identified, and other intermediates such as ethylene glycol and oxalic acid were also detected. Based on the analysis of degradation products, a partial degradation pathway was proposed.

**Keywords:** 1,4-Dioxane; Acinetobacter baumannii; Biodegradation; Pathway
Biodegradation of different tall oil soaps was studied in order to examine the behaviour of these bioproducts in natural environments and to study their biodegradation rates. The rates of biodegradation were studied by modelling the biodegradation phenomenon as a pseudo-first-order reaction. Biodegradation was studied in seven different environments. Four of these were water phases: groundwater in aerobic and anaerobic conditions, river water and Office of Environmental Compliance and Documentation (OECD) 301 F standard conditions. In addition, three solid phases, sand, acidic forest soil and topsoil, were used as a solid matrix. The results showed that the matrix and the concentration had a strong effect on both the rate and degree of the biodegradation reaction. As a result, all of the tall oil soaps were about 57–85% biodegradable in OECD 301 F conditions, but only moderately biodegradable in Finnish river water taken in the summer. When compared to the sample taken in the autumn, the biodegradation degree was considerably higher. In groundwater, biodegradation degree was low, even negligible in anaerobic conditions. With ten times less sample content, the biodegradation degrees in groundwater and surface water increased to 60% for all the tall oil soaps, with one soap, in particular, up to 80% during 100 days of measurement. In the topsoil, biodegradation was vague, and in slightly acidic forest soil, the decomposition reactions were complex. This is probably due to gas formation in the side reactions. In sand, tall oil soaps did not biodegrade at all.

**Keywords:** Manometric respirometric method; BOD; Tall oil soap; Biodegradation; Kinetics;


The main objective of this study was to evaluate the degradation of a ternary mixture of n-hexane, benzene, and methanol fed to two Trickle Bed Air Biofilters (TBABs) designated as “A” and “B”. Both TBABs were loaded with pelletized diatomaceous earth support media and run at an empty bed residence time (EBRT) of 120 s. TBABs “A” and “B” were operated at pH 4 and fed with n-hexane:benzene:methanol (C₆H₁₃:C₆H₆:C₃H₈) concentrations ratios of 1:3:10 and 1:3:6.6, respectively under different loading rates. The influent total loading rates varied from 39.2 to 117.7 g/m³h and from 32 to 96.4 g/m³h for TBABs “A” and “B”, respectively. In both TBABs, methanol and benzene were the most eliminated volatile organic compounds (VOCs), while the removal of n-hexane was controlled by the VOCs ratios. Higher removal efficiencies were obtained for the VOCs ratio of 1:3:6.6 corresponding to a total VOCs load of 96.4 g/m³h. The
addition of VOCs mixture to the TBABs resulted in change of the fungi community within the TBABs as compared to the fungi community when the TBABs were previously receiving only n-hexane as a sole substrate.

**Keywords:** Benzene; Biofilters; Biofiltration; Fungi; n-hexane; Methanol; Recalcitrant compounds; Trickle bed air biofilters (TBAB)

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The organophosphate pesticide chlorpyrifos (CP) has been used extensively since the 1960s for insect control. However, its toxic effects on mammals and persistence in environment necessitate its removal from contaminated sites, biodegradation studies of CP-degrading microbes are therefore of immense importance. Samples from a Pakistani agricultural soil with an extensive history of CP application were used to prepare enrichment cultures using CP as sole carbon source for bacterial community analysis and isolation of CP metabolizing bacteria. Bacterial community analysis (denaturing gradient gel electrophoresis) revealed that the dominant genera enriched under these conditions were *Pseudomonas*, *Acinetobacter* and *Stenotrophomonas*, along with lower numbers of *Sphingomonas*, *Agrobacterium* and *Burkholderia*. Furthermore, it revealed that members of Bacteroidetes, Firmicutes, α- and γ-Proteobacteria and Actinobacteria were present at initial steps of enrichment whereas β-Proteobacteria appeared in later steps and only Proteobacteria were selected by enrichment culturing. However, when CP-degrading strains were isolated from this enrichment culture, the most active organisms were strains of *Acinetobacter calcoaceticus*, *Pseudomonas mendocina* and *Pseudomonas aeruginosa*. These strains degraded 6–7.4 mg L⁻¹ day⁻¹ of CP when cultivated in mineral medium, while the consortium of all four strains degraded 9.2 mg L⁻¹ day⁻¹ of CP (100 mg L⁻¹). Addition of glucose as an additional C source increased the degradation capacity by 8–14 %. After inoculation of contaminated soil with CP (200 mg kg⁻¹) disappearance rates were 3.83–4.30 mg kg⁻¹ day⁻¹ for individual strains and 4.76 mg kg⁻¹ day⁻¹ for the consortium. These results indicate that these organisms are involved in the degradation of CP in soil and represent valuable candidates for in situ bioremediation of contaminated soils and waters.

**Keywords:** Chlorpyrifos; Organophosphates; Biodegradation; DGGE; Consortium; Enrichment

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Aflatoxin B1 (AFB1) is a highly toxic fungal metabolite having carcinogenic, mutagenic and teratogenic effects on human and animal health. Accidental feeding of aflatoxin-contaminated rice straw may be detrimental for ruminant livestock and can lead to transmission of this toxin or its metabolites into the milk of dairy cattle. White-rot basidiomycetous fungus *Pleurotus ostreatus* produces ligninolytic enzymes like laccase and manganese peroxidase (MnP). These extracellular enzymes have been reported to degrade many environmentally hazardous compounds. The present study examines the ability of *P. ostreatus* strains to degrade AFB1 in rice straw in the presence of metal salts and surfactants. Laccase and MnP activities were determined spectrophotometrically. The efficiency of AFB1 degradation was evaluated by high performance liquid chromatography. Highest degradation was recorded for both *P. ostreatus* MTCC 142 (89.14%) and *P. ostreatus* GHBBF10 (91.76%) at 0.5 µg mL\(^{-1}\) initial concentration of AFB1. Enhanced degradation was noted for *P. ostreatus* MTCC 142 in the presence of Cu\(^{2+}\) and Triton X-100, at toxin concentration of 5 µg mL\(^{-1}\). *P. ostreatus* GHBBF10 showed highest degradation in the presence of Zn\(^{2+}\) and Tween 80. Liquid chromatography-mass spectrometric analysis revealed the formation of hydrated, decarbonylated and O-dealkylated products. The present findings suggested that supplementation of AFB1-contaminated rice straw by certain metal salts and surfactants can improve the enzymatic degradation of this mycotoxin by *P. ostreatus* strains.

**Keywords:** Pleurotus ostreatus; Aflatoxin B1; Surfactants; Biodegradation; Laccase

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We live in a world dependent on fossil fuels which due to their excessive usage represent one of the major environmental pollutants. In sites with long history of fossil fuel usage it is expected to find microorganisms selected to thrive on such conditions. In the northern Adriatic Sea there are several sites with long history of exposure to fossil fuels – shipyards, oil refineries, tanker berths and a former coke plant. To exploit this long term pressure on marine habitats sediment samples were taken from 8 sites. For each site concentration of aliphatic and aromatic hydrocarbons was determined along with microbial diversity and abundance for each sample using DNA sequencing and analysis of 16s rDNA gene. Metagenomes from samples with lower microbial complexity and higher concentrations of hydrocarbons were sequenced to screen for genes involved in alkane degradation – namely AlkB family of alkane hydroxylases. To identify genes of interest in metagenomic sequences analysis platform based on KEGG and custom database containing hydrocarbon degrading genes was developed. Identified genes of interest were then synthesised, expressed in heterologous system and functionally screened.

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biodegradation and COD removal of 2-chlorophenol in a granular anoxic baffled reactor. 

The present work was designed to developed a granular anoxic baffled reactor (AnBR) and to investigate its performance in the treatment of 2-chlorophenol (2-CP). The acclimation and enrichment of microorganisms in activated sludge to degrade 2-CP was effectively accomplished. Then the influence of inlet 2-CP, hydraulic retention time (HRT), salinity, and type of electron acceptor (nitrate or organic compound) on the performance of AnBR in biodegradation of 2-CP was investigated. The increase of inlet 2-CP from 50 to 500 mg/L at a fixed HRT of 24 h did not adversely affected the AnBR where over 99% of inlet 2-CP was biodegraded. Decreasing the HRT stepwise from 24 to 4 h at an inlet 2-CP of 200 mg/L did not inhibit the rate of biodegradation (>99%). The increase of over 20 g/L salinity in the feed stream strongly inhibited the rate of 2-CP biodegradation in the AnBR, whereas the bioreactor could efficiently tolerate concentrations below 20 g/L NaCl. Moreover, the rate of 2-CP biodegradation under anoxic denitrifying metabolic conditions (presence of nitrate) was much greater than that under anaerobic metabolic conditions (absence of nitrate). Accordingly, the AnBR process is a feasible, simple, low-cost, and thus appropriate process for efficiently biodegrading toxic chlorinated organic compounds.

Keywords: Chlorophenols; Biodegradation; Anoxic metabolism; Baffled reactor; Granular biomass

Y.-P. Gai1,†, W.-T. Zhang2,†, Z.-M. Mu2 and, X.-L. Ji1,2,* (1State Key Laboratory of Crop Biology, Shandong Agricultural University, Taian, Shan-dong, China, 2College of Forestry, Shandong Agricultural University, Taian, Shandong, China. Xian-Ling Ji College of forestry, Shandong agricultural university, Taian, Shandong 271018, China. E-mail: xlji@sdau.edu.cn). Involvement of ligninolytic enzymes in degradation of wheat straw byTrametes trogii. Journal of Applied Microbiology, Volume 117(1) (2014):85–95

Wheat straw is generated in billions of tons around the world every year and has not been fully used. This study sought to evaluate the delignification capacity and enzyme production of Trametes trogii MT strain and to clarify the changes of structure and chemical composition of wheat straw during the decay process.

The results obtained revealed that the T. trogii MT strain has the ability to degrade lignin, cellulose as well as hemicellulose of wheat straw simultaneously. The strain can produce high activities of laccase, manganese peroxidase, xylanase, carboxymethylcellulase and feruloyl esterase but no lignin peroxidases during the decay process of a 60-day incubation period on wheat straw. Scanning electron microscopy observation and infrared spectroscopy analysis showed the lignin and carbohydrate of wheat straw were degraded with no obvious different levels. The low molecular mass fractions collected from the culture of the MT strains grown in wheat straw powder liquid medium showed high Fe$^{3+}$ chelating, reducing capacity and hydroxyl radical and hydrogen peroxide generation.

Trametes trogii MT has a complex mechanism to degrade lignocellulose, in addition to the extracellular enzymatic systems, and has great potential as an attractive micro-organism used for the biological degradation of waste straws.
This study revealed the dynamic changes of the ligninolytic enzymes of T. trogii MT during the degradation of wheat straw, and suggested that the decay patterns of wheat straw by T. trogii MT had some simultaneous type characteristics.

**Keywords:** biodegradation; ligninolytic enzymes; lignocellulose; *Trametes trogii*; wheat straw

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Correspondence: Lisa M. Gieg, Department of Biological Sciences, University of Calgary, 2500 University Dr. N.W., Calgary, AB, Canada T2N 1N4. Tel.: 403 210 7207; fax: 403 289 9311; e-mail: lmgieg@ucalgary.ca. Identification of toluene degraders in a methanogenic enrichment culture, FEMS Microbiology Ecology, Volume 89(3) (2014): 625–636

Methanogenic biodegradation involves the cooperative metabolism of syntrophic bacteria that catalyse the initial attack and subsequent degradation of hydrocarbons, and methanogens that convert intermediates such as hydrogen and carbon dioxide, formate, and/or acetate to methane. The identity of syntrophic microbes and the nature of their interactions with other syntrophs and methanogens are not well understood. Furthermore, it is difficult to isolate the organisms responsible for the initial activation and subsequent degradation of hydrocarbon substrates under methanogenic conditions due to the thermodynamic relationships that exist among microbes in methanogenic communities. We used time-resolved RNA stable isotope probing and RT-qPCR to identify the organisms involved in the initial attack on toluene and subsequent degradation reactions in a highly enriched toluene-degrading methanogenic culture. Our results reveal the importance of a *Desulfosporosinus* sp. in anaerobic toluene activation in the culture. Other organisms that appear to play roles in toluene degradation include *Syntrophaceae*, *Desulfovibrionales* and *Chloroflexi*. The high bacterial diversity observed in this culture and the extensive labelling of different phylogenetic groups over the course of the stable isotope probing experiment highlight the complexity of the relationships that exist in methanogenic ecosystems.

**Keywords:** stable isotope probing; RT-qPCR; anaerobic; methanogenesis; toluene; metabolism

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Benzo[a]pyrene (BaP), a five-ring polycyclic aromatic hydrocarbon (PAH), which has carcinogenic potency, is highly recalcitrant and resistant to microbial degradation. A novel fungus, *Lasiodiplodia theobromae* (*L. theobromae*), which can degrade BaP as a sole carbon source in liquid, was isolated in our laboratory. To prompt the further application of *L. theobromae* in remediation of sites polluted by BaP and other PAHs, the present study was
targeted toward the removal of BaP and PAHs from soil by *L. theobromae*. The degradation of BaP by *L. theobromae* was studied using a soil spiked with 50 mg/kg BaP. *L. theobromae* could remove 32.1% of the BaP after 35 days of cultivation. Phenanthrene (PHE) inhibited BaP degradation as a competitive substrate. The tested surfactants enhanced BaP degradation in soil by different extents, and a removal rate of 92.1% was achieved at a Tween-80 (TW-80) concentration of 5 g/kg. It was revealed that TW-80 could not only enhance BaP bioavailability by increasing its aqueous solubility and decreasing the size of its colloid particles but also increase enzyme secretion from *L. theobromae* and the population of *L. theobromae*. Moreover, ergosterol content together with the biomass C indicated the increase in *L. theobromae* biomass during the BaP biodegradation process in soils. Finally, a soil from a historically PAH-contaminated field at Beijing Coking Plant in China was tested to assess the feasibility of applying *L. theobromae* in the remediation of polluted sites. The total removal rate of PAHs by *L. theobromae* was 53.3%, which is 13.1% higher than that for *Phanerochaete chrysosporium* (*P. chrysosporium*), an effective PAH degrader. The addition of TW-80 to the field soil further enhanced PAH degradation to 73.2%. Hence, *L. theobromae* is a promising novel strain to be implemented in the remediation of soil polluted by PAHs.

**Keywords:** Bioremediation; *L. theobromae*; Benzo[a]pyrene (BaP); PAHs; Surfactants; Tween-80;

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This study investigates the ability of *Rhodococcus* sp. strain p52, a dioxin degrader, to biodegrade petroleum hydrocarbons. Strain p52 can use linear alkanes (tetradecane, tetracosane, and dotriacontane), branched alkane (pristane), and aromatic hydrocarbons (naphthalene and phenanthrene) as sole carbon and energy sources. Specifically, the strain removes 85.7% of tetradecane within 48 h at a degradation rate of 3.8 mg h⁻¹ g⁻¹ dry cells, and 79.4% of tetracosane, 66.4% of dotriacontane, and 63.9% of pristane within 9–11 days at degradation rates of 20.5, 14.7, and 20.3 mg day⁻¹ g⁻¹ dry cells, respectively. Moreover, strain p52 consumes 100% naphthalene and 55.3% phenanthrene within 9–11 days at respective degradation rates of 16 and 12.9 mg day⁻¹ g⁻¹ dry cells. Metabolites of the petroleum hydrocarbons by strain p52 were analyzed. Genes encoding alkane-hydroxylating enzymes, including cytochrome P450 (CYP450) enzyme (CYP185) and two alkane-1-monoxygenases, were amplified by polymerase chain reaction. The transcriptional activities of these genes in the presence of petroleum hydrocarbons were detected by reverse transcription-polymerase chain reaction. The results revealed potential of strain p52 to degrade petroleum hydrocarbons.

**Keywords:** Biodegradation; Tetradecane; Pristane; Naphthalene; Alkane-1-monoxygenase; Cytochrome P450; Rhodococcus sp

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Coking wastewater treatment plant (CWWTP) represents a typical point source of polycyclic aromatic hydrocarbons (PAHs) to the water environment and threatens the safety of drinking water in downstream regions. To enhance the removal of residual PAHs from bio-treated coking wastewater, a pilot-scale O$_3$/ultraviolet (UV) fluidized bed reactor (O$_3$/UV FBR) was designed and different operating factors including UV irradiation intensity, pH, initial concentration, contact time, and hydraulic retention time (HRT) were investigated at an ozone level of 240 g h$^{-1}$ and 25 ± 3 °C. A health risk evaluation and cost analysis were also carried out under the continuous-flow mode. As far as we know, this is the first time an O$_3$/UV FBR has been explored for PAHs treatment. The results indicated that between 41 and 75 % of 18 target PAHs were removed in O$_3$/UV FBR due to synergistic effects of UV irradiation. Both increased reaction time and increased pH were beneficial for the removal of PAHs. The degradation of the target PAHs within 8 h can be well fitted by the pseudo-first-order kinetics ($R^2$ > 0.920). The reaction rate was also positively correlated with the initial concentrations of PAHs. The health risk assessment showed that the total amount of carcinogenic substance exposure to surface water was reduced by 0.432 g day$^{-1}$. The economic analysis showed that the O$_3$/UV FBR was able to remove 18 target PAHs at a cost of US$0.34 m$^{-3}$. These results suggest that O$_3$/UV FBR is efficient in removing residuals from CWWTP, thus reducing the accumulation of persistent pollutant released to surface water.

Keywords: Coking wastewater; O3/UV; Fluidized bed reactor; Polycyclic aromatic hydrocarbons; Oxidation kinetics


The biodegradation of organic substances is an undoubtedly natural process; it is so common that we usually forget how hard it is to assess. Official references define the biodegradation process as ‘the biologically mediated degradation or transformation of chemicals usually carried out by microorganisms’ (ECHA, 2012). The International Union of Pure and Applied Chemistry (IUPAC) widens the definition to include the breakdown of a substance in vitro or in vivo as catalysed by enzymes (IUPAC, 1993).

The assessment of biodegradation (i.e. the biodegradation test or assay) can be conducted in many ways depending on the objective of the study. Biodegradability assays are designed to batch test a chemical substance (or polymer) as the sole carbon source in a mixed inoculum from different origins (river water, seawater, activated sludge and soil). Monitoring the biodegradation process remains a difficult task, ranging from the use of very basic sensors (oxygen or carbon dioxide) to very sophi ...

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Abstract Vol. No. 25, December 2014
Management (ILR), Justus Liebig University, Giessen, Germany, 2. Institute for Organic and Macromolecular Chemistry, Chair of Preparative Polymer Chemistry, Heinrich Heine University, Düsseldorf, Germany. Biodegradability of a polyacrylate superabsorbent in agricultural soil. Environmental Science and Pollution Research, Volume 21(16) (2014): 9453-9460

Superabsorbent polymers (SAP) are used, inter alia, as soil amendment to increase the water holding capacity of soils. Biodegradability of soil conditioners has become a desired key characteristic to protect soil and groundwater resources. The present study characterized the biodegradability of one acrylate based SAP in four agricultural soils and at three temperatures. Mineralisation was measured as the $^{13}$CO$_2$ efflux from $^{13}$C-labelled SAP in soil incubations. The SAP was either single-labelled in the carboxyl C-atom or triple-labelled including additionally the two C-atoms interlinked in the SAP backbone. The dual labelling allowed estimating the degradation of the polyacrylate main chain. The $^{13}$CO$_2$ efflux from samples was measured using an automated system including wavelength-scanned cavity ring-down spectroscopy. Based on single-labelled SAP, the mean degradation after 24 weeks varied between 0.45 % in loamy sand and 0.82 % in loam. However, the differences between degradation rates in different soils were not significant due to a large intra-replicate variability. Similarly, mean degradation did not differ significantly between effective temperature regimes of 20° and 30 °C after 12 weeks. Results from the triple-labelled SAP were lower as compared to their single-labelled variant. Detailed results suggest that the polyacrylate main chain degraded in the soils, if at all, at rates of 0.12–0.24 % per 6 months.

Keywords: Poly(acrylic acid); Superabsorbent polymer SAP; Biodegradation; $^{13}$CO$_2$ efflux


Human pharmaceutical active ingredients that are orally or parenterally administered may be metabolised in the body and after excretion may be further transformed in the receiving environmental compartments. The optimal outcome from an environmental point of view—complete mineralisation—is rarely observed. Small molecule pharmaceuticals are commonly not readily biodegradable according to Organisation for Economic Cooperation and Development (OECD) 301 tests. However, primary transformation is often observed. To gain information on the transformation of active ingredients in the environment, long-term studies like transformation in aquatic water/sediment systems according to OECD guideline 308 are required for the environmental risk assessment for human active pharmaceutical ingredients. Studies received until mid 2010 as part of the dossiers for marketing authorisation applications were evaluated concerning transformation products. The evaluation revealed that in 70 % of the studies, at least one transformation product (TP) is formed above 10 % of the originally applied dose, but in only 26 % of the studies are all TP identified. The evaluation also revealed that some TP of pharmaceutical active ingredients show a considerably longer DT$_{50}$ compared to the parent compound. An example is the TP (val)sartan acid that is formed from an antihypertensive compound.
**Keywords:** Transformation; Pharmaceuticals; Biodegradation; Activated sludge; Water/sediment system; OECD guidelines

Yu Zhang¹, Xin Gu¹, Jing Zhang¹, Min Yang¹. (¹ State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing, 100085, China). Degradation pathways of low-ethoxylated nonylphenols by isolated bacteria using an improved method. Environmental Science and Pollution Research, Volume 21(16)(2014): 9468-9476

Nonylphenol ethoxylates (NPEOs) with low ethoxylation degree (NP_{av2}EO; containing two ethoxy units on average) and estrogenic properties are the intermediate products of nonionic surfactant NPEOs. To better understand the environmental fate of low-ethoxylated NPEOs, phylogenetically diverse low-ethoxylated NPEO-degrading bacteria were isolated from activated sludge using gellan gum as the gelling reagent. Four isolates belonging to four genera, i.e., *Pseudomonas* sp. NP522b in γ-Proteobacteria, *Variovorax* sp. NP427b and *Ralstonia* sp. NP47a in β-Proteobacteria, and *Sphingomonas* sp. NP42a in α-Proteobacteria were acquired. *Ralstonia* sp. NP47 or *Sphingomonas* sp. NP42a, have not been reported for the degradation of low-ethoxylated NP_{av2}EOs previously. The biotransformation pathways of these isolates were investigated. The first three strains (NP522b, NP427b, and NP47a) exhibited high NP_{av2}EO oxidation ability by oxidizing the polyethoxy (EO) chain to form low-ethoxylated nonylphenoxy carboxylates, and then further oxidizing the alkyl chain to form carboxyalkylphenol polyethoxycarboxylates. Furthermore, *Sphingomonas* sp. NP42a degraded NP_{av2}EO through a nonoxidative pathway with nonylphenol monoethoxylate as the dominant product.

**Keywords:** Low-ethoxylated nonylphenol ethoxylates; Degrading bacteria; Biodegradation pathway; Gelling reagent

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Poly(lactic acid) nanocomposites containing Cloisite 15A, Cloisite 30B, and Dellite 43B were prepared by melt-mixing in a batch mixer and were exposed to UV radiation, temperature, and microorganism in solution and in a compost. Exposed samples, collected along the time, were characterized by several techniques. While the addition of organoclays had a positive effect on thermal stability, the degradation rate of nanocomposites increased when exposed to UV radiation and microorganism. Moreover, the degradation rate depends on the organoclay type. Even though the degradation rate is higher for nanocomposites, Fourier transform infrared spectrometry and gel permeation chromatography results demonstrated that the degradation mechanism is the same.

**Keywords:** PLA; Nanocomposites; Biodegradation; Degradation; Compost; Environment
Biodegradation tests with bacteria from activated sludge revealed the probable persistence of cyano-based ionic liquid anions when these leave waste water treatment plants. A possible biological treatment using bacteria capable of biodegrading similar compounds, namely cyanide and cyano-complexes, was therefore examined. With these bacteria from the genera *Cupriavidus*, the ionic liquid anions B(CN)$_4^-$, C(CN)$_3^-$, N(CN)$_2^-$ combined with alkaline cations were tested in different growth media using ion chromatography for the examination of their primary biodegradability. However, no enhanced biodegradability of the tested cyano-based ionic liquids was observed. Therefore, an in vitro enzymatic hydrolysis test was additionally run showing that all tested ionic liquid (IL) anions can be hydrolysed to their corresponding amides by nitrile hydratase, but not by nitrilase under the experimental conditions. The biological stability of the cyano-based anions is an advantage in technological application, but the occurrence of enzymes that are able to hydrolyse the parent compound gives a new perspective on future cyano-based IL anion treatment.

**Keywords:** Biodegradation; Ionic liquids; Cyano groups; Axenic culture; Biological treatment; Hazard assessment; Cupriavidus spp.; Nitrile hydratase; Nitrilase

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The increasing usage and the persistence of polyester polyurethane (PU) generate significant sources of environmental pollution. The effective and environmental friendly bioremediation techniques for this refractory waste are in high demand. In this study, three novel PU degrading bacteria were isolated from farm soils and activated sludge. Based upon 16S ribosomal RNA gene sequence blast, their identities were determined. Particularly robust activity was observed in *Pseudomonas putida*; it spent 4 days to degrade 92% of Impranil DLN™ for supporting its growth. The optimum temperature and pH for DLN removal by *P. putida* were 25 °C and 8.4, respectively. The degradation and transformation of DLN investigated by Fourier transformed infrared spectroscopy show the decrease in ester functional group and the emergence of amide group. The polyurethanolytic activities were both presented in the extracellular fraction and in the cytosol. Esterase activity was detected in the cell lysate. A 45-kDa protein bearing
polyurethanolytic activity was also detected in the extracellular medium. This study presented high PU degrading activity of P. putida and demonstrated its responsible enzymes during the PU degradation process, which could be applied in the bioremediation and management of plastic wastes.

**Keywords:** Polyurethane; Biodegradation; Pseudomonas putida; Fourier transformed infrared spectroscopy;


Agro-food, petroleum, textile, and leather industries generate saline wastewater with a high content of organic pollutants such as aromatic hydrocarbons, phenols, nitroaromatics, and azo dyes. Halophilic microorganisms are of increasing interest in industrial waste treatment, due to their ability to degrade hazardous substances efficiently under high salt conditions. However, their full potential remains unexplored. The isolation and identification of halophilic and halotolerant microorganisms from geographically unrelated and geologically diverse hypersaline sites supports their application in bioremediation processes. Past investigations in this field have mainly focused on the elimination of polycyclic aromatic hydrocarbons and phenols, whereas few studies have investigated N-aromatic compounds, such as nitro-substituted compounds, amines, and azo dyes, in saline wastewater. Information regarding the growth conditions and degradation mechanisms of halophilic microorganisms is also limited. In this review, we discuss recent research on the removal of organic pollutants such as organic matter, in terms of chemical oxygen demand (COD), dyes, hydrocarbons, N-aliphatic and N-aromatic compounds, and phenols, in conditions of high salinity. In addition, some proposal pathways for the degradation of aromatic compounds are presented.

**Keywords:** Halophilic; Biodegradation; Hydrocarbons; Dyes; Pollutants; PAH; Phenols

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The electrochemical degradation of the nonsteroidal anti-inflammatory drug ketoprofen in tap water has been studied using electro-Fenton (EF) and anodic oxidation (AO) processes with platinum (Pt) and boron-doped diamond (BDD) anodes and carbon felt cathode. Fast degradation of the parent drug molecule and its degradation intermediates leading to complete
mineralization was achieved by BDD/carbon felt, Pt/carbon felt, and AO with BDD anode. The obtained results showed that oxidative degradation rate of ketoprofen and mineralization of its aqueous solution increased by increasing applied current. Degradation kinetics fitted well to a pseudo-first-order reaction. Absolute rate constant of the oxidation of ketoprofen by electrochemically generated hydroxyl radicals was determined to be \((2.8 \pm 0.1) \times 10^9\) M\(^{-1}\) s\(^{-1}\) by using competition kinetic method. Several reaction intermediates such as 3-hydroxybenzoic acid, pyrogallol, catechol, benzophenone, benzoic acid, and hydroquinone were identified by high-performance liquid chromatography (HPLC) analyses. The formation, identification, and evolution of short-chain aliphatic carboxylic acids like formic, acetic, oxalic, glycolic, and glyoxylic acids were monitored with ion exclusion chromatography. Based on the identified aromatic/cyclic intermediates and carboxylic acids as end products before mineralization, a plausible mineralization pathway was proposed. The evolution of the toxicity during treatments was also monitored using Microtox method, showing a faster detoxification with higher applied current values.

**Keywords:** Ketoprofen; Electro-Fenton; Anodic oxidation; Hydroxyl radicals; Mineralization; Toxicity

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Roxarsone (3-nitro-4-hydroxy benzene arsenic acid) is an organoarsenic feed additive and has been widely used in the poultry industry to prevent coccidiosis and improve feed efficiency. The presence of roxarsone and its degradation products results in the instability of the anaerobic methanogenic process. This study investigated the degradation and speciation of roxarsone in an anaerobic granular sludge (AGS) system and the impacts of roxarsone and its degradation products on the structure of AGS. Roxarsone inhibited methane production, and the added roxarsone was rapidly degraded into 3-amino-4-hydroxyphenylarsenic acid (HAPA). After 240 days of incubation, the distribution of arsenic differed between the aqueous solution and the AGS in the assays of 20 and 350 mg/L roxarsone. Species analysis indicated that HAPA was completely degraded in all of the assays with roxarsone addition after 240 days of incubation. Species distribution was affected by the phases and the initial concentration of roxarsone added. The concentration of As(III) was higher than that of As(V) in both the aqueous solution and the AGS in all assays with roxarsone addition. The toxicity of roxarsone and its degradation products resulted in changes in the structure and the microorganism species in the AGS.

**Keywords:** Anaerobic digestion; Anaerobic granular sludge; Biodegradation; Inorganic arsenic; Roxarsone

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An aerobic Gram +ve bacterial strain capable of utilizing 2-Hydroxyquinoxaline (2-HQ) as sole source of carbon and energy was isolated from Chrysanthemum indicum Indian agricultural soil and named as HQ2. On the basis of morphology, physico-biochemical characteristics and 16S rRNA sequence analysis, strain HQ2 was identified as Bacillus sp. The generation time of Bacillus sp. in log phase during growth on 2-HQ is 0.79 h or 47.4 min. The optimal conditions for 2-HQ degradation by Bacillus sp. were inoculum density of 1.0 OD, pH of 6–8, temperature of 37–45 °C and 2-HQ concentration of 500 ppm. Among the additional carbon and nitrogen sources, carbon sources did not influence the degradation rate of 2-HQ, but nitrogen sources—yeast extract marginally enhanced the rate of degradation of 2-HQ. GC-MS analysis of the culture Bacillus sp. grown on 2-HQ indicated the formation of dimers from 2 molecules of 2-hydroxyquinoxaline. The formation of dimer for degradation of 2-HQ by the culture appears to be the first report to our scientific knowledge.

**Keywords:** 2-Hydroxyquinoxaline; Bacillus sp.; 16S rRNA sequence analysis; GC-MS analysis

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The potential of fungal co-culture of the filamentous Pestalotiopsis sp. NG007 with four different basidiomycetes – *Trametes versicolor* U97, *Pleurotus ostreatus* PL1, *Cerena* sp. F0607, and *Polyporus* sp. S133 – for accelerating biodegradation of petroleum hydrocarbons (PHCs) was studied using three different physicochemical characteristic PHCs in soil. All the combinations showed a mutual intermingling mycelial interaction on the agar plates. However, only NG007/S133 (50/50) exhibited an optimum growth rate and enzymatic activities that supported the degradation of asphalt in soil. The co-culture also degraded all fractions at even higher concentrations of the different PHCs. In addition, asphaltene, which is a difficult fraction for a single microorganism to degrade, was markedly degraded by the co-culture, which indicated that the simultaneous biodegradation of aliphatic, aromatic, resin, and asphaltene fractions had occurred in the co-culture. An examination of *in-vitro* degradation by the crude enzymes and the retrieval fungal culture from the soil after the experiment confirmed the accelerated biodegradation due to enhanced enzyme activities in the co-culture. The addition of piperonyl butoxide or AgNO\(_3\) inhibited biodegradation by 81–99%, which demonstrated the important role of P450 monoxygenases and/or dioxygenases in the initial degradation of the aliphatic and aromatic fractions in PHCs.

**Keywords:** Biodegradation; Dioxygenase; Fungal co-culture; Ligninolytic enzymes; Petroleum hydrocarbons

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Complex hydrocarbon and aromatic compounds degrading marine bacterial strains were isolated from deep sea sediment after enrichment on spent engine (SE) oil. Phenotypic characterization and phylogenetic analysis of 16S rRNA gene sequences showed the isolates were related to members of the *Pseudoalteromonas* sp., *Ruegeria* sp., *Exiguobacterium* sp. and *Acinetobacter* sp. Biodegradation using 1% (v/v) SE oil with individual and mixed strains showed the efficacy of SE oil utilization within a short retention time. The addition of non-ionic surfactant 0.05% (v/v) Tween 80 as emulsifying agent enhanced the solubility of hydrocarbons and renders them more accessible for biodegradation. The degradation of several compounds and the metabolites formed during the microbial oxidation process were confirmed by Fourier transform infrared spectroscopy and Gas chromatography–mass spectrometry analyses. The potential of this consortium to biodegrade SE oil with and without emulsifying agent provides possible application in bioremediation of oil contaminated marine environment.

**Keywords:** Aromatic hydrocarbons; Deep sea bacteria; Spent engine oil; Biodegradation; Bioremediation

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Three strains capable of rapidly degrading TBBPA by co-metabolism and utilizing formate as the carbon source, named as J-F-01, J-F-02, and J-F-03, respectively, were isolated from enrichment cultures, which have been treated with 0.5 mg/L TBBPA for 240 d. Based on morphology and 16S rRNA gene sequence analysis, both J-F-01 and J-F-02 were determined to *Pseudomonas* sp., while J-F-03 was identified as *Streptococcus* sp. A shorter half-life (6.1 d) of TBBPA was observed in pure culture of J-F-03 when compared with J-F-01 (22.5 d) and J-F-02 (13.6 d). Surprisingly, the degradation of TBBPA was significantly enhanced by the mixed culture of J-F-02 and J-F-03. The optimal degradation conditions for the mixed cultures were determined. Under the optimal conditions, TBBPA (0.5 mg/L) was completely metabolized by the mixed culture within ten days. Moreover, bromide and the metabolisms were detected, and a possible metabolic pathway was deduced from the detection of metabolite production patterns.

**Keywords:** TBBPA; Degradation; Formate; Co-metabolism


In this study, the role of sodium dodecyl sulfate (SDS) was explored for the removal of extracellular polymeric substance (EPS) from waste activated sludge (WAS) followed by
enzymatic bacterial pretreatment, which enhanced the subsequent anaerobic biodegradability. EPS was removed with 0.02 g/g SS of SDS. In the results of pretreatment, the suspended solids reduction and chemical oxygen demand solubilization were found to be 25.7% and 19.79% for deflocculated and bacterially pretreated sludge, whereas they were found to be 15.7% and 11% for flocculated sludge (without EPS removal and bacterially pretreated) and 7.85% and 6% for control sludge (raw sludge), respectively. Upon examining the anaerobic biodegradability, the biogas yield potential of deflocculated and bacterially pretreated, flocculated, deflocculated alone, and control sludges were found to be 0.467 L/(g VS), 0.355 L/(g VS), 0.315 L/(g VS), and 0.212 L/(g VS), respectively. Thus, the deflocculation and bacterial pretreatment improved the anaerobic biodegradability efficiently.

**Keywords:** Extracellular polymeric substance; Cell disruption; Enzyme activity; Anaerobic biodegradability; Waste activated sludge (WAS)

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The removal of four recalcitrant trace organic contaminants (TrOCs), namely carbamazepine, diclofenac, sulfamethoxazole and atrazine by laccase in an enzymatic membrane reactor (EMR) was studied. Laccases are not effective for degrading non-phenolic compounds; nevertheless, 22–55% removal of these four TrOCs was achieved by the laccase EMR. Addition of the redox-mediator syringaldehyde (SA) to the EMR resulted in a notable dose-dependent improvement (15–45%) of TrOC removal affected by inherent TrOC properties and loading rates. However, SA addition resulted in a concomitant increase in the toxicity of the treated effluent. A further 14–25% improvement in aqueous phase removal of the TrOCs was consistently observed following a one-off dosing of 3 g/L granular activated carbon (GAC). Mass balance analysis reveals that this improvement was not due solely to adsorption but also enhanced biodegradation. GAC addition also reduced membrane fouling and the SA-induced toxicity of the effluent.

**Keywords:** Enzymatic membrane reactor (EMR); Granular activated carbon (GAC); Laccase; Redox-mediator; Trace organic contaminants (TrOCs)

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*Abstract Vol. No. 25, December 2014*
The aim of this study was to evaluate the removal of linear alkylbenzene sulfonate (LAS) in an anaerobic fluidized bed reactor (AFBR) treating wastewater containing soap powder as LAS source. At Stage I, the AFBR was fed with a synthetic substrate containing yeast extract and ethanol as carbon sources, and without LAS; at Stage II, soap powder was added to this synthetic substrate obtaining an LAS concentration of 14 ± 3 mg L⁻¹. The compounds of soap powder probably inhibited some groups of microorganisms, increasing the concentration of volatile fatty acids (VFA) from 91 to 143 mg HAc L⁻¹. Consequently, the LAS removal rate was 48 ± 10% after the 156 days of operation. By sequencing, 16S rRNA clones belonging to the phyla Proteobacteria and Synergistetes were identified in the samples taken at the end of the experiment, with a remarkable presence of Dechloromonas sp. and Geobacter sp.

Keywords: Geobacter; Dechloromonas; Volatile fatty acids; Surfactant; 16S rRNA

The aim of this study was to evaluate the impact of short-term repeated exposure to a static magnetic field (induction 370 mT) on the Rhodococcus erythropolis cells. Specifically, it was ascertained the magnetic field’s potential to influence degradation of a phenol substrate, cell growth and respiration activity (oxygen consumption) during substrate biodegradation. The experiment took place over 3 days, with R. erythropolis exposed to the magnetic field for the first day. During the experiment, different recirculation rates between the reactor and the magnetic contactor has been tested. Use of the magnetic field at higher recirculation rates (residence time in contactor was less than 7 min) stimulated substrate (phenol) oxidation by around 34%; which, in turn, promoted R. erythropolis growth by around 28% by shortening the lag- and exponential-phases and increasing bacterial respiration activity by around 10%.

Keywords: Static magnetic field; Phenol biodegradation; Rhodococcus erythropolis; Fed-batch bioreactor; Bacterial degradation enhanced

High molecular weight (HMW) polynuclear aromatic hydrocarbons (PAHs) with more than three rings are inherently difficult to degrade. Degradation of HMW PAHs is primarily reported for actinomycetes, such as *Rhodococcus* and *Mycobacterium*. This study reports pyrene degradation by a *Pseudomonas aeruginosa* strain isolated from tank bottom sludge in a refinery. High cell surface hydrophobicity induced during growth on pyrene facilitated its utilization as sole carbon source. Specific growth rate (μ) in the range of 0.03–0.085 h⁻¹ could be achieved over the concentration range 25–500 mg/L. The specific growth rate and specific pyrene utilization rate increased linearly with increase in total pyrene concentration. Although various degradation intermediates were identified in the aqueous phase, accumulation of total organic carbon (TOC) in the aqueous phase was only a small fraction of TOC equivalents of pyrene lost from the cultures. The degradation pathway appears to be similar to that reported for *Mycobacterium* sp. PYR-I.

**Keywords:** HMW PAHs; Pyrene biodegradation kinetics; Growth rate on pyrene; Degradation intermediates


Recently, anaerobic digestion of lignocellulosic biomass for methane production has attracted considerable attention. However, there is little information regarding methane production from asparagus stem, a typical lignocellulosic biomass, by anaerobic digestion. In this study, alkaline pretreatment of asparagus stem was investigated for its ability to increase hydrolysis rate and methane production and to improve biodegradability (BD). The hydrolysis rate increased with increasing NaOH dose, due to higher removal rates of lignin and hemicelluloses. However, the optimal NaOH dose was 6% (w/w) according to the specific methane production (SMP). Under this condition, the SMP and the technical digestion time of the NaOH-treated asparagus stem were 242.3 mL/g VS and 18 days, which were 38.4% higher and 51.4% shorter than those of the untreated sample, respectively. The BD was improved from 40.1% to 55.4%. These results indicate that alkaline pretreatment could be an efficient method for increasing methane production from asparagus stem.

**Keywords:** Asparagus stem; Hydrolysis rate; Biodegradability; Methane production; Alkaline pretreatment

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Fluoranthene is highly toxic and ubiquitous in the environment. A study on degradation of 200 ppm of fluoranthene in MSM by two bacterial strains \textit{Pseudomonas aeruginosa} PSA5, \textit{Rhodococcus} sp. NJ2 and their consortium revealed that fluoranthene was degraded 74\% by \textit{Rhodococcus} sp. NJ2, 61\% by \textit{Pseudomonas} sp. PSA5 and 97\% by their consortium. Higher degradation in the consortium may be attributed to synergistic action between two bacteria. It was also observed that several degradative enzymes catechol 1,2 dioxygenase, catechol 2,3 dioxygenase, protocatechuate 2,3 dioxygenase, protocatechuate 3,4 dioxygenase, protocatechuate 4,5 dioxygenase, salicylate hydroxylase and 2-carboxybenzaldehyde dehydrogenase were differentially induced at different stages of fluoranthene degradation. Biodegradation kinetics indicated half life period of fluoranthene degradation. Besides, glycolipid, as a biosurfactant, was induced to facilitate the degradation process. Hence, both the bacteria may be used individually or in combination for effective decontamination of oil and sludge contaminated soil.

\textbf{Keywords:} Bacteria; Fluoranthene; Degradation; Degradative enzymes; Biosurfactant

\textbf{Biosensor}

Dylan P. Webster\textsuperscript{a}, Michaela A. TerAvest\textsuperscript{a}, Devin F.R. Doud\textsuperscript{b}, Arun Chakravorty\textsuperscript{a}, Eric C. Holmes\textsuperscript{a}, Caleb M. Radens\textsuperscript{a}, Swati Sureka\textsuperscript{a}, Jeffrey A. Gralnick\textsuperscript{b}, Largus T. Angenent\textsuperscript{a}. (\textsuperscript{a}Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY 14853, USA, \textsuperscript{b}BioTechnology Institute and Department of Microbiology, University of Minnesota—Twin Cities, St. Paul, MN 55108, USA). An arsenic-specific biosensor with genetically engineered \textit{Shewanella oneidensis} in a bioelectrochemical system. Biosensors and Bioelectronics, Volume 62 (2014): 320–324

Genetically engineered microbial biosensors have yet to realize commercial success in environmental applications due, in part, to difficulties associated with transducing and transmitting traditional bioluminescent information. Bioelectrochemical systems (BESs) output a direct electric signal that can be incorporated into devices for remote environmental monitoring. Here, we describe a BES-based biosensor with genetically encoded specificity for a toxic metal. By placing an essential component of the metal reduction (Mtr) pathway of \textit{Shewanella oneidensis} under the control of an arsenic-sensitive promoter, we have genetically engineered a strain that produces increased current in response to arsenic when inoculated into a BES. Our BES-based biosensor has a detection limit of ~40 \( \mu \)M arsenite with a linear range up to 100 \( \mu \)M arsenite. Because our transcriptional circuit relies on the activation of a single promoter, similar sensing systems may be developed to detect other analytes by the swap of a single genetic part.

\textbf{Keywords:} Microbial biosensor; Bioelectrochemical system (BES); Continuous monitoring; \textit{Shewanella}; Synthetic biology; Arsenic

Hee-Jo Lee, Jong-Gwan Yook (School of Electrical and Electronic Engineering, Yonsei University, Seoul, South Korea). Recent research trends of radio-frequency biosensors for biomolecular detection. Biosensors and Bioelectronics, Volume 61(2014): 448–459
This article reviews radio-frequency (RF) biosensors based on passive and/or active devices and circuits. In particular, we focus on RF biosensors designed for detection of various biomolecules such as biotin–streptavidin, DNA hybridization, IgG, and glucose. The performance of these biosensors has been enhanced by the introduction of various sensing schemes with diverse nanomaterials (e.g., carbon nanotubes, graphene oxide, magnetic and gold nanoparticles, etc.). In addition, the RF biosensing platforms that can be associated with an RF active system are discussed. Finally, the challenges of RF biosensors are presented and suggestions are made for their future direction and prospects.

Keywords: Biosensor; Biomolecule; Diagnosis; Radio-frequency; Detectable limit

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(Department of Chemistry, Center for Advanced Sensors & Environmental Systems (CASE), State University of New York at Binghamton, P.O. Box 6000, Binghamton, NY 13902-6000, United States). Biosensor for selective detection of E. coli in spinach using the strong affinity of derivatized mannose with fimbrial lectin. Biosensors and Bioelectronics, Volume 61(2014): 266–273

Escherichia coli (E. coli) contamination in foods and water resources represents a major threat for human health and the environment. This work exploits the strong affinity of mannose-containing oligosaccharides with the fimbrial lectin of E. coli to design novel biosensors. Modified carbohydrate ligands were synthesized by introducing phenyl residues and aliphatic chains to mannose via reductive amination in order to increase both the affinity and selectivity to E. coli compared to other pathogenic bacteria. The synthesized ligands include p-thiophenyl aminomannose (PTAM), p-carboxyphenyl aminomannose (PCAM), 1-deoxy-1-aminomannopyranoside (DAMP), glucosamine and low molecular weight chitosan bonded to mercapto undecanoic acid. The structures of the ligands were confirmed using 1H NMR and 1H, 13C, COZY NMR, and ESI/MS. The ligands were immobilized onto gold electrodes and SPR surfaces using-mercaptoundecanoic acid with glycine as deactivating agent. Two detection mechanisms were tested: (i) metal-enhanced electrochemical detection (MED) and (ii) label-free surface plasmon resonance (SPR) detection. The introduction of phenyl residues and aliphatic side groups to the mannose-containing oligosaccharides produced extremely high affinity for E. coli with detection limit of 1 cfu/mL. The relative selectivity of these ligands for E. coli, Citrobacter freundii, Staphylococcus epidermidis were 100%, 2.6% and 8.6% respectively. The biosensors were validated using spinach leaves at 3.0 cfu/mL. The work provides a generic biosensor for other pathogenic bacteria by enabling multivalent binding, immediate recognition for pathogens as well as inhibition of bacterial growth.

Keywords: Escherichia coli; FimH lectin; Mannose-binding protein; SPR; MED

Patthara Kongsuphola, Hui Hwee Ng, Joanna P. Pursey, Sunil K. Arya, Chee Chung Wong, Eugen Stulz, Mi Kyoung Park. (a Institute of Microelectronics, A*STAR (Agency for Science Technology and Research), 11 Science Park Road, Singapore Science Park II, 117685, Singapore, b School of Chemistry and Institute for Life Sciences, University of Southampton, Highfield, Southampton SO17 1BJ, UK). EIS-based biosensor

Serum background is a critical issue for biosensor development as it interferes with the detection of target molecules and may give rise to false positive signal. We present here highly sensitive and selective TNF-α biosensor which is able to detect TNF-α from non-diluted human serum using magnetic bead coupled antibody and electrochemical impedance spectroscopy (EIS) techniques. The process is designed to detect TNF-α from human serum in three stages; (1) abundant protein backgrounds are depleted from the serum using magnetic bead coupled albumin and IgG antibodies, (2) after background depletion TNF-α is captured using magnetic bead coupled TNF-α antibody, and (3) the captured TNF-α is eluted from the magnetic beads and measured using EIS technique in which comb structured gold microelectrodes array (CSGM) is utilized to enhance the detection sensitivity. The system is able to achieve the limit of detection (LOD) at 1 pg/ml (57 fM) and a linear relationship between increasing TNF-α concentrations and charge-transfer resistance in a dynamic range of 1–1000 pg/ml.

Keywords: Tumor necrosis factor; TNF-α; Electrochemical impedance spectroscopy; EIS; Serum; Non-diluted serum

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Continued progress in cell-phone devices has made them powerful mobile computers, equipped with sophisticated, permanent physical sensors embedded as the default configuration. By contrast, the incorporation of permanent biosensors in cell-phone units has been prevented by the multivocal nature of the stimuli and the reactions involved in biosensing and chemical sensing. Biosensing with cell phones entails the complementation of biosensing devices with the physical sensors and communication and processing capabilities of modern cell phones. Biosensing, chemical-sensing, environmental-sensing, and diagnostic capabilities would thus be supported and run on the residual capacity of existing cell-phone infrastructure. The technologies necessary to materialize such a scenario have emerged in different fields and applications. This article addresses the progress on cell-phone biosensing, the specific compromises, and the blend of technologies required to craft biosensing on cell phones.

Keywords: cell phones; biosensing; optical sensing; lab-on-a-chip; point-of-care; diagnostics


Amperometric hydrogen peroxide enzyme inhibition biosensors based on horseradish peroxidase (HRP) immobilised on electropolymerised neutral red (NR) or directly on the surface of carbon film electrodes (CFE) have been successfully applied to the determination of toxic Cr(III) and
Cr(VI). Parameters influencing the performance of the biosensor including the enzyme immobilisation method, the amount of hydrogen peroxide, applied potential and electrolyte pH were optimised. The inhibition of horseradish peroxidase by the chromium species was studied under the optimised conditions. Results from the quantitative analysis of chromium ions are discussed in terms of detection limit, linear range and sensitivity. The HRP kinetic interactions reveal mixed binding of Cr(III) with $I_{50} = 3.8 \mu M$ and inhibition binding constant $K_i = 11.3 \mu M$ at HRP/PNR/CFE biosensors and uncompetitive binding of Cr(VI) with $I_{50} = 3.9 \mu M$ and $K_i = 0.78 \mu M$ at HRP/CFE biosensors in the presence of $H_2O_2$ substrate. Interferences from other heavy metal ions were studied and the inhibition show very good selectivity towards Cr(III) and Cr(VI).

**Keywords:** Amperometric biosensor; Horseradish peroxidase; Poly(neutral red); Cr(III); Cr(VI); Enzyme inhibition

Li, L.\textsuperscript{a}, Xu, J.\textsuperscript{a}, Zheng, X.\textsuperscript{a}, Ma, C.\textsuperscript{a}, Song, X.\textsuperscript{b}, Ge, S.\textsuperscript{a}, Yu, J.\textsuperscript{a}, Yan, M.\textsuperscript{a}. (\textsuperscript{a} Key Laboratory of Chemical Sensing and Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, China, \textsuperscript{b} Cancer Research Center, Shandong Tumor Hospital, Jinan 250012, China). Growth of gold-manganese oxide nanostructures on a 3D origami device for glucose-oxidase label based electrochemical immunosensor. Biosensors and Bioelectronics, Volume 61(2014): 76-82

Flexible biosensors are of considerable current interest for the development of portable point-of-care medical products, minimally invasive implantable devices, and compact diagnostic platforms. Here, we reported an electrochemical paper based analytical device fabricated (EPADs) by sequentially growing gold nanoparticles (AuNPs) and manganese oxide (MnO\textsubscript{2}) nanowires networks on a freestanding three dimensional (3D) origami device. This fabricated through the growth of an AuNPs layer on the surfaces of cellulose fibers in the screen-printed paper working electrode (PWE), and thus developed a gold paper working electrode (Au-PWE). Subsequently, MnO\textsubscript{2} nanowires were successfully electrodeposited on Au-PWE to form a 3D network with large surface areas. Based on this novel EPADs and the principle of origami, we presented herein a simple immunosensing scheme using glucose oxidase (GOx) as an enzyme label, 3,3',5,5'-tetramethylbenzidine (TMB) as a redox terminator, and glucose as an enzyme substrate. The electrochemical enzymatic redox cycling was applied to the detection of prostate protein antigen (PSA), a biomarker of prostatic cancer. The proposed method successfully fulfilled the highly sensitive detection of PSA with a linear range of 0.005ngmL\textsuperscript{-1}-100ngmL\textsuperscript{-1} with a detection limit of 0.0012ngmL\textsuperscript{-1}. This EPADs exhibited high sensitivity, specificity and excellent performance in real human serum assay, and could be applied in point-of-care testing of other tumor markers for remote regions and developing countries © 2014 Elsevier B.V.

**Keywords:** Electrochemical immunosensor; Gold paper working electrode; Manganese oxide nanowire; Three dimensional origami device

Jie Wang\textsuperscript{a, b}, Yawan Zheng\textsuperscript{b}, Hui Jia\textsuperscript{a}, Hongwei Zhang\textsuperscript{a}. (\textsuperscript{a} State Key Laboratory of Hollow Fiber Membrane Materials and Processes, Tianjin Polytechnic University, Tianjin 300387, China, \textsuperscript{b} School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin 300387, China). Bioelectricity generation in an integrated system combining microbial fuel cell and tubular membrane reactor: Effects of operation

A bio-cathode microbial fuel cell (MFC) with tubular membrane was integrated to construct a microbial fuel cell–tubular membrane bioreactor (MFC–TMBR) system, in which the bio-cathode MFC was developed as a biosensor for COD real-time monitoring in TMBR and the performance was analyzed in terms of its current variation caused by operation parameters. With a constant anode potential, the effect of HRT demonstrated that higher rate of mass transport increased the response of the system. The system was further explored an inverse relationship between TMP and current peak by using EPS concentration under the different MLSS concentration. The sensor output had a linear relationship with COD up to 1000 mg/L (regression coefficient, $R^2 = 0.97$) and MLSS (regression coefficient, $R^2 = 0.94$). The simple and compact bio-cathode MFC biosensor for TMBR using MFC–TMBR integrated system showed promising potential for direct and economical COD online monitoring, and provided an opportunity to widen the application of MFC-based biosensor.

**Keywords:** Bio-cathode microbial fuel cell; COD; Biosensor; Membrane reactor

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**Bioengineering**

D Barrie Johnson. (College of Natural Sciences, Bangor University, Deiniol Road, Bangor LL57 2UW, UK). Biomining — biotechnologies for extracting and recovering metals from ores and waste materials. *Current Opinion in Biotechnology, Volume 30* (2014): 24–31

The abilities of acidophilic chemolithotrophic bacteria and archaea to accelerate the oxidative dissolution of sulfide minerals have been harnessed in the development and application of a biotechnology for extracting metals from sulfidic ores and concentrates. Biomining is currently used primarily to leach copper sulfides and as an oxidative pretreatment for refractory gold ores, though it is also used to recover other base metals, such as cobalt, nickel and zinc. Recent developments have included using acidophiles to process electronic wastes, to extract metals from oxidized ores, and to selectively recover metals from process waters and waste streams. This review describes the microorganisms and mechanisms involved in commercial biomining operations, how the technology has developed over the past 50 years, and discusses the challenges and opportunities for mineral biotechnologies in the 21st century.

Tianjie Yuan¹, Liping Xie¹, Baoquan Zhu¹ and Youjia Hu¹. (¹Shanghai Institute of Pharmaceutical Industry, 1111 Zhongshan Road (North No. 1), Shanghai, 200437, China. Youjia Hu: Email: bebydou@hotmail.com). Bioconversion of deoxysugar moieties to the biosynthetic intermediates of daunorubicin in an engineered strain of *Streptomyces coeruleobidus*. *Biotechnology Letters, Volume 36* (9) (2014): 1809-1818

Daunorubicin (DNR) is a representative anthracycline with anti-tumor bioactivity. Its convergent biosynthetic pathway has promoted the research on pursuing novel anthracyclines by combinatorial biosynthesis. SnoaL is a special polyketide cyclase that catalyzes the closure of nogalonic acid methyl ester with the C9-Sstereochemistry. In this study, the gene cluster of DNR was cloned, and snoaL was integrated into the DNR biosynthetic pathway for the substitution
of dnrD in *Streptomyces coeruleobidus* DM, which resulted in the production of epi-
alkaviketone. The biosynthetic pathway of NDP-4-deacetyl-L-chromose B was then expressed in
the engineered strain, which led to the production of corresponding glycosylated anthracycline
compounds. Finally, the bioactivities of these engineering strains were evaluated.

**Keywords:** Anthracyclines, Biosynthetic gene cluster, Combinatorial biosynthesis, 
Daunorubicin, Polyketide synthases

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**Pollen Biotechnology**

Concepción De Linares¹, ², Idoia Postigo³, Jordina Belmonte¹, ², Miguel Canela⁴ and Jorge Martínez⁵. (¹Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Spain, ². Institut de Ciència i Tecnologia Ambientals (ICTA), Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Spain, ³Department of Immunology, Microbiology and Parasitology, Faculty of Pharmacy, University of Basque Country, Vitoria, Spain, ⁴Department of Managerial Decision Sciences, IESE Business School, Barcelona, Spain. Concepción De Linares Email: concepcion.delinares@uab.cat). Optimization of the measurement of outdoor airborne allergens using a protein microarrays platform.  

Increased knowledge on allergenic molecules in the environmental air helps in the information on environmental air quality and in the prevention and treatment of allergies. The aim of this study is to develop and validate a new methodology for the simultaneous detection and quantification of several airborne allergens using protein microarray technology, which has been created for the clinical detection of allergens. The immunological method was performed with Immuno Solid-phase Allergen Chip (ISAC) inhibition assay. Reagents for the validation studies include the following: (1) three sera from patients allergic to grass pollen each with different IgE levels as the detection reagents, (2) recombinant Phl p 1 major allergen as the inhibitor for the inhibition assays, (3) “natural” Phl p 1 released by *Phleum pratense* (timothy grass) pollen grains as the “biologically” relevant aeroallergen and (4) samples of airborne pollens collected by a Multi-vial Cyclone Sampler for comparison of levels of pollen detection versus the protein allergen detection by the microarray assay. The results obtained showed that ISAC inhibition is a sensitive technique able to detect 2.1 pg/mL of Phl p 1 and the allergens released from 1 grain of natural pollen. Also, the airborne allergen samples analyzed showed a good correlation with the concentration of grass pollen in the air. The use of ISAC inhibition will greatly improve future airborne simultaneous allergen quantification, becoming a valuable option in air quality control.

**Keywords:** Airborne allergen; Microenvironment array chips; Pollen; Validation

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atmosphere of Rohtak city, Haryana (India): a pioneer study. Aerobiologia, Volume 30 (3) (2014): 229-238

An attempt has been made in the present study to assess the allergenicity of dominant pollen types recorded from the atmosphere of Rohtak city. Skin prick test was performed with the antigenic extracts of 22 pollen types on 150 local patients who visited Asthma Clinic, University of Health Sciences, Rohtak. Markedly positive skin reactions (2+ and above) varied from 4.6 to 20.6 % to various pollen antigens. *Cenchrus ciliaria* (20.6 %), *Zea mays* (20 %) and *Pennisetum typhoides* (19.3 %) were the pollen allergens exhibiting maximum sensitivity. Antigenic extract of *Cassia occidentalis*, *Cynodon dactylon* and *Ricinus communis*showed marked skin reactivity in 18.6 % of patients. *Prosopis juliflora*, *Chenopodium murale*, *Amaranthus spinosus*, *Cassia fistula* and *Cassia siamea* showed 2+ and above reactions in 16.6, 15.3, 14.6 and 14.0 % of the local patients, respectively. Least reactivity (4.6 %) was shown to the antigenic extract of *Cyperus rotundus*. Out of 52 sera screened for the presence of specific IgE antibodies against different antigenic extracts, only 5.5 % showed >60 % binding. About 30 % and above binding was shown to the antigenic extracts of *Z. mays*, *A. spinosus*, *R. communis* and *Xanthium strumarium*. The concordance between positive skin reaction and serum-specific IgE antibodies ranged from 15 to 69 %.

**Keywords:** Pollen; Aeroallergens; Bioassay; Immunoassay;

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The aim of this study was to determine the qualitative and quantitative composition of the airborne pollen of Santa Rosa city, La Pampa (Argentina), and to analyse the seasonal behaviour of the pollen types that have the highest representation in the atmosphere. The city, with temperate climate, is located in a cultivated area that corresponds phytogeographically to a xerophytic forest limiting with grasslands. The pollen sampling was performed using a Hirst-type volumetric spore trap located in the urban centre of the city, 15 m above ground level, from July 2007–June 2009. The annual pollen index was 51,647 pollen grains. The airborne pollen consisted of 73 pollen types, 42 of woody origin represented 66 % of the total and with winter-spring seasonality and 31 were of herbaceous origin, which represented 30 % of the total and with spring-summer seasonality. The composition of the woody airborne pollen reflected the formation of urban vegetation, consisting mainly of exotic taxa from tree species used in urban tree alignment. The most abundant types were as follows: Cupressaceae, Fraxinus, Ulmus, Olea europaea, Styrpholobium japonicum, Myrtaceae, Pinaceae, Platanus, Celtis-Morus and Populus. Native components such as Condalia microphylla were also found, indicating the ‘Espinal’ phytogeographical province that was typical of the area. The most abundant herbaceous airborne pollen types, in descending order, were as follows: Poaceae, Amaranthus-Chenopodiaceae, Urticaceae, Brassicaceae and Asteraceae. The emission sources of these pollen types were weeds that grew spontaneously in parks, waste grounds and flower beds of the city.
Abstract Vol. No. 25, December 2014

Keywords: Airborne pollen Urban monitoring station Volumetric sampler Native species Argentina


Airborne pollen calendars are useful to estimate the flowering season of the different plants as well as to indicate the allergenic potential present in the atmosphere at a given time. In this study, it is presented a 10-year survey of the atmospheric concentration of allergenic pollen types. Airborne pollen was performed, from 2003 to 2012, using a 7-day Hirst-type volumetric trap. The interannual variation of the daily mean concentration of the number of pollen grains and the main pollen season was determined as well as the hourly variations and correlation with meteorological parameters. During the study period, 18 different allergenic pollen types were considered based on its representativeness on the total annual airborne pollen concentration. The lowest annual concentrations were sampled in 2006 and the highest in 2007. The highest airborne pollen concentration was found during early spring and early summer. On the contrary, December was the month with the lowest pollen concentration. The major pollen sampled belongs to trees followed by weeds and grasses, being the most representative pollen types in the atmosphere: Urticaceae, Platanus, Poaceae, Pinaceae, Cupressaceae, Acer, Quercus, Castanea, Plantago, Alnus, Olea europaea, Betula, Myrtaceae and Populus. Intradaily distribution patterns of the pollen types studied presented differences with some taxa being predominantly sampled in the morning (9–11 a.m.) while others in first night hours (between 9 and 12 p.m.). Significantly correlations were found between the airborne pollen concentration and meteorological parameters.

Keywords: Aerobiology, Main pollen season, Meteorological parameters, Hourly distribution, Pollen spectrum

Biotechnology Policy Issue

Costanigro, M.a, Lusk, J.L.b. (a Colorado State University, United States, b Oklahoma State University, United States). The signaling effect of mandatory labels on genetically engineered food. Food Policy, Volume 49( P1) (2014): 259-267

It has been suggested that the adoption of mandatory labeling for genetically engineered food might send a signal to consumers that foods produced with biotechnology are unsafe or should be avoided. To date, however, there is little empirical evidence to substantiate this claim. This paper utilized data from two studies to explore whether consumers exposed to labels on genetically engineered foods expressed greater aversion to genetic engineering than consumers in control groups, who were exposed to decoy labels unrelated to the technology. We find little evidence of a signaling effect resulting from the mere exposure to labels. However, in Study 1, we find signaling operating in another fashion: there were stark differences in the implied
willingness-to-pay to avoid genetically engineered foods when consumers were exposed to mandatory "contains" labels vs. voluntary "does not contain" labels. In study 1, we also find aversion to a non-GE technology - ethylene ripening - that is comparable to aversion to biotechnology.

**Keywords:** Biotechnology; Experiment; GMO; Labeling; Signal; Survey

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Societal developments are hinged on the energy supplied by fossil fuels. However, the supply of these fuels is finite in the foreseeable future. This is aside the associated environmental degradation and economic sustainability of these fuels. These negative consequences and challenges spurred the search for sustainable energy sources such as biofuels. However, affordable feedstocks and efficient synthesis for renewable fuels remain indispensable and yet challenging line of research. Therefore, breakthroughs in plant biotechnology and mass production are essential prerequisites for ensuring the sustainability of biofuels as alternatives to petroleum-based energy. Conversely, public outcry concerning the food-for-fuel conflicts and land-use change hinder the popularity of such biofuel energy sources. Therefore, this paper reviewed the prospects of biogasoline production as sustainable alternative to ethanol and a compliment to biodiesel. Apart from reduction in greenhouse gas emissions, biogasoline promises to be cheaper and more environmental friendly. Further, inedible feedstocks such as microalgae and rubber seed oil would ensure higher net energy gain. Consequently, these will help resolve the food-for-fuel conflicts and land-use competitions. However, achieving the biofuel central policy depends on advances in processing the renewable energy sources.

**Keywords:** Biodiesel; Bioethanol; Biogasoline; Food-for-fuel; Microalgae; Rubber seed oil

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**Biogasoline:** An out-of-the-box solution to the food-for-fuel and land-use competitions. Energy Conversion and Management, Volume 89 (2015): 349-367

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Inoculation with exogenous white-rot fungi has been proven to be an efficient method to promote lignocellulose biodegradation during agricultural waste composting. Indigenous fungal communities, the most important organisms responsible for mineralization and decomposition of lignocellulosic materials in composts, can be affected by sample properties and other biotic factors. This research was conducted to determine the effects of the *Phanerochaete chrysosporium* inoculation on the indigenous fungal communities during agricultural waste composting. Fungal communities in samples with different inoculation regimes were investigated by sequencing and quantitative PCR. Results showed that *P. chrysosporium* inoculants produced significant negative effects on the indigenous fungal community abundance during the thermophilic stage. Samples inoculated during Phase II contained higher proportion of *Acremonium chrysogenum* and *Galactomyces geotrichum*, while those non-inoculated samples were dominated by *Coprinopsis cinerea* and *Scytalidium thermophilum*. Moreover, the indigenous fungal community abundance was significantly correlated with the C/N ratio, water soluble carbon and moisture content (*P* < 0.05). Redundancy analysis indicated that the most variation in distribution of indigenous fungal community structure was statistically explained by nitrate, C/N ratio, and moisture content, factors which solely explained 29.6 % (*F* = 30.316, *P* = 0.002), 25.6 % (*F* = 26.191, *P* = 0.002) and 10.0 % (*F* = 10.249, *P* = 0.002) of the variation in the indigenous fungal community structure, respectively.

**Keywords:** Composting; *Phanerochaete Chrysosporium*; Inoculation; Lignocellulose biodegradation; Indigenous fungal community; Redundancy analysis

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**Bioenergy**

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This study discussed the effects of different concentrations (0.625, 1.875 and 3.125 mM) of copper (Cu) in the form of CuSO4 on biomethane production and on the dynamics of microbial communities during the mesophilic anaerobic digestion (AD) of cow manure. The effects on biomethane production were found to depend on CuSO4 concentrations. After 50 days of AD, treatment A3 (3.125 mM) had lower cumulative biomethane production than the no-Cu control. The maximum value of cumulative biomethane production was detected under treatment A2 (1.875 mM). These results suggested that the stimulation or inhibition to biomethane production might be related to the concentration and chemical forms of Cu. Moreover, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to discuss the dynamics of microbial communities. Results revealed that different concentrations of CuSO4 had effects on the richness and diversity of bacterial and archaeal communities. The predominance of Bacteroidetes bacterium (GU339485.1) was verified through the sequencing of the dominant
DGGE bands. Furthermore, Bacteroidetes bacterium could be detected during the whole AD process and is adaptable to a certain concentration range of CuSO4.

**Keywords:** Copper, anaerobic digestion (AD), mesophilic, PCR-DGGE, bacterial community, archaeal community


The article investigates the performance of an integrated system for the energy recovery from biomass and waste based on anaerobic digestion, gasification and water treatment. In the proposed system, the organic fraction of waste of the digestible biomass is fed into an anaerobic digester, while a part of the combustible fraction of the municipal solid waste is gasified. Thus, the obtained biogas and syngas are used as a fuel for running a cogeneration system based on an internal combustion engine to produce electric and thermal power. The waste water produced by the integrated plant is recovered by means of both forward and inverse osmosis. The different processes, as well as the main components of the system, are modelled by means of a lumped and distributed parameter approach and the main outputs of the integrated plant such as the electric and thermal power and the amount of purified water are calculated. Finally, the implementation of the proposed system is evaluated for urban areas with a different number of inhabitants and the relating performance is estimated in terms of the main outputs of the system.

**Keywords:** Anaerobic digestion, gasification, water treatment, Combined Heat and Power system, plant performance simulation

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Anaerobic digestion of agro-industrial waste is of significant interest in order to facilitate a sustainable development of energy supply. Using of material and energy potentials of agro-industrial waste, in the framework of technical, economic, and ecological possibilities, contributes in increasing the share of energy generated from renewable energy sources. The paper deals with the benefits arising from the utilization of biogas produced by co-digestion of whey and cow manure. The advantages of this process are the profitability of the plant and the convenience in realizing an anaerobic digestion plant to produce biogas that is enabled by the benefits from the sale of electric energy at favorable prices. Economic aspects are related to the capital cost (€ 2,250,000) of anaerobic digestion treatment in a biogas plant with a 300 kW power and 510 kW heating unit in a medium size farm (450 livestock units). Considering the optimum biogas yield of 20.7 dm³ kg⁻¹ of wet substrate and methane content in the biogas obtained of 79%, the anaerobic process results in a daily methane production of 2,500 kg, with
the maximum power generation of 2,160,000 kWh y\(^{-1}\) and heat generation of 2,400,000 kWh y\(^{-1}\). The net present value (NPV), internal rate of return (IRR) and payback period for implementation of profitable anaerobic digestion process is evaluated. Ecological aspects related to carbon dioxide (CO\(_2\)) and methane (CH\(_4\)) emission reduction are assessed.

**Keywords:** Anaerobic digestion, agro-industrial waste, biogas, energy, economic, environmental

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In this study, the catalytic pyrolysis of waste furniture sawdust in the presence of ZSM-5, H-Y and MCM-41 (10 wt % of the biomass sample) was carried out in order to increase the quality of the liquid product at the various pyrolysis temperatures of 400, 450, 500 and 550°C. In the non-catalytic work, the maximum oil yield was obtained as 42% at 500°C in a fixed-bed reactor system. In the catalytic work, the maximum oil yield was decreased to 37.48, 30.04 and 29.23% in the presence of ZSM-5, H-Y and MCM-41, respectively. The obtained pyrolysis oils were analyzed by various spectroscopic and chromatographic techniques. It was determined that the use of a catalyst decreased acids and increased valuable organics found in the bio-oil. The removal of oxygen from bio-oil was confirmed with the results of the elemental analysis and gas chromatography-mass spectrometry.

**Keywords:** Catalytic pyrolysis, sawdust, ZSM-5, H-Y, MCM-41

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In this study, we propose the use of tung cake for the production of organic acids, with an emphasis on citric acid by solid-state fermentation. We evaluated the conditions of production and the by-products from the biodiesel chain as raw materials involved in this bioprocess. First, we standardized the conditions of solid-state fermentation in tung cake with and without residual fat and with different concentrations of glycerine using the fungus *Aspergillus niger*. The solid-state fermentation process was monitored for 7 days considering the biomass growth and pH level. Citric acid production was determined by high-performance liquid chromatography. Fungal development was better in the crude tung cake, consisting of 20% glycerine. The highest citric acid yield was 350 g kg\(^{-1}\) of biomass. Therefore, the solid-state fermentation of the tung cake with glycerine led to citric acid production using the *Aspergillus niger* fungus.
**Keywords:** Citric acid, by-products, Aspergillus niger, glycerine, tung cake, solid-state fermentation, SSF, biodiesel


Dark fermentation for bio-hydrogen (bio-H₂) production is an easily operated and environmentally friendly technology. However, low bio-H₂ production yield has been reported as its main drawback. Two strategies have been followed in the past to improve this fact: genetic modifications and adjusting the reaction conditions. In this paper, the second one is followed to regulate the bio-H₂ release from the reactor. This operating condition alters the metabolic pathways and increased the bio-H₂ production twice. Gas release was forced in the continuous culture to study the equilibrium in the mass transfer between the gaseous and liquid phases. This equilibrium depends on the H₂, CO₂, and volatile fatty acids production. The effect of reducing the bio-H₂ partial pressure (bio-H₂ pp) to enhance bio-H₂ production was evaluated in a 30 L continuous stirred tank reactor. Three bio-H₂ release strategies were followed: uncontrolled, intermittent, and constant. In the so called uncontrolled fermentation, without bio-H₂ pp control, a bio-H₂ molar yield of 1.2 mol/mol glucose was obtained. A sustained low bio-H₂ pp of 0.06 atm increased the bio-H₂ production rate from 16.1 to 108 mL/L/h with a stable bio-H₂ percentage of 55 % (v/v) and a molar yield of 1.9 mol/mol glucose. Biogas release enhanced bio-H₂ production because lower bio-H₂ pp, CO₂ concentration, and reduced volatile fatty acids accumulation prevented the associated inhibitions and bio-H₂ consumption.

**Keywords:** Controlled continuous culture; Continuous intermittent gas release; Hydrogen partial pressure; Dark fermentation


Anaerobic digestion (AD) process is a well-established method to generate energy from the organic wastes both from the environmental and economical perspectives. The purpose of present study is to evaluate energy production from potato wastes by incorporating cow manure into the process. Firstly, a laboratory pilot of one-stage biogas production was designed and built according to continuously stirred tank reactor (CSTR) system. The setup was able to automatically control the environmental conditions of the process including temperature, duration, and rate of stirring. AD experiment was exclusively performed on co-digestion of potato peel (PP) and cow manure (CM) in three levels of mixing ratio including 20:80, 50:50, 80:20 (PP:CM), and 0:100 as control treatment based on the volatile solid (VS) weight without adding initial inoculums. After hydraulic retention time (HRT) of 50 days on average 193, 256,
348, and 149 norm liter (LN) (kg VS)\(^{-1}\), methane was produced for different mixing ratios, respectively. Statistical analysis shows that these gas productions are significantly different. The average energy was determined based on the produced methane which was about 2.8 kWh (kg VS)\(^{-1}\), implying a significant energy production potential. The average chemical oxygen demand (COD) removal of treatments was about 61 %, showing that it can be leached significantly with high organic matter by the employed pilot. The energy efficiency of 92 % of the process also showed the optimum control of the process by the pilot.

**Keywords:** Anaerobic digestion; Renewable energy; Biogas; Potato peel; CSTR system; Biowaste; Food industry waste

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Sweet sorghum juice was fermented into ethanol using *Saccharomyces cerevisiae* (ATCC 24858). Factorial experimental design, regression analysis and response surface method were used to analyze the effects of the process parameters including juice solid concentration from 6.5 to 26% (by mass), yeast load from 0.5 g L\(^{-1}\) to 2 g L\(^{-1}\) and fermentation temperature from 30 °C to 40 °C on the ethanol yield, final ethanol concentration and fermentation kinetics. The fermentation temperature, which had no significant effect on the ethanol yield and final ethanol concentration, could be set at 35 °C to achieve the maximum fermentation rate. The yeast load, which had no significant effect on the final ethanol concentration and fermentation rate, could be set at 1 g L\(^{-1}\) to achieve the maximum ethanol yield. The juice solid concentration had significant inverse effects on the ethanol yield and final ethanol concentration but a slight effect on the fermentation rate. The raw juice at a solid concentration of 13% (by mass) could be directly used during fermentation. At the fermentation temperature of 35 °C, yeast solid concentration of 1 g L\(^{-1}\) and juice solid concentration of 13%, the predicted ethanol yield was 101.1% and the predicted final ethanol concentration was 49.48 g L\(^{-1}\) after 72 h fermentation. Under this fermentation condition, the modified Gompertz’s equation could be used to predict the fermentation kinetics. The predicted maximum ethanol generation rate was 2.37 g L\(^{-1}\) h\(^{-1}\).

**Keywords:** Sweet sorghum juice; Ethanol; Fermentation; Response surface method; Biofuel; Optimization

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Recently interest has been revived in the use of plant-derived waste oils as renewable replacements for fossil diesel fuel. Olive-pomace oil (OPO) extracted from alperujo (by-product of processed olives for olive oil extraction), and produced in considerable quantities throughout the Mediterranean countries, can be used for biodiesel production. A steam treatment of alperujo is being implemented in OPO extraction industry. This steam treatment improves the solid–liquid separation by centrifugation and facilitates the drying for further extraction of OPO. It has been verified that the steam treatment of this by-product also increases the concentration of OPO in the resulting treated solid, a key factor from an economic point of view. In the present work, crude OPO from steam-treated alperujo was found to be good source for producing biodiesel. Oil enrichment, acidity, biodiesel yield and fatty acid methyl ester composition were evaluated and compared with the results of the untreated samples. Yields and some general physicochemical properties of the quality of biodiesel were also compared to those obtained with other oils commonly used in biodiesel production. As for biodiesel yield no differences were observed. A transesterification process which included two steps was used (acid esterification followed by alkali transesterification). The maximum biodiesel yield was obtained using molar ratio methanol/triglycerides 6:1 in presence of sodium hydroxide at a concentration of 1% (w/w), reaction temperature 60 °C and reaction time 80 min. Under these conditions the process gave yields of about 95%, of the same order as other feedstock using similar production conditions.

**Keywords:** Alperujo; Biodiesel; Steam treatment; Methyl esters; Olive-pomace oil; Transesterification

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In arid regions, reductions in the amount of available agricultural water are fueling interest in alternative, low water-use crops. Perennial grasses have potential as low water-use biofuel crops. However, little is known about which perennial grasses can produce high quantity, high quality yields with low irrigation on formerly high-input agricultural fields in arid regions. We monitored biomass production, weed resistance, rooting depth, and root architecture of nine perennial grasses under multiple irrigation treatments in western Nevada. Under a low irrigation treatment (71 ± 9 cm irrigation water annually), cool-season grasses produced more biomass and were more weed-resistant than warm-season grasses. With additional irrigation (120 ± 12 cm water annually), warm- and cool-season grasses had similar biomass production, but cool-season species remained more weed-resistant. Among species within each grass type, we observed high variability in performance. Two cool-season species (Elytrigia elongata and Leymus cinereus) and one warm-season species (Bothriochloa ischaemum) performed better than the other tested species. Root depth was not correlated with biomass production, but species with deeper roots had fewer weeds. Abundance of fine roots (but not large roots) was correlated with increased biomass and fewer weeds. Both L. cinereus and E. elongata had deep root systems dominated by fine roots, while B. ischaemum had many fine roots in shallow soil but few roots in deeper soil. Cool-season grasses (particularly E. elongata, L. cinereus, and other species with abundant fine roots) may be worthy of further attention as potential biofuel crops for cold desert agriculture.
**Keywords:** Walker lake; *Panicum virgatum*; Cellulosic biofuel crops; Root diameter; Deficit irrigation; Arid ecosystems

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In order to improve the biotransformation rate of *Spartina alterniflora* (SA) and evaluate the feasibility of NaOH-treatment as a pretreatment of digested SA (DSA) for advanced anaerobic biogasification, two experiments were conducted under lab-scale conditions. First, SA was directly digested under 35 ± 1 °C with initial total solid (TS) loading of 20% (the first stage). After 90 day's reaction, cumulative biogas yield of SA and average methane content were 388 ± 22 ml g⁻¹ TS\(_{\text{added}}\) and 59.62 ± 1.88%, respectively. Then the DSA was treated with 5% NaOH solution and was used for advanced biogasification (the second stage). The cumulative biogas yield of NaOH-treatment and control were 210 ± 11 ml g⁻¹ TS\(_{\text{added}}\) and 103 ± 16 ml g⁻¹ TS\(_{\text{added}}\), respectively, with 70.78 ± 2.11% and 76.92 ± 1.94% of average methane content. The total cumulative biogas yield (sum of the first stage and the second stage of NaOH-treatment) and TS removal rate of SA were 495 ± 24 ml g⁻¹ TS\(_{\text{added}}\) and 76.85 ± 1.94%. After NaOH treatment, surface lignin and some carbohydrate of DSA were destructed into lignin fragment, organic acids, and some other small molecular organic matter; while the skeleton structure of lignin and cellulose were not destructed significantly. The results of \(^{13}\)C NMR showed that methyl and carboxylic C groups of DSA were decomposed significantly. The results indicated that NaOH-treatment was a good pretreatment for DSA.

**Keywords:** Advanced biogasification; NaOH-treatment; *Spartina alterniflora*; Anaerobic digestion; Biogas

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Enzymatic biodiesel production kinetics under previously optimized conditions were investigated. Waste frying oil (WFO) was used as the raw material, Novozym 435 as catalyst, methanol as acyl acceptor and tert-butanol as co-solvent. To investigate pure transesterification kinetics improving product properties, 3 Å molecular sieves were incorporated into the reaction to provide an anhydrous medium avoiding the side reactions of hydrolysis and esterification. The effects of either WFO or methanol on the reaction rate were analyzed separately. The reaction was described by a Ping Pong mechanism and competitive inhibition by methanol. The results obtained in the kinetics study were applied in the operation of a semi-continuous reactor for
biodiesel production. The operational conditions of each reaction cycle were: methanol-to-oil ratio 8/1 (mol/mol), 15% (wt) Novozym 435, 0.75% (v/v) of tert-butanol, 44.5°C, 200 rpm and 4 h of reaction time. The enzymes were successively reused by remaining in the reactor during all the cycles. Under these conditions, biodiesel production yields higher than 80% over 7 reaction cycles were observed. Both the kinetics study and the reactor operation showed that Novozym 435 was not inhibited at high methanol concentrations and that the kinetics of the proposed enzymatic process could be comparable to the conventional chemical process.

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Fermentative production of bio-hydrogen (bio-H₂) from organic residues has emerged as a promising alternative for providing the required electron source for hydrogen driven remediation strategies. Unlike the widely used production of H₂ by bacteria in fresh water systems, few reports are available regarding the generation of biogenic H₂ and optimisation processes in marine systems. The present research aims to optimise the capability of an indigenous marine bacterium for the production of bio-H₂ in marine environments and subsequently develop this process for hydrogen driven remediation strategies. Fermentative conversion of organics in marine media to H₂ using a marine isolate, Pseudoalteromonas sp. BH11, was determined. A Taguchi design of experimental methodology was employed to evaluate the optimal nutritional composition in batch tests to improve bio-H₂ yields. Further optimisation experiments showed that alginate-immobilised bacterial cells were able to produce bio-H₂ at the same rate as suspended cells over a period of several weeks. Finally, bio-H₂ was used as electron donor to successfully dehalogenate trichloroethylene (TCE) using biogenic palladium nanoparticles as a catalyst. Fermentative production of bio-H₂ can be a promising technique for concomitant generation of an electron source for hydrogen driven remediation strategies and treatment of organic residue in marine ecosystems.

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Microorganisms play a significant role in bioethanol production from lignocellulosic material. A challenging problem in biocconversion of rice bran is the presence of toxic inhibitors in lignocellulosic acid hydrolysate. Various strains of Zymomonas mobilis (ZM4, TISTR 405, 548, 550 and 551) grown under biofilm or planktonic modes were used in this study to examine their potential for biocconversion of rice bran hydrolysate and ethanol production efficiencies. Z. mobilis readily formed bacterial attachment on plastic surfaces, but not on glass surfaces. Additionally, the biofilms formed on plastic surfaces steadily increased over time, while those formed on glass were speculated to cycle through accumulation and detachment phases. Microscopic analysis revealed that Z. mobilis ZM4 rapidly developed homogeneous biofilm
structures within 24 hours, while other Z. mobilis strains developed heterogeneous biofilm structures. ZM4 biofilms were thicker and seemed to be more stable than other Z. mobilis strains. The percentage of live cells in biofilms was greater than that for planktonic cells (54.32 ± 7.10% vs. 28.69 ± 3.03%), suggesting that biofilms serve as a protective niche for growth of bacteria in the presence of toxic inhibitors in the rice bran hydrolysate. The metabolic activity of ZM4 grown as a biofilm was also higher than the same strain grown planktonically, as measured by ethanol production from rice bran hydrolysate (13.40 ± 2.43 g/L vs. 0.432 ± 0.29 g/L, with percent theoretical ethanol yields of 72.47 ± 6.13% and 3.71 ± 5.24% respectively). Strain TISTR 551 was also quite metabolically active, with ethanol production by biofilm and planktonically grown cells of 8.956 ± 4.06 g/L and 0.0846 ± 0.064 g/L (percent theoretical yields were 48.37 ± 16.64% and 2.046 ± 1.58%, respectively). This study illustrates the potential for enhancing ethanol production by utilizing bacterial biofilms in the bioconversion of a readily available and normally unusable low value by-product of rice farming.


The desire to reduce dependence on the ever diminishing fossil fuel reserves coupled with the impetus towards green energy has seen increased research in biofuels as alternative sources of energy. Lignocellulose materials are one of the most promising feedstocks for advanced biofuels production. However, their utilisation is dependent on the efficient hydrolysis of polysaccharides, which in part is dependent on cost-effective and benign pretreatment of biomass to remove or modify lignin and release or expose sugars to hydrolytic enzymes. Laccase is one of the enzymes that are being investigated not only for potential use as pretreatment agents in biofuel production, mainly as a delignifying enzyme, but also as a biotechnological tool for removal of inhibitors (mainly phenolic) of subsequent enzymatic processes. The current review discusses the major advances in the application of laccase as a potential pretreatment strategy, the underlying principles as well as directions for future research in the search for better enzyme-based technologies for biofuel production. Future perspectives could include synergy between enzymes that may be required for optimal results and the adoption of the biorefinery concept in line with the move towards the global implementation of the bioeconomy strategy.

Keywords: Laccase; Lignocellulose; Pretreatment; Biofuels


In vitro degradation of hexacosane (C26H54), a HMW n-alkane, was studied in MSM by two bacterial strains i.e., Pseudomonas sp. BP10 and Stenotrophomonas nitritireducens E9, isolated from petroleum sludge, in isolation and combination. The results revealed that both the strains were able to metabolize hexacosane by 82% in isolation and 98% in their consortium after 7 days. An enhancement of 16% in hexacosane degradation by the consortium indicated an additive action of bacterial strains. However, in control, a degradation of 21% was attributed to
abiotic factors. During incubation with hexacosane, both the bacteria continued to multiply in isolation and consortium, which reflected that hexacosane was utilized by bacteria as a carbon and energy source. Activities of alkane hydroxylase and alcohol dehydrogenase were differentially expressed in isolation and combination, indicating their involvement in hexacosane degradation. Enhanced cell surface hydrophobicity and emulsification index and reduced surface tension also supported the degradation process.

**Keywords:** Hexacosane; Bacteria; Degradative enzymes; Surface tension; Cell surface hydrophobicity

Jun Cheng*, Rui Huang, Tao Li, Junhu Zhou, Kefa Cen. (State Key Laboratory of Clean Energy Utilization, Zhejiang University, Hangzhou 310027, China). Biodiesel from wet microalgae: Extraction with hexane after the microwave-assisted transesterification of lipids. Bioresource Technology, Volume 170(2014): 69–75

A chloroform-free novel process for the efficient production of biodiesel from wet microalgae is proposed. Crude biodiesel is produced through extraction with hexane after microwave-assisted transesterification (EHMT) of lipids in wet microalgae. Effects of different parameters, including reaction temperature, reaction time, methanol dosage, and catalyst dosage, on fatty acids methyl esters (FAMEs) yield are investigated. The yield of FAME extracted into the hexane from the wet microalgae is increased 6-fold after the transesterification of lipids. The yield of FAME obtained through EHMT of lipids in wet microalgae is comparable to that obtained through direct transesterification of dried microalgae biomass with chloroform; however, FAME content in crude biodiesel obtained through EHMT is 86.74%, while that in crude biodiesel obtained through the chloroform-based process is 75.93%. EHMT ensures that polar pigments present in microalgae are not extracted into crude biodiesel, which leads to a 50% reduction in nitrogen content in crude biodiesel.

**Keywords:** Wet microalgae; Biodiesel; Hexane; Transesterification; Microwave


Aim of this work is to introduce an alternative to the standard biodiesel production chain, presenting an innovative in situ system. It is based on the chemical conversion of vegetable oil from oleaginous crops in synergy with the gasification of the protein cake disposed by the seed press. The syngas from the gasifier is here used to produce electrical power while part of it is converted into methanol. The methanol is finally used to transform the vegetable oil into biodiesel. Through a coupled use of ASPEN PLUS™ and MATLAB™ codes, a rapeseed, soy and sunflower rotation, with a duration of three year, was simulated considering 15 ha of soil. This surface resulted sufficient to feed a 7 kWel power plant. Simulation outputs proven the system to be self-sustainable. In addition, economical NPV of the investment is presented. Finally the environmental, economical and social advantages related to this approach are discussed.
Keywords: Crop rotation; Gasification; Biodiesel; Syngas; Methanol

D. Hernández\textsuperscript{a}, M. Solana\textsuperscript{b}, B. Riaño\textsuperscript{a}, M.C. García-González\textsuperscript{a}, A. Bertucco\textsuperscript{b}. \textsuperscript{a}Agricultural Technological Institute of Castilla y León, Ctra. Burgos, km. 119, 47071 Valladolid, Spain, \textsuperscript{b}Department of Industrial Engineering, University of Padova, Via Marzolo 9, 35131 Padova, Italy). Biofuels from microalgae: Lipid extraction and methane production from the residual biomass in a biorefinery approach. Bioresource Technology, Volume 170(2014): 370–378

Renewable fuels and energy are of major concern worldwide and new raw materials and processes for its generation are being investigated. Among these raw materials, algae are a promising source of lipids and energy. Thus, in this work four different algae have been used for lipid extraction and biogas generation. Lipids were obtained by supercritical CO\textsubscript{2} extraction (SCCO\textsubscript{2}), while anaerobic digestion of the lipid-exhausted algae biomass was used for biogas production. The extracted oil composition was analyzed (saturated, monounsaturated and polyunsaturated fatty acids) and quantified. The highest lipid yields were obtained from \textit{Tetraselmis} sp. (11\%) and \textit{Scenedesmus almeriensis} (10\%), while the highest methane production from the lipid-exhausted algae biomass corresponded to \textit{Tetraselmis} sp. (236 mL CH\textsubscript{4}/g VS\textsubscript{added}).

Keywords: Microalgae; Lipid extraction; Supercritical carbon dioxide; Anaerobic digestion; Biorefinery

Hongqin Wu\textsuperscript{a,c}, Xiaoling Miao\textsuperscript{a,b,c}. \textsuperscript{a}State Key Laboratory of Microbial Metabolism and School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China, \textsuperscript{b}State Key Laboratory of Motor Vehicle Biofuel Technology, Nanyang 473000, China, \textsuperscript{c}Biomass Energy Research Center, Shanghai Jiao Tong University, Shanghai 200240, China). Biodiesel quality and biochemical changes of microalgae \textit{Chlorella pyrenoidosa} and \textit{Scenedesmus obliquus} in response to nitrate levels. Bioresource Technology, Volume 170(2014): 421–427

Biodiesel quality associated with biochemical components of \textit{Chlorella pyrenoidosa} and \textit{Scenedesmus obliquus} under different nitrate levels were investigated. The highest lipid contents of 54.5\% for \textit{C. pyrenoidosa} and 47.7\% for \textit{S. obliquus} were obtained in nitrate absence. Carbohydrate peaked at 0.3 g L\textsuperscript{-1} with values of 40.7\% for \textit{C. pyrenoidosa} and 42.5\% for \textit{S. obliquus}. Protein content seemed species dependent, which decreased substantially to 11.2\% in \textit{C. pyrenoidosa} and 8.8\% in \textit{S. obliquus} under nitrate absence in present research. Better biodiesel quality (e.g. cetane number >58, iodine value <69) could be obtained from \textit{C. pyrenoidosa} in nitrate absence and \textit{S. obliquus} in 0.3 g L\textsuperscript{-1}, where the highest saturated fatty acids (39.5 for \textit{C. pyrenoidosa}, 31.2 for \textit{S. obliquus}) and the lowest unsaturated fatty acids (60.5 for \textit{C. pyrenoidosa}, 68.8 for \textit{S. obliquus}) were obtained. These results suggest that microalgae grown in the presence of nitrogen may limit biodiesel quality.

Keywords: \textit{Chlorella pyrenoidosa}; \textit{Scenedesmus obliquus}; Nitrate levels; Fatty acid compositions; Biodiesel quality
Sundaravadivelnathan Ponnesamy\textsuperscript{a}, Harvind Kumar Reddy\textsuperscript{a}, Tapaswy Muppaneni\textsuperscript{a}, Cara Meghan Downes\textsuperscript{b}, Shuguang Deng\textsuperscript{a} \textsuperscript{a} Chemical & Materials Engineering Department, New Mexico State University, Las Cruces, NM 88003, USA; \textsuperscript{b} Economics, Applied Statistics \& International Business Department, New Mexico State University, Las Cruces, NM 88003, USA). Life cycle assessment of biodiesel production from algal bio-crude oils extracted under subcritical water conditions. Bioresource Technology, Volume 170(2014): 454–461

A life cycle assessment study is performed for the energy requirements and greenhouse gas emissions in an algal biodiesel production system. Subcritical water (SCW) extraction was applied for extracting bio-crude oil from algae, and conventional transesterification method was used for converting the algal oil to biodiesel. 58 MJ of energy is required to produce 1 kg of biodiesel without any co-products management, of which 36\% was spent on cultivation and 56\% on lipid extraction. SCW extraction with thermal energy recovery reduces the energy consumption by 3–5 folds when compared to the traditional solvent extraction. It is estimated that 1 kg of algal biodiesel fixes about 0.6 kg of CO\textsubscript{2}. An optimized case considering the energy credits from co-products could further reduce the total energy demand. The energy demand for producing 1 kg of biodiesel in the optimized case is 28.23 MJ.

\textbf{Keywords:} Life cycle assessment; LCA; Subcritical water extraction; Biodiesel; Greenhouse gas emissions

Wassa Tongprawhan, Sirasit Srinuanpan, Benjamas Cheirsilp. (Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand). Biocapture of CO\textsubscript{2} from biogas by oleaginous microalgae for improving methane content and simultaneously producing lipid. Bioresource Technology, Volume 170(2014): 90–99

This study aimed to use oleaginous microalgae to capture CO\textsubscript{2} from biogas for improving methane content and simultaneously producing lipid. Several microalgae were screened for their ability to grow and produce lipid using CO\textsubscript{2} in biogas. A marine \textit{Chlorella} sp. was the most suitable strain for capturing CO\textsubscript{2} and producing lipid using biogas (50\% v/v CO\textsubscript{2} in methane) as well as using 50\% v/v CO\textsubscript{2} in air. The medium and operating conditions were optimized through response surface methodology (RSM). The optimal concentrations of KNO\textsubscript{3} and K\textsubscript{2}HPO\textsubscript{4} were 0.80 g L\textsuperscript{-1} and 0.06 g L\textsuperscript{-1}, respectively. The optimal operating conditions were: initial pH of 7.8, initial cell concentration of 10\textsuperscript{7.5} cells mL\textsuperscript{-1}, light intensity of 4500 lux and gas flow rate of 0.03 L min\textsuperscript{-1}. After optimization, 89.3\% of CO\textsubscript{2} was removed from biogas and the methane content was increased up to 94.7\%. The lipid productivity was 94.7 mg L\textsuperscript{-1} day\textsuperscript{-1}.

\textbf{Keywords:} Biogas; CO\textsubscript{2} capture; Lipid; Methane; Oleaginous microalgae

Dang P. Ho\textsuperscript{a}, Huu Hao Ngo\textsuperscript{b}, Wenshan Guo\textsuperscript{b}. (\textsuperscript{a} Advanced Water Management Centre, School of Chemical Engineering, University of Queensland, Brisbane, Australia, \textsuperscript{b} Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology, Sydney, P.O. Box 123, Broadway, NSW 2007, Australia). A mini review on renewable sources for biofuel. Bioresource Technology, Volume 169(2014): 742–749
Rapid growth in both global energy demand and carbon dioxide emissions associated with the use of fossil fuels has driven the search for alternative sources which are renewable and have a lower environmental impact. This paper reviews the availability and bioenergy potentials of the current biomass feedstocks. These include (i) food crops such as sugarcane, corn and vegetable oils, classified as the first generation feedstocks, and (ii) lignocellulosic biomass derived from agricultural and forestry residues and municipal waste, as second generation feedstocks. The environmental and socioeconomic limitations of the first generation feedstocks have placed greater emphasis on the lignocellulosic biomass, of which the conversion technologies still faces major constraints to full commercial deployment. Key technical challenges and opportunities of the lignocellulosic biomass-to-bioenergy production are discussed in comparison with the first generation technologies. The potential of the emerging third generation biofuel from algal biomass is also reviewed.

Keywords: Bioenergy; Agricultural residues; Organic wastes; Biomass; Energy crops

Haiying Yu\textsuperscript{a}, Hongjun Lin\textsuperscript{a}, Meijia Zhang\textsuperscript{a}, Huachang Hong\textsuperscript{a}, Yiming He\textsuperscript{b}, Fangyuan Wang\textsuperscript{a}, Leihong Zhao\textsuperscript{c}. \textsuperscript{a}College of Geography and Environmental Sciences, Zhejiang Normal University, Jinhua 321004, PR China, \textsuperscript{b}Department of Materials Physics, Zhejiang Normal University, Jinhua 321004, PR China, \textsuperscript{c}Institute of Physical Chemistry, Zhejiang Key Laboratory for Reactive Chemistry on Solid Surfaces, Zhejiang Normal University, Jinhua 321004, PR China). Membrane fouling in a submerged membrane bioreactor with focus on surface properties and interactions of cake sludge and bulk sludge. Bioresource Technology, Volume 169(2014): 213–219

In this study, the fouling behaviors and surface properties of cake sludge and bulk sludge in a submerged membrane bioreactor (MBR) were investigated and compared. It was found that the specific filtration resistance (SFR) of cake sludge was about 5 times higher than that of bulk sludge. Two types of sludge possessed similar extracellular polymeric substances (EPS) content, particle size distribution (PSD) and zeta potential. However, their surface properties in terms of surface tensions were significantly different. Further analysis showed that cake sludge was more hydrophilic and had worse aggregation ability. Moreover, cake sludge surface possessed more hydrocarbon, less oxygen and nitrogen moieties than bulk sludge surface. It was suggested that, rather than EPS and PSD differences, the differences in the surface composition were the main cause of the great differences in SFR and adhesion ability between cake sludge and bulk sludge.

Keywords: Membrane fouling; Specific filtration resistance; Surface tension; Interaction energy

Xumeng Ge\textsuperscript{a}, Tracie Matsumoto\textsuperscript{b}, Lisa Keith\textsuperscript{b}, Yebo Li\textsuperscript{a}. \textsuperscript{a}Department of Food, Agricultural and Biological Engineering, The Ohio State University/Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691-4096, USA, \textsuperscript{b}USDA, ARS, DKI US PBARC, Plant Genetic Resources and Disease Research, 64 Nowelo Street, Hilo, HI 96720, USA). Biogas energy production from tropical biomass wastes by anaerobic digestion. Bioresource Technology, Volume 169(2014): 38–44

Anaerobic digestion (AD) is an attractive technology in tropical regions for converting locally abundant biomass wastes into biogas which can be used to produce heat, electricity, and transportation fuels. However, investigations on AD of tropical forestry wastes, such as albizia
biomass and food wastes, such as taro, papaya, and sweet potato, are limited. In this study, these tropical biomass wastes were evaluated for biogas production by liquid AD (L-AD) and/or solid-state AD (SS-AD), depending on feedstock characteristics. When albizia leaves and chips were used as feedstocks, L-AD had greater methane yields (161 and 113 L kg$^{-1}$ VS, respectively) than SS-AD (156.8 and 59.6 L kg$^{-1}$ VS, respectively), while SS-AD achieved 5-fold higher volumetric methane productivity than L-AD. Mono-digestion and co-digestion of taro skin, taro flesh, papaya, and sweet potato achieved methane yields from 345 to 411 L kg$^{-1}$ VS, indicating the robustness of AD technology.

**Keywords:** Tropical; Biomass waste; Anaerobic digestion; Biogas; Albizia

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The aim of this study was to investigate the specific methane production and the energy balance at a small farm scaled mesophilic biogas plant in a cold climate area. The main substrate was dairy cow slurry. Fish silage was used as co-substrate for two of the three test periods. Energy production, substrate volumes and thermal and electric energy consumption was monitored.

Methane production depended mainly on type and amount of substrates, while energy consumption depended mainly on the ambient temperature. During summer the main thermal energy consumption was caused by heating of new substrates, while covering for thermal energy losses from digester and pipes required most thermal energy during winter. Fish silage gave a total energy production of 1623 kWh/m$^3$, while the dairy cow slurry produced 79 kWh/m$^3$ slurry. Total energy demand at the plant varied between 26.9% and 88.2% of the energy produced.

**Keywords:** Biogas; Dairy cow slurry; Fish silage; Energy balance

**Toyokazu Miura**$^{a,c}$, **Akihisa Kita**$^{a,c}$, **Yoshiko Okamura**$^{a,c}$, **Tsunehiro Akî**$^{a,c}$, **Yukihiko Matsumura**$^{b,c}$, **Takahisa Tajima**$^{a,c}$, **Junichi Kato**$^{a}$, **Yutaka Nakashimada**$^{a,c}$ ($^{a}$ Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8530, Japan, $^{b}$ Division of Energy and Environmental Engineering, Institute of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima 739-8527, Japan, $^{c}$ CREST, JST, Japan). Evaluation of marine sediments as microbial sources for methane production from brown algae under high salinity. Bioresource Technology, Volume 169(2014): 362–366

Various marine sediments were evaluated as promising microbial sources for methane fermentation of *Saccharina japonica*, a brown alga, at seawater salinity. All marine sediments tested produced mainly acetate among volatile fatty acids. One marine sediment completely converted the produced volatile fatty acids to methane in a short period. Archaeal community analysis revealed that acetoclastic methanogens belonging to the *Methanosarcina* genus dominated after cultivation. Measurement of the specific conversion rate at each step of methane
production under saline conditions demonstrated that the marine sediments had higher conversion rates of butyrate and acetate than mesophilic methanogenic granules. These results clearly show that marine sediments can be used as microbial sources for methane production from algae under high-salt conditions without dilution.

**Keywords:** Brown algae; Marine macroalgae; Marine sediments; Methane production; *Saccharina japonica*

Xu Xu a, Ji Young Kim b, Yu Ri Oh b, Jong Moon Park a,b,c. ( a School of Environmental Science and Engineering, Pohang University of Science and Technology, San 31, Hyoja-dong, Pohang 790-784, South Korea, b Department of Chemical Engineering, Pohang University of Science and Technology, San 31, Hyoja-dong, Pohang 790-784, South Korea, c Division of Advanced Nuclear Engineering, Pohang University of Science and Technology, San 31, Hyoja-dong, Pohang 790-784, South Korea). Production of biodiesel from carbon sources of macroalgae, *Laminaria japonica*. Bioresource Technology, Volume 169: (2014): 455–461

As aquatic biomass which is called “the third generation biomass”, *Laminaria japonica* (also known as *Saccharina japonica*) consists of mannitol and alginate which are the main polysaccharides of algal carbohydrates. In this study, oleaginous yeast (*Cryptococcus curvatus*) was used to produce lipid from carbon sources derived from *Laminaria japonica*. Volatile fatty acids (VFAs) were produced by fermentation of alginate extracted from *L. japonica*. Thereafter, mannitol was mixed with VFAs to culture the oleaginous yeast. The highest lipid content was 48.30%. The composition of the fatty acids was similar to vegetable oils. This is the first confirmation of the feasibility of using macroalgae as a carbon source for biodiesel production.

**Keywords:** *Laminaria japonica*; *Cryptococcus curvatus*; Volatile fatty acids (VFAs); Fatty acid methyl esters (FAMEs); Biodiesel


Dried milled biomass samples are frequently utilised in small-scale batch digestion tests. However, herbage chemical composition can be altered by thermal drying, and this may affect specific methane (CH$_4$) yields. Thus, the specific CH$_4$ yield of herbage pre- and post-ensiling, prepared by two preparation methods were compared. Perennial ryegrass samples were either non-thermally dried (i.e. subject to cryogenic conditions, −196 °C) or thermally dried (40 °C), prior to milling. Specific CH$_4$ yield was subsequently determined in a small-scale batch digestion test. Herbage pre-ensiling yielded 204 and 243 L CH$_4$ kg$^{-1}$ VS$_{added}$ and herbage post-ensiling yielded 212 and 188 L CH$_4$ kg$^{-1}$ VS$_{added}$ with non-thermal dried and thermal dried sample preparation methods, respectively. Due to opposing effects of thermal drying on CH$_4$ yields of herbage either pre- or post-ensiling, it is not recommended to use thermal drying.
Instead, it is recommended that non-thermal dried herbage samples are used in small-scale batch digestion tests.

**Keywords:** Grass; Silage; Thermal drying; Anaerobic digestion; Methane


This study concerns in-house development of cellulases from a mutant *Penicillium janthinellum* EMS-UV-8 and its application in separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) processes for bioethanol production from pre-treated wheat straw. In a 5 L fermentor, the above strain could produce cellulases having activity of 3.1 FPU/mL and a specific activity of 0.83 FPU/mg of protein. In-house developed cellulase worked more efficiently in case of SSF as ethanol concentration of 21.6 g/L and yield of 54.4% were obtained which were higher in comparison to SHF (ethanol concentration 12 g/L and 30.2% yield). This enzyme preparation when compared with commercial cellulase for hydrolysis of pre-treated wheat straw was found competitive. This study demonstrates that *P. janthinellum* EMS-UV-8 is a potential fungus for future large-scale production of cellulases.

**Keywords:** Cellulase; *Penicillium*; Bioethanol; SHF; SSF


Alkaline pretreatment was studied to analyze the influence on waste activated sludge (WAS) reduction, methane production and microbial community structure during anaerobic digestion. Methane production from alkaline pretreated sludge (A-WAS) (pH = 12) increased from 251.2 mL/L d to 362.2 mL/L d with the methane content of 68.7% compared to raw sludge (R-WAS). Sludge reduction had been improved, and volatile suspended solids (VSS) removal rate and protein reduction had increased by ~10% and ~35%, respectively. The bacterial and methanogenic communities were analyzed using 454 pyrosequencing and clone libraries of 16S rRNA gene. Remarkable shifts were observed in microbial community structures after alkaline pretreatment, especially for *Archaea*. The dominant methanogenic population changed from *Methanosaeta* for R-WAS to *Methanosarcina* for A-WAS. In addition to the enhancement of solubilization and hydrolysis of anaerobic digestion of WAS, alkaline pretreatment showed significant impacts on the enrichment and syntrophic interactions between microbial communities.

**Keywords:** Waste activated sludge (WAS); Alkaline pretreatment; Methane production; Microbial community; 454 pyrosequencing

Francesco G. Gentili. (Department of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden). Microalgal biomass and lipid

The aim of the study was to grow microalgae on mixed municipal and industrial wastewater to simultaneously treat the wastewater and produce biomass and lipids. All algal strains grew in all wastewater mixtures; however, *Selenastrum minutum* had the highest biomass and lipids yields, up to 37% of the dry matter. Nitrogen and phosphorus removal were high and followed a similar trend in all three strains. Ammonium was reduced from 96% to 99%; this reduction was due to algal growth and not to stripping to the atmosphere, as confirmed by the amount of nitrogen in the dry algal biomass. Phosphate was reduced from 91% to 99%. In all strains used the lipid content was negatively correlated to the nitrogen concentration in the algal biomass. Mixtures of pulp and paper wastewater with municipal and dairy wastewater have great potential to grow algae for biomass and lipid production together with effective wastewater treatment.

**Keywords:** Algae; Nitrogen removal; Phosphorus removal; Total lipids; Wastewater

Thomas Schmidt\textsuperscript{a,b}, Michael Nelles\textsuperscript{a,b}, Frank Scholwin\textsuperscript{b}, Jürgen Pröter\textsuperscript{a}. (\textsuperscript{a} Department of Biochemical Conversion, Deutsches Biomasseforschungszentrum gemeinnützige GmbH, Leipzig 04347, Germany, \textsuperscript{b} Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock 18059, Germany). Trace element supplementation in the biogas production from wheat stillage – Optimization of metal dosing. Bioresource Technology, Volume 168(2014): 80–85

A trace element dosing strategy for the anaerobic digestion of wheat stillage was developed in this study. Mesophlic CSTR reactors were operated with the sulfuric substrate wheat stillage in some cases under trace element deficiency. After supplementing trace elements during the start-up, one of the elements of Fe, Ni, Co, Mo, and W were depleted in one digester while still augmenting the other elements to determine minimum requirements for each element. The depletion of Fe and Ni resulted in a rapid accumulation of volatile fatty acids while Co and W seem to have a long-term effect. Based on the results it was possible to reduce the dosing of trace elements, which is positive with reference to economic and environmental aspects.

**Keywords:** Anaerobic digestion; Biogas; Trace elements

Qiyong Xu\textsuperscript{a}, Xiao Jin\textsuperscript{a}, Zeyu Ma\textsuperscript{a}, Huchun Tao\textsuperscript{b}, Jae Hac Ko\textsuperscript{b}. (\textsuperscript{a} Key Laboratory for Eco-efficient Polysilicate Materials, School of Environment and Energy, Peking University Shenzhen Graduate School, Guangdong 518055, China, \textsuperscript{b} Key Laboratory for Heavy Metal Pollution Control and Reutilization, School of Environment and Energy, Peking University Shenzhen Graduate School, Guangdong 518055, China). Methane production in simulated hybrid bioreactor landfill. Bioresource Technology, Volume 168(2014): 92–96

The aim of this work was to study a hybrid bioreactor landfill technology for landfill methane production from municipal solid waste. Two laboratory-scale columns were operated for about ten months to simulate an anaerobic and a hybrid landfill bioreactor, respectively. Leachate was recirculated into each column but aeration was conducted in the hybrid bioreactor during the first stage. Results showed that leachate pH in the anaerobic bioreactor maintained below 6.5, while in the hybrid bioreactor quickly increased from 5.6 to 7.0 due to the aeration. The temporary
aeration resulted in lowering COD and BOD₃ in the leachate. The volume of methane collected from the hybrid bioreactor was 400 times greater than that of the anaerobic bioreactor. Also, the methane production rate of the hybrid bioreactor was improved within a short period of time. After about 10 months’ operation, the total methane production in the hybrid bioreactor was 212 L (16 L/kg waste).

**Keywords:** Bioreactor landfill; Aeration; Leachate recirculation; Methane production

Four 15-L lab-scale continuous stirred tank reactors were operated under mesophilic conditions to investigate the effect of ammonia inhibition. Stable isotope fingerprinting of biogas was applied as a process monitoring tool. Ammonia inhibition was initiated by amendment of chicken manure to maize silage fed reactors. During the accumulation of ammonia, the concentration of volatile fatty acids increased while the biogas production and pH decreased. However, in one reactor, an inhibited steady state with stable gas production even at high ammonia levels was achieved, while the other reactor proceeded to complete process failure. A depletion of the δ¹³CH₄ and δ¹³CO₂ values preceded the process inhibition. Moreover, the stable isotope composition of biogas also forecasted the complete process failure earlier than other standard parameters. The stable isotope analyses of biogas have a potential for mechanistic insights in anaerobic processes, and may be used to pre-warn process failure under stress conditions.

**Keywords:** Biogas; Process stability; Ammonia inhibition; Stable isotope fingerprinting

In the present work four algae were tested for their biomass production potential in neat livestock wastewater. *Chroococcus* sp.1 was found to be the best for biomass production under controlled (2.13 g L⁻¹) and outdoor conditions (4.44 g L⁻¹) with >80% of nutrients removal. The produced biomass was then digested with cattle dung as cosubstrate. Interestingly, up to 291.83 ± 3.904 mL CH₄ g⁻¹ VS_fed was produced during codigestion studies (C/N ≈ 13.0/1). In contrast to this, only 202.49 ± 11.19 and 141.70 ± 2.57 mL CH₄ g⁻¹ VS_fed was recorded with
algae (C/N ≈ 9.26/1) and cattle dung (C/N ≈ 31.56/1) alone, respectively. The estimated renewable power generation potential of the investigated coupled process was around 333.79–576.57 kWh d⁻¹ for a dairy farm with 100 adult cattle. However, further scale-up and testing is needed to make this process a reality.

**Keywords:** Livestock wastewater; Biomethane; Algae; Cattle dung; Codigestion


This study evaluates the production of biodiesel and ethanol from spent coffee grounds (SCG). The extraction of oil from SCG, biodiesel production and ethanol production processes were studied. The liquid-to-solid ratio and temperature were evaluated in the ultrasound-assisted extraction of the oil from SCG. The highest yield (12%) was obtained using 4 mL g⁻¹ liquid-to-solid ratio at 60 °C for 45 min. The process to produce biodiesel showed a yield of 97% into fatty acid methyl esters (FAME). The highest glucose yield (192 mg g⁻¹ SCG⁻¹) was obtained by hydrolysis with 0.4 mol L⁻¹ sulfuric acid at 121 °C for 15 min. The hydrolysate was used as fermentation medium for ethanol production by *Saccharomyces cerevisiae* obtaining 19.0 g L⁻¹ at 10 h of process of ethanol with a yield of ethanol and productivity of 0.50 g g⁻¹ and 1.90 g L⁻¹ h⁻¹, respectively. Spent coffee grounds were considered a potential feedstock for biodiesel and ethanol production.

**Keywords:** Spent coffee ground; Ethanol; Biodiesel; Ultrasound; Fatty acid

Hai-Bo Shen⁵, Xiao-Yu Yong⁵, Yi-Lu Chen⁵, Zhi-Hong Liao⁶, Rong-Wei Si⁶, Jun Zhou⁵, Shu-Ya Wang⁵, Yang-Chun Yong⁵, Ping-Kai OuYang⁵, Tao Zheng⁵. (⁵ College of Biotechnology and Pharmaceutical Engineering, Nanjing TECH University, Nanjing 210009, China, ⁶ Bioenergy Research Institute, Nanjing TECH University, Nanjing 210009, China, Biofuels Institute, School of the Environment, Jiangsu University, Zhenjiang 212013, China). Enhanced bioelectricity generation by improving pyocyanin production and membrane permeability through sophorolipid addition in *Pseudomonas aeruginosa*-inoculated microbial fuel cells. Bioresource Technology, Volume 167(2014): 490–494

Improvement on electron shuttle-mediated extracellular electron transfer (EET) is of great potential to enhance the power output of MFCs. In this study, sophorolipid was added to enhance the performance of *Pseudomonas aeruginosa*-inoculated MFC by improving the electron shuttle-mediated EET. Upon sophorolipid addition, the current density and power density increased ~ 1.7 times and ~ 2.6 times, respectively. In accordance, significant enhancement on pyocyanin production (the electron shuttle) and membrane permeability were observed. Furthermore, the conditions for sophorolipid addition were optimized to achieve maximum pyocyanin production.
(14.47 ± 0.23 µg/mL), and 4 times higher power output was obtained compared to the control. The results substantiated that enhanced membrane permeability and pyocyanin production by sophorolipid, which promoted the electron shuttle-mediated EET, underlies the improvement of the energy output in the P. aeruginosa-inoculated MFC. It suggested that addition of biosurfactant could be a promising way to enhance the energy generation in MFCs.

**Keywords:** Microbial fuel cell (MFC); Sophorolipid; Pyocyanin; *Pseudomonas aeruginosa*; Permeability

Yubin Ma¹, Zhiyao Wang¹, Changjiang Yu, Yehu Yin, Gongke Zhou. (Key Laboratory of Biofuels, Shandong Provinical Key Laboratory of Energy Genetics, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China). Evaluation of the potential of 9 *Nannochloropsis* strains for biodiesel production. Bioresource Technology, Volume 167(2014): 503–509

*Nannochloropsis* have attracted sustained interest from algal biodiesel researchers due to their high biomass accumulation rate and high lipid content. There are six recognized species in the *Nannochloropsis* genus that are phylogenetically divided into *Nannochloropsis gaditana*, *Nannochloropsis salina*, *Nannochloropsis granulata*, *Nannochloropsis limnetica*, *Nannochloropsis oceanica* and *Nannochloropsis oculata*. In this study, the potential of 9 *Nannochloropsis* species from the 6 genus for biodiesel production was evaluated by determining their growth rate, biomass accumulation, lipid productivity, lipid composition, fatty acid profiles and biodiesel properties. The results showed that the best strain was *N. oceanica* IMET1, with lipid productivity of 158.76 ± 13.83 mg L⁻¹ day⁻¹, TAG production of 1.67 ± 0.20 g/L, favorable fatty acid profiles of C16–C18 (56.62 ± 1.96%) as well as suitable biodiesel properties of higher cetane number (54.61 ± 0.25), lower iodine number (104.85 ± 2.80 gI²/100 g) and relative low cloud point (3.45 ± 0.50 °C). *N. oceanica* IMET1 could be consider as valuable feedstock for microalgal biodiesel production.

**Keywords:** Biodiesel quality; Fatty acid profiles; Lipid productivity; *Nannochloropsis*; Microalgae


This study proposes a method to produce biodiesel from wet wastewater sludge. Xylene was used as an alternative cosolvent to hexane for transesterification in order to enhance the biodiesel yield from wet wastewater sludge. The water present in the sludge could be separated during transesterification by employing xylene, which has a higher boiling point than water. Xylene enhanced the biodiesel yield up to 8.12%, which was 2.5 times higher than hexane. It was comparable to the maximum biodiesel yield of 9.68% obtained from dried sludge. Xylene could reduce either the reaction time or methanol consumption, when compared to hexane for a similar yield. The fatty acid methyl esters (FAMEs) content of the biodiesel increased approximately two fold by changing the cosolvent from hexane to xylene. The transesterification method using
xylene as a cosolvent can be applied effectively and economically for biodiesel recovery from wet wastewater sludge without drying process.

**Keywords:** Biodiesel; Wet wastewater sludge; Cosolvent; *In situ* transesterification; Xylene

Zhen Wang¹, Zhe Lv¹, Jiiliang Du, Chunling Mo, Xiushan Yang, Shen Tian. (College of Life Science, Capital Normal University, Beijing 100048, China). Combined process for ethanol fermentation at high-solids loading and biogas digestion from unwashed steam-exploded corn stover. Bioresource Technology, Volume 166(2014): 282–287

A combined process was designed for the co-production of ethanol and methane from unwashed steam-exploded corn stover. A terminal ethanol titer of 69.8 g/kg mass weight (72.5%) was achieved when the fed-batch mode was performed at a final solids loading of 35.5% (w/w) dry matter (DM) content. The whole stillage from high-solids ethanol fermentation was directly transferred in a 3-L anaerobic digester. During 52-day single-stage digester operation, the methane productivity was 320 mL CH₄/g volatile solids (VS) with a maximum VS reduction efficiency of 55.3%. The calculated overall product yield was 197 g ethanol + 96 g methane/kg corn stover. This indicated that the combined process was able to improve overall content utilization and extract a greater yield of lignocellulosic biomass compared to ethanol fermentation alone.

**Keywords:** High solids fed batch SSF; Anaerobic digestion; Ethanol and methane co-production; Mass balance

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The biomethane yield of various energy crops, selected among potential alternatives to maize in the Greater Region, was assessed. The biomass yield, the volatile solids (VS) content and the biochemical methane potential (BMP) were measured to calculate the biomethane yield per
hectare of all plant species. For all species, the dry matter biomass yield and the VS content were the main factors that influence, respectively, the biomethane yield and the BMP. Both values were predicted with good accuracy by linear regressions using the biomass yield and the VS as independent variable. The perennial crop Miscanthus appeared to be the most promising alternative to maize when harvested as green matter in autumn and ensiled. Miscanthus reached a biomethane yield of $5.5 \pm 1 \times 10^3 \text{ m}^3 \text{ ha}^{-1}$ during the second year after the establishment, as compared to $5.3 \pm 1 \times 10^3 \text{ m}^3 \text{ ha}^{-1}$ for maize under similar crop conditions.

**Keywords:** Biochemical methane potential (BMP); Lignocellulose; Anaerobic digestion; Biomethanation; Energy crops

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The hemicelluloses fraction of black liquor is an underutilized resource in many chemical pulp mills. It is possible to extract and separate the lignin and hemicelluloses from the black liquor and use the hemicelluloses for biochemical conversion into biofuels and chemicals. Precipitation of the lignin from the black liquor would consequently decrease the thermal load on the recovery boiler, which is often referred to as a bottleneck for increased pulp production. The objective of this work is to techno-economically evaluate the production of sodium-free lignin as a solid fuel and butanol to be used as fossil gasoline replacement by fractionating black liquor. The hydrolysis and fermentation processes are modeled in Aspen Plus to analyze energy and material balances as well as to evaluate the plant economics. A mathematical model of an existing pulp and paper mill is used to analyze the effects on the energy performance of the mill subprocesses.

**Keywords:** Techno-economic analysis; Black liquor fractionation; Butanol fermentation; Process integration; Pulp and paper mill

Gozde Duman\textsuperscript{a}, Md. Azhar Uddin\textsuperscript{b}, Jale Yanik\textsuperscript{a}. (\textsuperscript{a} Faculty of Science, Department of Chemistry, Izmir Institute of Technology, 35430 Urla, Izmir, Turkey, \textsuperscript{b} Department of Environmental Chemistry and Materials, Okayama University, 3-1-1 Tsushima Naka, Okayama 700-8530, Japan). Hydrogen production from algal biomass via steam gasification. Bioresource Technology, Volume 166(2014): 24–30

Algal biomasses were tested as feedstock for steam gasification in a dual-bed microreactor in a two-stage process. Gasification experiments were carried out in absence and presence of catalyst. The catalysts used were 10% Fe\textsubscript{2}O\textsubscript{3}–90% CeO\textsubscript{2} and red mud (activated and natural forms). Effects of catalysts on tar formation and gasification efficiencies were comparatively investigated. It was observed that the characteristic of algae gasification was dependent on its components and the catalysts used. The main role of the catalyst was reforming of the tar derived from algae pyrolysis, besides enhancing water gas shift reaction. The tar reduction levels were in the range of 80–100% for seaweeds and of 53–70% for microalgae. Fe\textsubscript{2}O\textsubscript{3}–CeO\textsubscript{2} was found to be the most effective catalyst. The maximum hydrogen yields obtained were 1036 cc/g algae for *Fucus serratus*, 937 cc/g algae for *Laminaria digitata* and 413 cc/g algae for *Nannochloropsis oculata*. 

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**Keywords:** Algae; Steam gasification; Hydrogen; Iron catalyst


This study investigated the potential of enhancing the activity of iron-reducing bacteria (IRBs) to increase the biomethanation rate of waste activate sludge (WAS). The effects of biostimulation by ferric oxyhydroxide (Phase 2) and bioaugmentation with an enriched IRB consortium (Phase 3) were examined in a continuous anaerobic reactor treating WAS. Compared to the control operation (Phase 1), significant rises in methane yield (10.8–59.4%) and production rate (24.5–52.9%) were demonstrated by the biostimulation and bioaugmentation treatments. Visible structural changes were observed in bacterial community with the phases while not in archaeal community. Acinetobacter- and Spirochaetales-related populations were likely the major players driving anaerobic iron respiration and thus leading to enhanced biomethanation performance, in Phases 2 and 3, respectively. Our results suggest an interesting new potential for enhancing biomethanation of WAS.

**Keywords:** Biomethanation; Iron oxyhydroxide; Iron-reducing bacteria; Microbial community structure; Waste activated sludge

A. Arumugam*, M. Sandhya, V. Ponnusami†. (School of Chemical & Biotechnology, SASTRA University, Thirumalaisamudram, Thanjavur 613 401, India). Biohydrogen and polyhydroxyalkanoate co-production by Enterobacter aerogenes and Rhodobacter sphaeroides from Calophyllum inophyllum oil cake. Bioresource Technology, Volume 164(2014): 170–176

The feasibility of coupled biohydrogen and polyhydroxyalkanoate production by Enterobacter aerogenes and Rhodobacter sphaeroides using Calophyllum inophyllum oil cake was studied under dark and photo fermentation conditions. The utilization of a non-edible acidic oil cake (C. inophyllum), and exploitation of a modified minimal salt media led to reduction in the cost of media. Cost of fermentation is reduced by implementation of alternate dark-photo fermentative periods and through the use of a co-culture consisting of a dark fermentative (E. aerogenes) and a photo fermentative (R. sphaeroides) bacterium. The biohydrogen and polyhydroxyalkanoate produced were 7.95 L H$_2$/L media and 10.73 g/L media, respectively, under alternate dark and photo fermentation and were 3.23 L H$_2$/L media and 5.6 g/L media, respectively under complete dark fermentation. The characteristics of the oil cake and alternate dark (16 h) and photo (8 h) fermentative conditions were found to be supportive in producing high biohydrogen and polyhydroxyalkanoate (PHA) yield.

**Keywords:** Biohydrogen; Polyhydroxyalkanoate; Dark fermentation; Photo fermentation; Calophyllum inophyllum oil cake

Jinlan Cheng$^a$,$^b$, Shao-Yuan Leu$^{c,}$,$^b$, J.Y. Zhu$^{b,}$,$^d$, Thomas W. Jeffries$^e$. (a Jiangsu Provincial Key Lab of Pulp and Paper Science and Technology, Nanjing Forestry University, Nanjing,
Sulfite pretreatment to overcome the recalcitrance of lignocelluloses (SPORL) was applied to an empty fruit bunches (EFB) for ethanol production. SPORL facilitated delignification through lignin sulfonation and dissolution of xylan to result in a highly digestible substrate. The pretreated whole slurry was enzymatically saccharified at a solids loading of 18% using a relatively low cellulase loading of 15 FPU/g glucan and simultaneously fermented without detoxification using *Saccharomyces cerevisiae* of YRH400. An ethanol yield of 217 L/tonne EFB was achieved at titer of 32 g/L. Compared with literature studies, SPORL produced high ethanol yield and titer with much lower cellulase loading without detoxification.

**Keywords:** Empty fruit bunches (EFB); Pretreatment; High solids Enzymatic hydrolysis/saccharification; Fermentation; High titer ethanol

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**Nano Biotechnology**


In this paper the preparation of chitosan nanoparticles was carried out using methacrylic acid (MAA) and studied by both ultraviolet (UV)- visible transmission spectrophotometry and transmission electron microscopy (TEM). Nanoparticles with sizes as small as 17 to 25 nm were achieved. The obtained nanoparticles had a homogeneous morphology showing a quite uniform particles size distribution with a spherical shape. The solution was pH-sensitive, due to swelling and aggregation of the nanoparticles. The use of slow release fertilizer has become a new trend to save fertilizer consumption and to reduce environmental pollution. In this work, chitosan (CS) nanoparticles were obtained for the loading of NPK fertilizers. The stability of the CS-PMAA colloidal suspension was higher with the addition of nitrogen and potassium than with the addition of phosphorus, due to the higher anion charge from the calcium phosphate than the anion charges from the potassium chloride and urea. The mean diameter increase of the CS-PMAA nanoparticles in suspension with the addition of different compounds was P > K > N which indicates that the elements are being aggregated and loaded on the surface of the chitosan nanoparticles.

**Keywords:** Nanoparticles formation, chitosan, fertilizers, nanosolution.

Aparna J. Padman¹, Janey Henderson¹, Simon Hodgson¹ and Pattanathu K. S. M. Rahman¹. (¹School of Science and Engineering, Teesside University, Middlesbrough, TS13BA, UK. Email: p.rahman@tees.ac.uk). Biomediated synthesis of silver

Biomediated silver nanoparticles were synthesized using a cell free extract of a soil bacterium, *Exiguobacterium mexicanum* PR 10.6. The silver nanoparticles were characterised using UV–Vis spectroscopy, energy dispersive spectroscopy, Fourier transform infrared spectroscopy, and transmission electron microscopy. The nanoparticles ranged from 5 to 40 nm. Extracellular polymeric substance played a critical role in the reduction of silver ion and nanoparticle stabilisation when using the cell free extract. The synthesis using *E. mexicanum* is an effective eco-friendly, rapid method for silver nanoparticle synthesis within 1 h.

**Keywords:** Biomediated synthesis, Cell-free extract, *Exiguobacterium mexicanum*, Extracellular polymeric substance, Nanoparticles, Silver nanoparticles

Nano-biotechnology is a cutting-edge field with a wide range of applications and clear industrial prospects in medicine and health. Especially drug delivery, biosensors and imaging technology, intelligent medical equipment, etc. will play an important role in the diagnosis, treatment and health care. Compared to the developed countries focusing nano-biotechnology as a 21st century research priorities, the development of nanotechnology and nanomedicine in China will also be very extensive, state 973 Program and “Strategic Priority Research Program” classify nanobiotechnology as special projects and give priority to support its development.

Over the past 5 years, three joint symposia in Nanotechnology and Nanomedicine (2008, 2010 and 2012) have been convened by the National Center for Nanoscience and Technology (NCNST), Chinese Academy of Sciences (CAS), and the National Institutes of Health (NIH), taking the important step in the development of a mutually productive relationship between Chinese and American scientists — focused on nano-biotechnology and nanomedicine and building partnerships to benefit fellow scientists and to advance the pace of scientific knowledge.

In December 2012, the third China–U.S. Symposium on Nanotechnology and Nanomedicine was hosted by NCNST in Fragrant Hills, Beijing. This meeting focused on two topics of a strong mutual interest to U.S. and Chinese researchers, including drug delivery for cancer therapy and developing new imaging tools for diagnosing cancer and/or assisting with the surgical removal of cancer tissues. During three days of discussion, not only the Chinese scientists fully understood the research progress of American scientists in the latest nanotechnology of tumor therapy, but also American counterparts were introducedf of the important achievements of China, resulting in a significant positive impact. Through this meeting, it effectively consolidates the existing mutual cooperation and exchange mechanism for researchers and further promotes the development of China–U.S. nanobiotechnology and nanomedicine.
In this Special Issue, we collect a series of reviews from several excellent participants that address important themes highlighted during the conference. It is hoped that this Special Issue would contribute to the continued better communication, understanding, and collaboration among scientists working in the different countries with the great scientific output of nanotechnology research for medical application. We sincerely thank those who have supported and participated in the symposia, and particularly those who contributed articles to this Special Issue. We also would like to thank those who have reviewed and edited the manuscripts.

Yu Gao\textsuperscript{a,1}, Jingjing Xie\textsuperscript{a,1}, Haijun Chen\textsuperscript{a,b}, Songen Gu\textsuperscript{a}, Rongli Zhao\textsuperscript{a}, Jingwei Shao\textsuperscript{a}, Lee Jia\textsuperscript{a} \textsuperscript{1} (\textsuperscript{a} Cancer Metastasis Alert and Prevention Institute, College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350002, China, \textsuperscript{b} Department of Pharmaceutical Engineering, College of Chemistry and Chemical Engineering, Fuzhou University, Fujian 350108, China). Nanotechnology-based intelligent drug design for cancer metastasis treatment. Biotechnology Advances, Volume 32 (2014): 761–777

Traditional chemotherapy used today at clinics is mainly inherited from the thinking and designs made four decades ago when the Cancer War was declared. The potency of those chemotherapy drugs on in-vitro cancer cells is clearly demonstrated at even nanomolar levels. However, due to their non-specific effects in the body on normal tissues, these drugs cause toxicity, deteriorate patient's life quality, weaken the host immunosurveillance system, and result in an irreversible damage to human's own recovery power. Owing to their unique physical and biological properties, nanotechnology-based chemotherapies seem to have an ability to specifically and safely reach tumor foci with enhanced efficacy and low toxicity. Herein, we comprehensively examine the current nanotechnology-based pharmaceutical platforms and strategies for intelligent design of new nanomedicines based on targeted drug delivery system (TDDS) for cancer metastasis treatment, analyze the pros and cons of nanomedicines versus traditional chemotherapy, and evaluate the importance that nanomaterials can bring in to significantly improve cancer metastasis treatment.

\textbf{Keywords:} Nanotechnology; Nanomedicine; Targeted drug delivery system; Cancer metastasis therapy; Nanoparticle platform

Shutao Guo, Leaf Huang: (Division of Molecular Pharmaceutics and Center for Nanotechnology in Drug Delivery, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA). Nanoparticles containing insoluble drug for cancer therapy. Biotechnology Advances, Volume 32(4) (2014): 778–788

Nanoparticle drug formulations have been extensively researched and developed in the field of drug delivery as a means to efficiently deliver insoluble drugs to tumor cells. By mechanisms of the enhanced permeability and retention effect, nanoparticle drug formulations are capable of greatly enhancing the safety, pharmacokinetic profiles and bioavailability of the administered treatment. Here, the progress of various nanoparticle formulations in both research and clinical applications is detailed with a focus on the development of drug/gene delivery systems. Specifically, the unique advantages and disadvantages of polymeric nanoparticles, liposomes, solid lipid nanoparticles, nanocrystals and lipid-coated nanoparticles for targeted drug delivery will be investigated in detail.

\textbf{Keywords:} Nanoparticle; Drug delivery; Lipid; Calcium phosphate; Nanocrystals; siRNA; Polymer; Gold nanoparticle; Liposome
Zhiyong Wang\textsuperscript{a,b}, Gang Liu\textsuperscript{a,c,d}, Hairong Zheng\textsuperscript{b}, Xiaoyuan Chen\textsuperscript{c} (\textsuperscript{a}Center for Molecular Imaging and Translational Medicine, School of Public Health, Xiamen University, Xiamen, Fujian 361102, China, \textsuperscript{b}Paul C. Lauterbur Research Center for Biomedical Imaging, Shenzhen Key Laboratory for MRI, Institute of Biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China, \textsuperscript{c}State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Xiamen University, Xiamen 361102, China, \textsuperscript{d}MOE key Lab of Spectrochemical Analysis & Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China, \textsuperscript{e}Laboratory of Molecular Imaging and Nanomedicine (LOMIN), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH), Bethesda, MD 20892, USA). Rigid nanoparticle-based delivery of anti-cancer siRNA: Challenges and opportunities. Biotechnology Advances, Volume 32(2014): 831–843

Gene therapy is a promising strategy to treat various genetic and acquired diseases. Small interfering RNA (siRNA) is a revolutionary tool for gene therapy and the analysis of gene function. However, the development of a safe, efficient, and targetable non-viral siRNA delivery system remains a major challenge in gene therapy. An ideal delivery system should be able to encapsulate and protect the siRNA cargo from serum proteins, exhibit target tissue and cell specificity, penetrate the cell membrane, and release its cargo in the desired intracellular compartment. Nanomedicine has the potential to deal with these challenges faced by siRNA delivery. The unique characteristics of rigid nanoparticles mostly inorganic nanoparticles and allotropes of carbon nanomaterials, including high surface area, facile surface modification, controllable size, and excellent magnetic/optical/electrical properties, make them promising candidates for targeted siRNA delivery. In this review, recent progresses on rigid nanoparticle-based siRNA delivery systems will be summarized.

Keywords: Gene therapy; RNA interference (RNAi); Small-interfering RNA (siRNA); Gene delivery; Nanoparticles

Hamid R. Taghiyari\textsuperscript{a}, Bahman Moradi-Malek\textsuperscript{b}, Maryam Ghorbani Kookandeh\textsuperscript{b}, Omid Farajpour Bibalan\textsuperscript{a}. (\textsuperscript{a}Wood Science and Technology Department, Faculty of Civil Engineering, Shahid Rajae Teacher Training University, Lavizan, Shabanloo St., Tehran, Iran, \textsuperscript{b}Faculty of Natural Resources, Sari Agricultural Sciences and Natural Resources University, Sari, Iran). Effects of silver and copper nanoparticles in particleboard to control Trametes versicolor fungus. International Biodeterioration & Biodegradation, Volume 94(2014): 69–72

This study looked at commercial particleboard treatment with 200 ppm nanosilver (NS) and nanocopper (NC) suspensions (10–80 nm) to assess the effects on hardness and resistance to the white-rot fungus \textit{Trametes versicolor}. Suspensions were added to mats at two levels (100 and 150 ml kg\textsuperscript{-1} dry weight wood particles) and comparison was made with control boards. Both metal nanoparticles had significantly improved resistance to \textit{T. versicolor}, resulting in decreasing mass loss (ML). Nanocopper was more effective at lower levels. Fungal exposure was associated with reduced hardness in NS-control and NS100 treatments, whereas NS150 produced significant reduction in fungal growth. Copper more effectively protected panels from fungal attack. High significant correlation (85\%) was found between ML and hardness values.
Keywords: Biological resistance; Composite board; Heat transferring property; Metal nanoparticles; Particleboard


Silver nanoparticulate enhanced aqueous silane/siloxane emulsions and their efficacy against biofouling mechanisms are presented within this study. Different concentrations of silver nanoparticulates were added into viscous shear-thinning aqueous treatments and key attributes required for facade remedial applications assessed. Water repellence, biofouling resistance, and aesthetical alteration were studied to assess key treatment attributes. In addition, assessment of the porosity, sorptivity, and treatment depth was used to identify penetration and facade protection efficacy and morphological alteration of the masonry substrate. Results showed that silver nanoparticulate incorporation did not impede treatment penetration, better water repellent attributes were achieved with increased concentration while effectively conserving the morphology and aesthetics of the substrate. It was concluded that the reduced bioreceptivity observed primarily stemmed from the silver nanoparticulates ability to sanitise the surface, and that only small concentrations (<0.5%wt) were required to attain significantly beneficial improvements. Treatments were deemed practically and commercially viable for retrofit and heritage projects.

Keywords: Algae; Emulsion; Biofouling; Masonry; Nanoparticulate; Silver

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This study investigated the cytotoxicity, genotoxicity, and growth inhibition effects of four different inorganic nanoparticles (NPs) such as aluminum (nAl), iron (nFe), nickel (nNi), and zinc (nZn) on a dibenzofuran (DF) degrading bacterium Agrobacterium sp. PH-08. NP (0–1,000 mg L−1)-treated bacterial cells were assessed for cytotoxicity, genotoxicity, growth and biodegradation activities at biochemical and molecular levels. In an aqueous system, the bacterial cells treated with nAl, nZn and nNi at 500 mg mL−1 showed significant reduction in cell viability (30–93.6%, p < 0.05), while nFe had no significant inhibition on bacterial cell viability. In the presence of nAl, nZn and nNi, the cells exhibited elevated levels of reactive oxygen species (ROS), DNA damage and cell death. Furthermore, NP exposure showed significant (p < 0.05) impairment in DF and catechol biodegradation activities. The reduction in DF biodegradation was ranged about 71.7–91.6% with single NPs treatments while reached up to 96.3% with a mixture of NPs. Molecular and biochemical investigations also clearly revealed that NP exposure drastically affected the catechol-2,3-dioxygenase activities and its gene (c23o)
expression. However, no significant inhibition was observed in nFe treatment. The bacterial extracellular polymeric materials and by-products from DF degradation can be assumed as key factors in diminishing the toxic effects of NPs, especially for nFe. This study clearly demonstrates the impact of single and mixed NPs on the microbial catabolism of xenobiotic-degrading bacteria at biochemical and molecular levels. This is the first study on estimating the impact of mixed NPs on microbial biodegradation.

**Keywords:** Nanotoxicity; Agrobacterium sp. PH-08; Bacterial viability; Dibenzofuran degradation; Catechol oxidation


For a full estimation of the risk related with the presence of engineered nanoparticles (ENPs) in the environment, the use of the current ecotoxicological methods may prove insufficient. In the study presented herein, various methods of assessment of ecotoxicity were applied to compare the phytotoxicity of three ENPs: nano-ZnO, nano-TiO₂ and nano-Ni. The toxicity was assayed both for aqueous solutions of the ENPs (the germination/elongation test and Phytotestkit F™) and for ENPs added to soil (Phytotoxkit F™ and modified Phytotoxkit F™). *Lepidium sativum* was used as a test plant. The scope of the study also included the assessment of the effect of the method of ENP application to the soil (as powder and aqueous suspension) on their phytotoxicity. In the course of the study, no effect of the studied ENPs and their bulk counterparts on the germination of seeds was observed. The root growth inhibition of *L. sativum* depended on the kind of test applied. The trend between concentration of ENPs and effect depended on the method used and kind of ENPs. For most nanoparticles (despite of the method used), the differences in phytotoxicity between nano and bulk particles were observed. Depending on the kind of ENPs, their phytotoxicity differs between water and soil. ZnO (nano and bulk) and nano-Ni were more toxic in soil than in water. For TiO₂ and bulk-Ni, reverse trend was observed. A different method of ENP application to soil differently affects the phytotoxicity.

**Keywords:** Phytotoxicity; Engineered nanoparticles; Methods; Lepidium sativum

R. Emmanuelb, Chelladurai Karuppihab, Shen-Ming Chena, Selvakumar Palanisamya, S. Padmavathyc, P. Prakashb. (a Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei 106, Taiwan, ROC, b Post Graduate and Research Department of Chemistry, Thiagarajar College, Madurai 625009, Tamil Nadu, India, c Department of Zoology and Microbiology, Thiagarajar College, Madurai 625009, Tamil Nadu, India). Green synthesis of gold nanoparticles for trace level detection of a hazardous pollutant (nitrobenzene) causing Methemoglobinemia. Journal of Hazardous Materials, Volume 279(2014): 117–124

The present study involves a green synthesis of gold nanoparticles (Au-NPs) using *Acacia nilotica* twig bark extract at room temperature and trace level detection of one of the hazardous
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materials, viz. nitrobenzene (NB) that causes Methemoglobinemia. The synthesis protocol demonstrates that the bioreduction of chloroaauric acid leads to the formation of Au-NPs within 10 min, suggesting a higher reaction rate than any other chemical methods involved. The obtained Au-NPs have been characterized by UV–vis spectroscopy, X-ray diffraction, transmission electron microscopy, Energy-Dispersive X-ray Spectroscopy and Fourier Transform Infrared Spectroscopy. The electrochemical detection of NB has been investigated at the green synthesized Au-NPs modified glassy carbon electrode by using differential pulse voltammetry (DPV). The Au-NPs modified electrode exhibits excellent reduction ability toward NB compared to unmodified electrode. The developed NB sensor at Au-NPs modified electrode displays a wide linear response from 0.1 to 600 µM with high sensitivity of 1.01 µA µM⁻¹ cm⁻² and low limit of detection of 0.016 µM. The modified electrode shows exceptional selectivity in the presence of ions, phenolic and biologically coactive compounds. In addition, the Au-NPs modified electrode exhibits an outstanding recovery results toward NB in various real water samples.

Keywords: Gold nanoparticles; Green synthesis; Nitrobenzene; Electrocatalysis; Differential pulse voltammetry

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There has been extensive growth in nanoscale technology in the last few decades to such a degree that nanomaterials (NMs) have become a constituent in a wide range of commercial and domestic products. With NMs already in use in several consumer products, concerns have emerged regarding their potential adverse environmental impacts. Although research has been undertaken in order to minimise the gaps in our understanding of NMs in the environment, little is known about their bioavailability and toxicity in the aquatic environment. Nano-toxicology is defined as the study of the toxicity of nanomaterials. Nano-toxicology studies remain poorly and unevenly distributed. To date most of the research undertaken has been restricted to a narrow range of test species such as daphnids. Crabs are bio-indicators that can be used for toxicological research on NMs since they occupy a significant position in the aquatic food chain. In addition, they are often used in conventional ecotoxicological studies due to their high sensitivity to environmental stressors and are abundantly available. Because they are benthic organisms they are prone to contaminant uptake and bioaccumulation. To our knowledge the crab has never been used in nano-toxicological studies. In this context, an extensive review on published scientific literature on the ecotoxicity of silver NPs (AgNPs) on aquatic organisms was conducted. Some of the most common biomarkers used in ecotoxicological studies are described. Emphasis is placed on the use of biomarker responses in crabs as monitoring tools, as well as on its limitations. Additionally, the gaps in nano-toxicological research and recommendations for future research initiatives are addressed.

Keywords: Biomarkers; crabs; ecotoxicity; nanomaterials; Potamanuates warren; silver nanoparticles

The fields of metallic nanoparticle study and synthetic biology have a great deal to offer one another. Metallic nanoparticles as a class of material have many useful properties. Their small size allows for more points of contact than would be the case with a similar bulk compound, making nanoparticles excellent candidates for catalysts or for when increased levels of binding are required. Some nanoparticles have unique optical qualities, making them well suited as sensors, while others display para-magnetism, useful in medical imaging, especially by magnetic resonance imaging (MRI). Many of these metallic nanoparticles could be used in creating tools for synthetic biology, and conversely the use of synthetic biology could itself be utilised to create nanoparticle tools. Examples given here include the potential use of quantum dots (QDs) and gold nanoparticles as sensing mechanisms in synthetic biology, and the use of synthetic biology to create nanoparticle-sensing devices based on current methods of detecting metals and metalloids such as arsenate. There are a number of organisms which are able to produce a range of metallic nanoparticles naturally, such as species of the fungus, *Phoma* which produces anti-microbial silver nanoparticles. The biological synthesis of nanoparticles may have many advantages over their more traditional industrial synthesis. If the proteins involved in biological nanoparticle synthesis can be put into a suitable bacterial chassis then they might be manipulated and the pathways engineered in order to produce more valuable nanoparticles.

**Biomimicry**

Jun Ma\(^a,b\), Jianglin Wang\(^c\), Xin Ai\(^b\), Shengmin Zhang\(^a,b\). (\(^a\) Advanced Biomaterials and Tissue Engineering Center, Huazhong University of Science and Technology, Wuhan 430074, PR China, \(^b\) Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan 430074, PR China, \(^c\) Department of Chemistry and Biochemistry, Stephenson Life Sciences Research Center, University of Oklahoma, Norman, OK 73019, USA). Biomimetic self-assembly of apatite hybrid materials: From a single molecular template to bi-/multi-molecular templates. Biotechnology Advances, Volume 32(4) (2014): 744–760

The self-assembly of apatite and proteins is a critical process to induce the formation of the bones and teeth in vertebrates. Although hierarchical structures and biomineralization mechanisms of the mineralized tissues have been intensively studied, most researches focus on the self-assembly biomimetic route using one single-molecular template, while the natural bone is an outcome of a multi-molecular template co-assembly process. Inspired by such a mechanism in nature, a novel strategy based on multi-molecular template co-assembly for fabricating bone-like hybrid materials was firstly proposed by the authors. In this review article we have summarized the new trends from single-molecular template to bi-/multi-molecular template systems in biomimetic fabrication of apatite hybrid materials. So far, many novel apatite hybrid materials with controlled morphologies and hierarchical structures have been successfully achieved using bi-/multi-molecular template strategy, and are found to have multiple common
features in comparison with natural mineralized tissues. The carboxyl, carbonyl and amino groups of the template molecules are identified to initiate the nucleation of calcium phosphate during the assembling process. For bi-/multi-molecular templates, the incorporation of multiple promotion sites for calcium and phosphate ions precisely enables to regulate the apatite nucleation from the early stage. The roles of acidic molecules and the synergetic effects of protein templates have been significantly recognized in recent studies. In addition, a specific attention is paid to self-assembling of apatite nanoparticles into ordered structures on tissue regenerative scaffolds due to their promising clinical applications ranging from implant grafts, coatings to drug and gene delivery.

Keywords: Mineralization; Apatite; Single molecular template; Bi-/multi-molecular template; Nucleation; Crystal growth; Bone repair; Dental repair; Controlled assembly
Name of Journals

1. Acta Biotechnologica
2. Aerobiologia
3. Annual Review-Plant Pathology
4. Annual Review- Ecology and Systematics
5. Annual Review-Biochemistry
6. Annual Review-Biomedical Engineering
7. Annual Review-Biophysics and Biomolecular Structure
8. Annual Review-Microbiology
9. Annual Review-Pharmacology and Toxicology
10. Annual Review-Phytopathology
11. Annual Review-Physiology
12. Annual Review-Plant Physiology
13. Annual Review-Public Health
15. Applied and Environmental Microbiology
16. Applied Microbiology & Biotechnology
17. Aquaculture
18. Allergy
19. Australian Journal of Plant Physiology
20. Biocatalysis and Transformation
21. Biocontrol
22. Biocontrol Potential and its exploitation in sustainable Agriculture
23. Biodegradation
24. Biodeterioration & Biodegradation
25. Biodiversity and Conservation
26. Biological Agriculture and Horticulture
27. Biomass and Bioenergy
28. Biomedical and Environmental Sciences
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30. Bioresource Technology
31. Bioscience, Biotechnology and Biochemistry
32. Biosensors-and –Bioelectronics
33. Bioseperation
34. Biotechnology Letters
35. Biotechnology Advances
36. Biotechnology and Applied Biochemistry
37. Biotechnology and Bioengineering
38. Botanical Review
39. Canadian Journal of Microbiology
40. Cell & Tissue Banking
41. Clinical Microbiology Reviews
42. Critical Reviews in Biotechnology
43. Crop Research Hisar
44. Current Microbiology
45. Current Opinion in Biotechnology
46. Current Science
47. Cytotechnology
48. Ecology and Environmental Corner
49. Ecological Engineering
50. Ecotoxicology
51. Environmental Conservation
52. Environmental Research
53. Environmental Pollution
54. Enzyme and Microbial Technology
55. Every Man’s Science
56. Environmental Impact Assessment Review
57. Fems Microbiology & Ecology
58. Food & Agricultural Immunology
59. Global Environmental Change
60. Hydrometallurgy
61. Immunological Research
62. Indian Agriculturist
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65. Indian Journal of Agricultural Science
66. Indian Journal of Biotechnology
67. Indian Journal of Ecology
68. Indian Journal of Experimental Biology
69. Indian Journal of Environmental Toxicology
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72. International Biodeterioration & Biodegradation
73. International Journal of Biotechnology
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76. Journal of Agriculture and Environmental Ethics
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79. Journal of Chemical Technology & Biotechnology
80. Journal of Environmental Management
81. Journal of Food Science and Technology-Mysore
82. Journal of Hazardous Materials
83. Journal Indian Association Environment Management
84. Journal Indian Pollution Control
85. Journal of Indian Soil Science
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87. Journal of Scientific and Industrial Research
88. Microbial Review
89. Microbiological Research
90. Molecular Biotechnology
91. Mycological Research
92. Mycorrhizal Biology
93. Nature
94. Nature Biotechnology
95. New Biotechnology
96. Perspectives-in-Biotechnology
97. Pesticide research Journal
98. Pestology
99. Plants and Soil
100. Process Biochemistry
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103. Reviews in Environmental Science and Biotechnology
104. Research Journal Chemistry & Environment
105. Sciences
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107. Shaspa
108. The Indian Forester
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