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BACKGROUND

Environmental Information System (ENVIS) is established in the year 1984 as a network of Information Centre. It is planned by the Ministry of Environment and Forest. Aim of this centre is to provide descriptive data and environmental subject related numerical data. Now 35 centres are working under this network on various subjects area in the country. The focal point of this network is at the Ministry of Environmental and Forest, Government of India, New Delhi.

EMCB-ENVIS Centre (27) is established for studies on Environmental Biotechnology as Pollutant Degradation at the University of Kalyani, Department of Environmental Science, Nadia-741235, West Bengal.

The objective of this centre is to collect data, related to the above mentioned subject, from different major libraries in Kolkata with different journals, Annual reviews, Internet and to generate a database and to create a website with this database. View point of this journal abstract is to help the interested research workers, scientist, administrator and the public.

This is the Second publication of this ENVIS Centre. This contains the abstract of research papers collected in the area of Environmental Biotechnology from various journal published during June 2000 onwards. Here various topics like Bio-engineering, Bio-degradation, Bio-remediation, Bio-transformation etc. are covered. We are grateful to the various libraries and their staff for their extended cooperation in the collection of the articles.

Abstract Format

The format of the abstract is as follows:

Abstract : The abstracts were arranged in alphabetic orders 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 name. When the address of any other author is found, it is written after wards delimited by stop(.).

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

GENERAL INFORMATION

Abstract have been taken directly from source document like research report, journals, Internet, seminars, proceedings, standards and patents. All the resources published within the year 2000-2003.

Abstract are broadly classified and arranged under the following heads:

Bioaccumulation: It studies address the buildup of bioaccumulative compounds through biomagnification and/or bioconcentration. 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 any time they are taken up and stored 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. These 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.

Because the microorganisms already occur naturally in the environment they pose no contamination risk.

Bio-Transformation: This is a process of Biological changes of complex compound to simpler toxic to non-toxic or vice-versa. Several microorganism are capable of transforming a variety of compound founding nature but generally with respect to synthetic compound they are unable to show any appropriate action. Biotransfer appears to be one of the major detoxication method known so far.

Biomarker: It is a biological response to a chemical that gives a measure of exposure and, sometimes, of toxic effect. Biological markers found in crude oils and source rock extracts can provide molecular evidence of the correlation among oils and their sources.

Bioenergy: In recent decades, efforts were made for evolving were non-polluting bioenergy sources or energy generation from organic waste or biomass. These are all ecofriendly solution. Biomass energy supply demand balances have become a component of energy sector analysis and planning and assumed greater importance in countries. These are variety of biological energy sources. Biomass, Biogas, Hydrogen are the example of Bioenergy.

Biofertilizer: To reduce the impact of excess chemical fertilizers in the field of agriculture the biofertilizer is 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 were 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 involves combining organic materials under conditions that enables them to decompose more quickly than they would in nature.

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 nature, there is no 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 will 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 will 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. A major focus for bioengineering is to improve the quality of life of people with medical

conditions that restrict independent living and integration within the community.

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

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

Agricultural Biotechnology: Over the years tremendous success was made in diverse fields 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. Crop production against pest and disease stress resistance of crops also considered to be an emerging area of Agricultural Biotechnology.

ABBREVIATIONS USED IN ADDRESSES AND CITED JOURNALS

Acad	Academy	Chem	Chemistry
Adm	Administration	Chemi	Chemical
Admn	Administrative	Clini	Clinical
Adv	Advance	Co	Company
Agric	Agriculture	Coil	College
Agrici	Agricultural	Comm	Committee
Amer,	American	Commn	Commission
An	Annual	Comp	Comparative
Analyt	Analytical	Conf	Conference
Anat	Anatomy	Conv	Convention
Anim	Animal	Conserv	Conservation
Ann	Annals	Conti	Control
Appt	Applied	Contam	Contamination
Arch	Archives	Corpn	Corporation
Archaeo	Archaeology	Coun	Council
Archaeol	Archaeological	Cult	Culture
Architect	Architecture	Cultl	Cultural
Assoc	Association	Curr	Current
Asst	Assistant	Dept	Department
Atom	Atomic	Dev	Development

Bacterio	Bacteriology	Develop	Developmental
Bacteriol	Bacteriological	Dig	Digest
Bd	Board	Div	Division
Bio	Biology	Divl	Divisional
Biochem	Biochemistry	Dte	Directorate
Biochemi	Biochemical	Dy	Deputy
Bioengng	Bioengineering	Eco	Ecology
Biol	Biological	Ecol	Ecological
BiometeO	Biometeorology	Econ	Economics
Biophys	Biophysics	Ecosys	Ecosystem
Biometeol	Biometeorological	Exotoxico	Ecotoxicology
Biotech	Biotechnology(s)	Endocrinol	Endocrinological
Biotechno	Biotechnology	Engng	Engineering
Biotechnol	Bitechnological	Engrs	Engineers
Bidg	Building	Env	Environment
Bot	Botany	Environ	Environmental
Boti	Botanical	Epidemic	Epidemiology
Br	Branch	Epidemiol	Epidemiological
Bull	Bulletin	Estb	Establishment
Cent	Centre	Ethnopharmaco	Ethnopharmacology
Centl	Central	Exot	Experiment

Expti	Experimental	Microbiol	Microbiological
Fac	Faculty	Min	Ministry
Fd	Food	Monit	Monitoring
Fedn	Federation	Myco	Mycology
Fert	Fertiliser	Mycol	Mycological
Fmg	Farming	Nat	Natural
Gaz	Gazette	Natl	National
Genet	Genetics	N-E	North Eastern
Geo	Geology	Nut	Nutrition
Geogr	Geography	No	Number
Geogr	Geographical	Occ	Occasional
Geol	Geological	Occupl	Occupational
Geosci	Geoscience	Oceanogr	Oceanography
Govt	Government	Org	Organic
Hist	History	Orgn	Organisation
Hlth	Health	Pharmaco	Pharmacology
Hort	Horticulture	Pharmacol	Pharmacological
Hosp	Hospital	Phyl	Physical
Hydro	Hydrology	Patho	Pathology
Hydrol	Hydrological	Pathol	Pathological
Immuno	Immunology	Petrochemi	Petrochemical
Immunol	Immunological	Petro	Petrology
Ind	Industry	PG	Post Graduate
Inf	Information	Phys	Physics
Inst	Institute	Physio	Physiology

Instn	Institution	Phytopath	Phytopathology
Int	International	Phytopathol	Phytopathological
Irrig	Irrigation	Plang	Planning
J	Journal	Polln	Pollution
Lab	Laboratory	Proc	Proceedings
Lett	Letter(s)	Prot	Protection
Ltd	Limited	Pub	Publication
Malario	Malariology	Pvt	Private
Malariol	Malariological	Qlty	Quality
Manag	Management	Qr	Quarter
Med	Medicine	Rad	Radiation
Medl	Medical	Radio	Radiology
Metab	Metabolism	Radiol	Radiological
Metall	Metallurgy	Rd	Road
Metallurg	Metallurgical	Recd	Received
Meteo	Meteorology	Reg	Region
Meteol	Meteorological	Regl	Regional
Microbio	Microbiology		

Rep	Report	Stud	Studies
Reptr	Reporter	Surv	Survey
Res	Research	Syst	System
Rev	Review	Tax	Taxonomy
Sch	School(s)	Techi	Technical
Sci	Sciences(s)	Techno	Technology
Scient	Scientific	Technol	Technological
S-E	South East	Toxico	Toxicology
Sec	Section	Toxicol	Toxicological
Sect	Sector	Trans	Transcations
Semin	Seminar	Trans	Transportation
Ser	Services	Tmg	Training
Soc	Society	Trop	Tropical
Sod	Social	Univ	University
Stat	Statistics	Util	Utilisation
Stati	Statistical	Vet	Veterinary
Stand	Standard(s)	Zoo	Zoology
Std	Study	Zool	Zoological

Bioaccumulation

Anastasios I Zouboulis, Nick K Lazaridis, Kostas A Matis. (School of Chemistry, Division of Chemical Technology, Aristotle University (Box 116), GR-54006 Thessaloniki, Greece). **Removal of toxic metal ions from aqueous systems by biosorptive flotation.** Journal of Chemical Technology & Biotechnology, 77(8) (2002), 958-964.

Biosorptive flotation was used as a combined operation for the simultaneous abstraction of nickel, copper and zinc ions from aqueous streams. Laboratory-scale batch experiments, as well as pilot-scale continuous experiments, have been conducted. Grape stalks, a by-product of the winery industry, were used as sorbent material. The experimental procedure consisted of two consecutive stages: (i) biosorption, and (ii) flotation. The possibility of reusing biomass, after appropriate elution, was also examined. The main parameters examined were biomass concentration, particle size of sorbent, surfactant concentration, pH and flocculation. Flotation removals, following laboratory-scale experiments, were found to be in the order of 100, 85 and 70% for copper, zinc and nickel, respectively. In pilot-scale experiments, biomass sorption capacities were determined as 25 for copper, 81 for zinc and $7 \mu\text{mol dm}^{-3}$ for nickel. The order of biomass affinity regarding the studied metals was $\text{Cu} > \text{Zn} > \text{Ni}$. Short retention time and high effectiveness suggest that biosorptive flotation is a promising treatment process for the removal of toxic metals from contaminated aqueous solutions.

Bogdana Koumanova, P Peeva, Stephen J Allen, K A Gallagher, M G Healy. (Department of Chemical Engineering, University of Chemical Technology and Metallurgy, Sofia 1756, Bulgaria. School of Chemical Engineering, Queens University of Belfast, David Keir Building, Stranmillis Road, Belfast, UK). **Biosorption from aqueous solutions by eggshell membranes and *Rhizopus oryzae*: equilibrium and kinetic studies.** Journal of Chemical Technology & Biotechnology, 77(5) (2002), 539-545.

This study assesses the use of eggshell membranes and *Rhizopus oryzae* as media for the biosorption of *p*-chlorophenol (*p*-CP), 2,4-dichlorophenol (2,4-DCP), 3,5-dichlorophenol (3,5-DCP), reactive dye and cadmium from aqueous solutions. The performance of the adsorbents was quantified by measuring the equilibrium uptake and the batch rate kinetics from solutions. The constants in the Freundlich, Langmuir and Redlich-Peterson isotherm models were calculated through the linearization of the equations and linear regression. The kinetics of the adsorption systems for cadmium and a reactive dye have been assessed in a batch stirred adsorber. The effect of the process parameters such as pH, adsorbate concentration, adsorbent dosage, adsorbent particle size, temperature and agitation speed are reported. The external mass transfer coefficients are reported for some different system conditions. Both materials are determined to be effective adsorbents and could find application in the treatment of contaminated wastestreams.

D. Karunasagar, J. Arunachalam, K. Rashmi, J. Naveena Lavanya Latha, P. Maruthi Mohan. (National Center for Compositional Characterization of Materials, Department of Atomic Energy, ECIL Road, Hyderabad 500 062, AP, India. Department of Biochemistry, Osmania University, Hyderabad 500 007, AP, India). **Biosorption of inorganic and methyl mercury by a biosorbent from *Aspergillus niger*.** World Journal of Microbiology and Biotechnology, 19(3) (2003) 291-295.

A biosorbent prepared by alkaline extraction of *Aspergillus niger* biomass was evaluated for its potential to remove mercury species – inorganic (Hg^{2+}) and methyl mercury (CH_3Hg^+) – from aqueous solutions. Batch experiments were carried out to determine the pH and time profile of sorption for both species in the pH range 2–7. The Hg^{2+}

exhibited more rapid sorption and higher capacity than the CH_3Hg^+ . Further, removal of both mercury species from spiked ground water samples was efficient and not influenced by other ions. Sorption studies with esterified biosorbent indicated loss of binding of both mercury species (>80%), which was regained when the ester groups were removed by alkaline hydrolysis, suggesting the involvement of carboxyl groups in binding. Further, no interconversion of sorbed species occurred on the biomass. The biosorbent was reusable up to six cycles without serious loss of binding capacity. Our results suggest that the biosorbent from *Aspergillus niger* can be used for removal of mercury and methyl mercury ions from polluted aqueous effluents.

Dirk F. Wenderoth, Petra Rosenbrock, Dietmar Pieper, Manfred G. Höfle. (GBF-German Research Centre for Biotechnology, Department for Environmental Microbiology, Braunschweig, Germany). **Assessment of Population Dynamics of Specific Strains in Groundwater Bioaugmentation Experiments by Two Different Molecular Techniques.** Water, Air and Soil Pollution, 2(3)(2002), 195-203.

A set of microcosm experiments was performed to understand the behaviour of special degraders in bioaugmentation experiments. In the experiments the following chlorobenzene degraders were used: the genetically modified *Pseudomonas putida* F1ΔCC, and the two wild-type strains *Pseudomonas putida* GJ31 and *Pseudomonas aeruginosa* RHO1. These strains were used at an initial cell density of 10^5 cells mL^{-1} groundwater which had been spiked with 1,2-dichlorobenzene (1,2-DCB), 1,4-dichlorobenzene (1,4-DCB) and, as main contaminant, chlorobenzene (CB). The population dynamics and behaviour of the three special degraders within the groundwater microcosms were studied by single-strand conformation polymorphism (SSCP) analysis of 16S rDNA fragments amplified from directly extracted community DNA and fluorescent *in situ* hybridization (FISH) with species-specific probes. RHO1 disappeared after 4 days as detected by FISH in contrast to SSCP-detection where RHO1 could be found during the whole incubation time. Whereas GEM F1ΔCC and wild-type strain GJ31 survived the whole incubation for 20 days. With both methods we were able to detect all strains with high specificity among the indigenous microbial community. The data sets obtained from SSCP analysis and FISH were highly correlated. Specific band intensity within the SSCP fingerprints and the cell counts determined by FISH gave a quantitative overview about the introduced strains.

H. Shahandeh, L. R. Hossner. (Department of Soil and Crop Sciences, 618 Heep Center, Texas A&M University, College Station, TX, U.S.A. Department of Soil and Crop Sciences, 618 Heep Center, Texas A&M University, College Station, TX, U.S.A.). **Role of Soil Properties in Phytoaccumulation of Uranium.** Water, Air, and Soil Pollution, 141(1-4) (2002), 165-180.

Thirty four plant species were screened for uranium (U) accumulation from U contaminated soil. There was a significant difference in U accumulation among plant species. Sunflower (*Helianthus annuus*) and Indian mustard (*Brassica juncea*) accumulated more U than other plant species. Sunflower and Indian mustard were selected as potential U accumulators for further study in one U mine tailing soil and eight cultivated soils (pH range 4.7 to 8.1) contaminated with different rates (100 to 600 mg U (VI) kg^{-1}) of uranyl nitrate ($\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$). Uranium fractions of contaminated soils [(exchangeable, carbonate, manganese (Mn), iron (Fe), organic, and residual)] were determined periodically over an 8-week incubation period. Uranium accumulated mainly in the roots of plant species. The highest concentration of U was 102 mg U kg^{-1} in plant shoots and 6208 mg U kg^{-1} in plant roots. Plant performance was affected by U contamination rates, especially in calcareous soils. Plants grown in soils with high carbonate-U fractions accumulated the most U in shoots and roots. The lowest plant U occurred in clayey acid soils with high Fe, Mn and organic U-fractions. The effectiveness

of U remediation of soils by plants was strongly influenced by soil type. Soil properties determined the tolerance and accumulation of U in plants.

Hila Elifantz, Elisha Telor. (Department of Agriculture Botany, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel). **Heavy Metal Bieserption by Plant Biomass of the Macrophyte *Ludwigia stolonifera***. Water, Air, and Soil Pollution, 141(1-4) (2002), 207-218.

Ludwigia stolonifera biomass of roots, floating loots and leaves ware tested for their performance as heavy metal biofilters. Cadmium (Cd) and nickel (Ni) (50 ppm) solutions were filtered through 0.5-1.5 g packed columns with each biomaterial, to determine their metal removal efficiency. Root column was more efficient in removing Ni (as low as 6 ppb m the effluent) than of Cd (as low as to 22 ppb in the effluent). This tendency was also observed upon treatment of a mixed solution of both metals. Floating roots column reduced Cd content to the same level as the root column, but its metal binding capacity was higher; 93 mg Cd g⁻¹ DW in floating roots in comparison to 43 mg Cd g⁻¹ DW in the roots biofilter. Leaf biomass column demonstrated the best metal binding capacity; 128 mg Cd g⁻¹ DW, and Cd concentration in the effluent was 17 ppb. Pectin content was 5, 8 and 10% W/W in roots, leaves and floating roots biofilters, respectively. It seems that ion exchange is the major mechanism by which the metal is biosorbed. Evidence for the exchange of the bound heavy metal ions against the discharge of light metal ions such as calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) was provided.

Hui Niu, Bohumil Volesky. (Department of Chemical Engineering, McGill University, 3610 University St, Montreal, Canada H3A 2B2). **Gold-cyanide biosorption with L-cysteine**. Journal of Chemical Technology & Biotechnology, 75(6) (2000), 436-442.

L-Cysteine increased gold-cyanide biosorption by protonated *Bacillus subtilis*, *Penicillium chrysogenum* and *Sargassum fluitans* biomass. At pH 2, the maximum Au uptakes were 20.5 μmol g⁻¹, 14.2 μmol g⁻¹ and 4.7 μmol g⁻¹ of Au, respectively, approximately 148-250% of the biosorption performance in the absence of cysteine. Au biosorption mainly involved anionic AuCN₂⁻ species adsorbed by ionizable functional groups on cysteine-loaded biomass carrying a positive charge when protonated [(biomass-cysteine-H⁺)-(AuCN₂⁻)]. Deposited gold could be eluted from Au-loaded biomass at pH 3-5. The elution efficiencies were higher than 92% at pH 5.0 with the Solid-to-Liquid ratio, S/L, = 4. Increasing solution ionic strength (NaNO₃) decreased Au uptake. FTIR analyses indicated that the main functional groups involved in gold biosorption in the presence of L-cysteine are probably N-, S- and O-containing groups. The present results confirm that certain waste microbial biomaterials are capable of effectively removing and concentrating gold from solutions containing residual cyanide if applied under appropriate conditions.

Ioannis Savvaidis, Martin N. Hughes, Robert K. Poole. (Department of Chemistry, University of Ioannina, Ioannina 45110, Greece. Department of Molecular Biology and Biotechnology, The University of Sheffield, Firth Court, Western Bank, Sheffield S10 2 TN, UK). **Copper biosorption by *Pseudomonas cepacia* and other strains**. World Journal of Microbiology and Biotechnology, 19(2) (2003), 117-121.

Biosorption of copper by *Pseudomonas cepacia* was found to be dependent on added copper concentration. Copper uptake by the cells was rapid over the range of copper concentrations tested and complete within the first 10 min of incubation time. The effect of pH on copper uptake by *P. cepacia* was determined using overlapping buffers over the pH range 3-8, and copper biosorption from a 10 mM copper solution was greatest at pH 7. Copper uptake (measured by analysis of cell digests) was unaffected by cyanide and

azide (up to 30 mM) and by incubation of cells with a 10 mM copper solution at 4 °C. Evidence from these results suggested that copper uptake by *P. cepacia* cells involves surface binding and not intracellular accumulation by active transport. Biosorption of copper by various *Pseudomonas* isolates from metal-contaminated environments agreed well with copper biosorption by *Pseudomonas* strains from the National Collection of Type Cultures (NCTC).

J. T. Moragham, J. Padilla, J.D. Etchevers, K. Grafton, J.A. Acosta-Gallegos. (Department of Soil Science and Corresponding. Department of Plant Sciences, North Dakota State University, Fargo, ND 58103, USA. Department of Plant Sciences, North Dakota State University, Fargo, ND 58105. USA. Instituto de Recursos Naturales, Colegio de Postgraduados, C. P. 56230, Montecillo, Mexico. INIFAP. Chapingo 56230, Mexico). **Iron accumulation in seed of common bean.** *Plant and soil*, 246(2) (2002), 175-183.

The effect of soil and genotype on iron concentration (Fe) in common bean (*Phaseolus vulgaris* L.) seed was studied in the greenhouse. Liming an acid soil increased soil pH from 6.0-7.3 but had no effect on seed (Fe) of three bean genotypes (Voyager, T39, UI911) from the Middle American gene pool in North Dakota. However, liming decreased seed-manganese concentration [Mn]. The influence of FeEDDHA on Fe accumulation in seed of the three bean genotypes, grown on acid (pH=6.0) and naturally calcareous (pH=8.2) soils, was also studied in North Dakota. Seed from the acid soil contained 25% higher [Fe] than seed from the calcareous soil. FeEDDHA increased seed [Fe] only on the calcareous soil, but reduced seed [Mn] on both soils. Voyager seed, characterized by a relatively low [Fe] in the seed coat, had a higher seed [Fe] than the other two genotypes. The hypothesis that high seed [Fe] is characterized by a low seed-coat [Fe] was next investigated. Voyager, T39 and 18 diverse Latin American genotypes from the Middle American gene pool were grown on a soil (pH=7.0) with Andic properties in Mexico in the presence and absence of FeEDTA. FeEDTA increased seed [Fe]. Seed of Voyager and a Mexican genotype (Bayo 400) had the highest seed [Fe]. However, Bayo 400, unlike Voyager, contained a high percentage of its seed Fe in the seed coat. Consequently, a high seed [Fe] genotype does not necessarily have a low seed-coat [Fe]. Both soil and genotype affect Fe accumulation in bean seed.

K S Dolt, G V Reddy, M P Shah, P D Kunjadia and I L Kothari. (Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat 388 120, India). **Bioaccumulation of Neem limonoids in *Pleurotus sajor-caju*.** *Journal of Scientific & Industrial Research*, 60 (2001), 937-940.

Pleurotus sajor-caju, an, important edible mushroom, is cultivated on various combinations of neem fruit components for bioaccumulation of active principles of neem fruit that enrich its medicinal value. Husk and hull: kernel (1: 1) results in the best cultivation with biological efficiency up to 45.75 per cent, which is comparable to its cultivation on banana pseudostem (47.32 per cent), a potent lignocellulosic substrate. To find out the intensity of accumulation of medicinally important limonoids in the fruiting bodies through HPLC, *Azadirachtin*, one of the limonoids, is taken as a reference compound and it is found to be accumulated in the fruiting bodies of *P. sajor-caju* (4.19 ppm). The presence of limonoids in these mushrooms makes them a health food.

Nikolaus Nestle. (TU München, Institut für Wasserchemie, Marchioninistraße 17, D-81377 München, Germany). **NMR studies on heavy metal immobilization in biosorbents and mineral matrices.** *Reviews in Environmental Science and Biotechnology*, 1(3) (2002), 215-225.

Magnetic resonance imaging and other NMR methods have a potential for the non-

destructive observation of environmentally relevant processes with both spatial and temporal resolution. Among other applications, such methods can be used to study transport and immobilization of paramagnetic heavy metal ions in biosorbents and other matrices. This overview covers various NMR approaches to study such processes and illustrates them with examples of imaging on alginate-based biosorbents and on heavy-metal doped gypsum pastes. Experimental challenges in studies of other matrices are shortly addressed as well.

P Vasudevan, V Padmavathy, N Tewari, S C Dhingra. (Centre for Rural Development & Technology, Indian Institute of Technology, Delhi 110016, India). **Biosorption of Heavy Ions**. Journal of Scientific & Industrial Research. 60 (2001), 112-120.

Increasing environmental pollution by metal ions has led to the necessity of evolving efficient and cost-effective treatment technologies. Biosorption of metal ions using microbial biomass could be useful, especially for wastewater treatment. The metal uptake capacity of various biosorbents and the mechanism of uptake are reviewed. Based on the above facts it is observed that biosystems have good adsorption capacity with the metal ion uptake varying between 0.01 to 1.0 mmol/g biomass. In certain systems the metal ion uptake capacity is even one order higher. The kinetics of adsorption also seems favourable as the percentage uptake within the first few minutes is high (upto 90 percent). It is of interest to compare biosorption efficiency with the current methods employed for removal of selected metal ions from effluents by the industries. Coagulation and sedimentation process are commonly used for various metal ions. In depth studies on various metal ion biosorbent systems for specific effluents have to be carried at different concentration levels, establishing the uptake kinetics and optimized for various process parameters.

Pinaki Sar, Stanislaus F D'Souza. (Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India). **Biosorptive uranium uptake by a *Pseudomonas* strain: characterization and equilibrium studies**. Journal of Chemical Technology & Biotechnology, 76(12) (2001), 1286-1294.

The biosorptive uranium(VI) uptake capacity of live and lyophilized *Pseudomonas* cells was characterized in terms of equilibrium metal loading, effect of solution pH and possible interference by selected co-ions. Uranium binding by the test biomass was rapid, achieving >90% sorption efficiency within 10 min of contact and the equilibrium was attained after 1 h. pH-dependent uranium sorption was observed for both biomass types with the maximum being at pH 5.0. Metal uptake by live cells was not affected by culture age and the presence of an energy source or metabolic inhibitor. Sorption isotherm studies at a solution pH of either 3.5 or 5.0 indicated efficient and exceptionally high uranium loading by the test biomass, particularly at the higher pH level. At equilibrium, the lyophilized *Pseudomonas* exhibited a metal loading of $541 \pm 34.21 \text{ mg g}^{-1}$ compared with a lower value by the live organisms ($410 \pm 25.93 \text{ mg g}^{-1}$). Experimental sorption data showing conformity to both Freundlich and Langmuir isotherm models indicate monolayered uranium binding by the test biomass. In bimetallic combinations a significant interference in uranium loading was offered by cations such as thorium(IV), iron(II and III), aluminium(III) and copper(II), while the anions tested, except carbonate, were ineffective. Uranium sorption studies in the presence of a range of Fe^{3+} and SO_4^{2-} concentrations indicate a strong inhibition (80%) by the former at an equimolar ratio while more than 70% adsorption efficiency was retained even at a high sulfate level ($30\,000 \text{ mg dm}^{-3}$). Overall data indicate the suitability of the *Pseudomonas* sp biomass in developing a biosorbent for uranium removal from aqueous waste streams.

Ping Yong, Neil A Rowson, J Peter G Farr, I Rex Harris, Lynne E Macaskie. (School of Biosciences, The University of Birmingham, Birmingham B15 2TT, UK. School of Chemical Engineering, The University of Birmingham, Birmingham B15 2TT, UK. School of Metallurgy and Materials Science, The University of Birmingham, Birmingham B15 2TT, UK). **Bioaccumulation of palladium by *Desulfovibrio desulfuricans***. Journal of Chemical Technology & Biotechnology, 77(5) (2002), 593-601.

Palladium uptake by resting cell suspensions of *Desulfovibrio desulfuricans* NCIMB 8307 was studied without or with electron donor (formate), which gave metal uptake attributable to biosorption of Pd(II) and bioreduction of Pd(II) to Pd(0), respectively. The maximum biosorption capacity of palladium (at pH 2) was up to 196 mg Pd g⁻¹ dry cells (1.85 mmol g⁻¹; approx 20% of the dry weight). Biosorption was to 85% of the maximum in less than 10 min and the biomass was saturated within 30 min. Biosorption of Pd(II) was greater from the chloro- than the ammine complex and was inhibited in the presence of excess chloride ion. Bioreductive accumulation of Pd(II) from Pd(NH₃)₄²⁺ was achieved in the presence of electron donor (formate) but was also inhibited by excess Cl⁻. Up to 100% of Pd(II) reduction to Pd(0) was achieved within 5 min anaerobically at pH 7 and 30 min at pH 3. Pd(0) was localized on the biomass surface using electron microscopy and was characterized using energy dispersive X-ray microanalysis (EDAX) and X-ray diffraction analysis (XRD). Biosorption was Pd-specific with respect to Pt and Rh using test solutions and acid (aqua regia) leachates from spent automotive catalysts. The total Pd removed from the latter was only 15%, attributable to the inhibitory effect of residual chloride ion from the acidic extractant. Pd biorecovery is limited by the need for an improved extraction technology to minimize the formation of PdCl₄²⁻ in solution rather than by constraints of the Pd-accumulating biomass.

Remígio Machado, Jorge R Carvalho, M Joana Neiva Correia. (Department of Chemical Engineering, Instituto Superior Técnico, Av Rovisco Pais, 1049-001, Lisbon, Portugal). **Removal of trivalent chromium(III) from solution by biosorption in cork powder**. Journal of Chemical Technology & Biotechnology, 77(12) (2002), 1340-1348.

The removal of trivalent chromium from solutions using biosorption in cork powder is described. The adsorption isotherm was determined, along with the effect of different variables, such as biomass particle size, solid-liquid ratio, reaction time, metal concentration and pH, on the efficiency of chromium removal. It was concluded that the adsorption is slow and favoured by an increase in pH. Therefore, using a solid-liquid ratio of 4 g dm⁻³ it is possible to reduce the chromium concentration in the solution from 10 mg dm⁻³ to less than 1.5 mg dm⁻³ in 2 h at 22 °C. The kinetic studies verified that the sorption of chromium by cork was described by a second-order model. The elution results showed that 50% of the chromium bound to the cork was eluted using 0.5 mol dm⁻³ H₂SO₄ and that cork maintains its binding capacity over four cycles of biosorption/elution.

Thrassyvoulos Manios, Edward I. Stentiford, Paul A. Millner. (Department of Agricultural Technology, Technological Education Institute of Crete, Heraklion, 71110, Crete, Greece. School of Civil Engineering, Leeds University, Leeds LS2 9JT, UK. School of Biochemistry and Molecular Biology, Leeds University, Leeds LS2 9JT UK). **The effect of heavy metals accumulation on the chlorophyll concentration of *Typha latifolia* plants, growing in a substrate containing sewage sludge compost and watered with metaliferous water**. Ecological Engineering, 20(1) (2003), 65-74.

Typha latifolia plants, commonly known as cattails, were grown in a mixture of sewage sludge compost, commercial compost and perlite. Four groups (A, B, C and U) were irrigated (once every 2 weeks) with a solution containing different concentrations of Cd, Cu, Ni, Pb and Zn, where in the fifth (group M) tap water was used. At the end of the 10

weeks experimental period the mean concentration of Ni, Cu and Zn in the roots and leaves of the plants in the four groups was significantly larger to that of the plants of group M. A linear regression test satisfactorily correlated the metals' concentrations in the irrigation solutions with the metals concentration in the leaves and roots of groups A, B, C and D). The concentration of total chlorophyll, chlorophyll a (chl_a) and chlorophyll b (chl_b) in (he leaves of the developing plants was also monitored in 2 weeks intervals. Groups A, B, C and M presented an increasing concentration of total chlorophyll, with lime. In group U (stronger solution), the mean total chlorophyll concentration was reduced from 1080.69 µg/g fresh weight (f.w.) in the 8th week to 715.14 µg/g f.w., in the 10th week, a probable evidence of inhibition. When statistically tested, it was suggested that there was no significant difference between the mean chlorophyll values of the groups in each set of samples, concluding that no significant toxic action was imposed in the plants by the metals. However, when similar statistical analysis was implemented in the ratios of chl_a and chl_b, there was significant reduction of the ratios in group's D plants, suggesting .some increase in chlorophyll hydrolysis due to the metals accumulation (toxic effect) in comparison with the other groups.

Ufuk Alkan, Siddik Cindoruk, Yücel Tademir, Christopher Colby. (Uluda University, Department of Environmental Engineering, 16059 Görükle, Bursa, Turkey. University of Adelaide, Department of Chemical Engineering, Adelaide, Australia). **Influence of an aerobic selector on copper and hexavalent chromium biosorption by activated sludge.** Journal of Chemical Technology & Biotechnology, 77(10) (2002), 1141-1148.

The influence of an aerobic selector on biosorption of Cu and Cr(VI) by activated sludge was studied. *In-vitro* batch adsorption tests were performed using sludge harvested from bench-scale activated sludge systems. Metal biosorption by activated sludge was rapid with equilibrium usually reached within an hour. Adsorption behaviour closely followed a Freundlich isotherm model. Experimental data suggested that an aerobic selector increased the biosorption of the metal ions by activated sludge, confirming observations by others in a similar study but with different heavy metals. Freundlich isotherms indicated that the biosorption capacity of activated sludge was increased by 15% for Cu and 30% for Cr(VI). Activated sludge from both systems had a greater biosorption capacity for Cu than for Cr(VI). The effects of pH and sludge concentration were also investigated. The results indicate that these parameters may influence the biosorption characteristics of activated sludge.

Biocomposting

Ranjith Jayasekara, Greg T Lonergan, Ian Harding, Ian Bowater, Peter Halley, Gregor B Christie. (Centre for Applied Colloid and BioColloid Science, Swinburne University of Technology, Hawthorn, Victoria 3122, Australia. Cooperative Research Centre for International Food Manufacture and Packaging Science, Swinburne University of Technology, Hawthorn, Victoria 3122, Australia. Materials Characterisation and Processing Centre, The University of Queensland, Queensland, 4072, Australia). **An automated multi-unit composting facility for biodegradability evaluations.** Journal of Chemical Technology & Biotechnology, 76(4) (2001), 411-417.

A system has been developed for studying the biodegradation of natural and synthetic polymeric material. The system is based on standard methods developed by the European Committee for Standardisation (CEN TC 261) (ISO/DIS 14855) and the American Society of Testing Materials, ASTM Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials under Controlled Composting Conditions (ASTM D 5338-92). A new low-cost compost facility has been used which satisfies the requirements of these standards. The system has been automated for data collection and

has been run under the conditions specified by the standards. In the system, cellulose, newspaper and two starch-based polymers were treated with compost in a series of 3 dm³ vessels at 52 °C and under conditions of optimum moisture and pH. The degradation was followed over time by measuring the amount of carbon released as carbon dioxide.

Sharma Deepa, ChauhanUK. (Mici-obio Biotech Lab, Sch Env Bio, APS Univ, Rewa MP). **Biomanagement of cellulose waste.** *J Indian Assoc Environ Manag*, 27(3) (2000), 221-223.

Attempt was made at adopting cellulose as the base of disposal of garbage. The addition of nutrient-rich supplements, supported the growth of the inoculum culture of *Aspergillus terreus* (M₃). Booster inoculation of the culture maintained cellulose activity of the enzyme which in turn facilitated a faster degradation of the waste. Therefore the inoculated substrate was degraded to a higher extent as compared to the substrate of the uninoculated control.

Biodegradation

Abd El-Rahman Mansy, Ebtessam El-Bestawy. (Agricultural Research Center at Sabahia, Central Laboratory of Pesticides, Alexandria, Egypt. Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt). **Toxicity and biodegradation of fluometuron by selected cyanobacterial species.** *World Journal of Microbiology and Biotechnology*, 18(2) (2002), 125-131.

The Biodegradation capabilities of six selected cyanobacterial species for fluometuron, a phenylurea herbicide, as well as its inhibitory effect on chlorophyll a content were investigated. The selected species (three strains of *Microcystis aeruginosa*, *Anabaena cylindrica*, *A. flos-aquae* and *A. spiroides*) were subjected to three elevated concentrations of fluometuron (0.14, 0.7 and 1.4 mg/ml) for different exposure times (1–5 days). Results revealed that biodegradation of fluometuron is species-dependent and positively correlated with the exposure time, reaching maximum efficiency after 5 days at all the investigated concentrations. All the species tested showed generally great ability to degrade the compound even at the highest concentration with specific variations among them. Biodegradation efficiencies of fluometuron by the selected species were in the following ranges; 39.2–99.9; 87.5–100; and 93.2–100 at 0.14; 0.7 and 1.4 mg fluometuron/ml respectively. It was noticed that the gradual increase in the pesticide concentration enhances its biodegradability by the selected algal species. Variations according to species as well as exposure time were discussed. The highest fluometuron concentration (1.4 mg/l) showed the highest inhibition of chlorophyll a content in the tested species and toxicity was also species- and time-dependent.

A M EI-Masry, M F Ghaly, M A Khalafallah, Y A El-Fayed. (Chemistry and Botany Department. Faculty of Science. Zagazig University, Zagazig, Egypt). **Chemical and Biochemical Degradation of Waste Cellulosic Materials.** *Journal of Scientific & Industrial Research*, 61 (2002), 719-725.

Some of the agricultural wastes such as pith bagasse, rice husk and corn maize are subjected to both chemical and biochemical degradation. Different acid concentrations as well as cellulase-producing microorganisms are tested. Comparative studies between the chemical hydrolysis and enzymatic degradation, for the selected cellulosic materials, are discussed. Cellulase(s) enzyme activity, 50.25 ug/mL is obtained (pH 5-5 using 2g/L carbon source) in shaking culture. Results indicate that the optimum conc. of HCl, which

gives the highest yield of reducing sugars from the used cellulose materials is 5 per cent. Byproducts formed by acid degradation and enzymatic hydrolysis reveal that the residue of chemical hydrolysis is the most appropriate substrate for enzyme degradation rather than the raw materials. Screening of cellulase producing-organisms in the soil, show that actinomyces and fungi are predominant. The most efficient fungal isolate is identified as *Aspergillus ochraceus*. Occurrence of free reducing sugars in the growth medium as well as prolonged incubation times, both drastically affect cellulase(s) yield. Reduction in sugar content and cellulose remaining are inversely proportional.

A. M. Langenhoff, J. J. M. Staps, C. Pijls, A. Alphenaar, G. Zwiep, H. H. M. Rijnaarts. (TNO Environmental, Energy and Process Innovation, Department of Environmental Biotechnology, Apeldoorn, The Netherlands. Tauw Milieu Consultancy, Deventer, The Netherlands. Akzo Nobel Chemicals, Hengelo, The Netherlands). **Intrinsic and Stimulated *In Situ* Biodegradation of Hexachlorocyclohexane (HCH)**. *Water, Air and Soil Pollution: Focus*, 2(3) (2002), 171-181.

The feasibility of the biodegradation of HCH and its intermediates has been investigated. A recent characterisation of two sites in The Netherlands has shown intrinsic biodegradation of HCH. At one site, breakdown products (monochlorobenzene, benzene and chlorophenol) were found in the core of the HCH-plume, whereas the HCH-concentration decreased over time and space. Characterisation of a second, industrial site indicated less intrinsic biodegradation and the need to stimulate biodegradation. In the laboratory, enhanced HCH degradation was tested with soil and groundwater material from both sites, and the required conversion to the intermediates benzene and monochlorobenzene was demonstrated. Furthermore, the biodegradation of these intermediates could be initiated by adding low amounts of oxygen (<5%). Adding nitrate enhanced this degradation. We hypothesise that this occurs through anaerobic nitrate reducing conversion of oxidised intermediates. At the non-industrial other site, intrinsic degradation took place, as shown in the laboratory experiments. Interpretation of the field data with computer codes Modflow and RT3D was performed. As a result of the modelling study, it has been proposed to monitor natural attenuation for several years before designing the final approach. At the industrial site, the results of the batch experiments are applied. Anaerobic HCH degradation to monochlorobenzene and benzene is stimulated via the addition of an electron donor. Infiltration facilities have been installed at the site to create an anaerobic infiltration zone in which HCH will be degraded, and these facilities are combined with the redevelopment of the site.

Alan François, Hugues Mathis, Davy Godefroy, Pascal Piveteau, Françoise Fayolle,* and Frédéric Monot. (Institut Français du Pétrole, Département de Microbiologie, 92852 Rueil-Malmaison Cedex, France). **Biodegradation of Methyl *tert*-Butyl Ether and Other Fuel Oxygenates by a New Strain, *Mycobacterium austroafricanum* IFP 2012**. *Applied and Environmental Microbiology*, 68(6) 2002, 2754-2762.

A strain that efficiently degraded methyl *tert*-butyl ether (MTBE) was obtained by initial selection on the recalcitrant compound *tert*-butyl alcohol (TBA). This strain, a gram-positive methylotrophic bacterium identified as *Mycobacterium austroafricanum* IFP 2012, was also able to degrade *tert*-amyl methyl ether and *tert*-amyl alcohol. Ethyl *tert*-butyl ether was weakly degraded. *tert*-Butyl formate and 2-hydroxy isobutyrate (HIBA), two intermediates in the MTBE catabolism pathway, were detected during growth on MTBE. A positive effect of Co²⁺ during growth of *M. austroafricanum* IFP 2012 on HIBA was demonstrated. The specific rate of MTBE degradation was 0.6 mmol/h/g (dry weight) of cells, and the biomass yield on MTBE was 0.44 g (dry weight) per g of MTBE. MTBE, TBA, and HIBA degradation activities were induced by MTBE and TBA, and TBA was a good inducer. Involvement of at least one monooxygenase during degradation of MTBE and TBA was shown by (i) the requirement for oxygen, (ii) the production of propylene

epoxide from propylene by MTBE- or TBA- grown cells, and (iii) the inhibition of MTBE or TBA degradation and of propylene epoxide production by acetylene. No cytochrome P-450 was detected in MTBE- or TBA-grown cells. Similar protein profiles were obtained after sodium dodecyl sulfate-polyacrylamide gel electrophoresis of crude extracts from MTBE- and TBA-grown cells. Among the polypeptides induced by these substrates, two polypeptides (66 and 27 kDa) exhibited strong similarities with known oxidoreductases.

Albert L. Juhasz, Grant A. Stanley and Margaret L. Britz. (Centre for Bioprocessing and Food Technology, Victoria University of Technology, P.O. Box 14428 MCMC, Melbourne, Australia 8001. School of Life Sciences and Technology, Victoria University of Technology, P.O. Box 14428 MCMC, Melbourne, Australia 8001). **Degradation of High Molecular Weight PAHs in Contaminated Soil by a Bacterial Consortium: Effects on Microtox and Mutagenicity Bioassays.** *Bioremediation Journal*, 4(4) (2000), 271-283.

Bioaugmentation of polycyclic aromatic hydrocarbon (PAH)-contaminated soil was investigated using a mixed bacterial culture (community five) isolated from an abandoned industrial site. Community five was inoculated into contaminated soil containing a total PAH (two- to five-ring compounds) concentration of approximately 820 mg/kg soil. PAH degradation by the indigenous microbial population was restricted to the lower molecular weight compounds (naphthalene, acenaphthene, fluorene and phenanthrene) even with yeast extract addition: these compounds decreased by 14 to 37%, in soil hydrated to 50% water capacity, following 91 days of incubation at 24°C. Inoculation of community five into this PAH-contaminated soil resulted in significant decreases in the concentration of all PAHs over the incubation period: greater than 86% of naphthalene, acenaphthene, fluorene, and phenanthrene were degraded after 91 days, while anthracene, fluoranthene, and pyrene were degraded to lesser extents (51.7 to 57.6%). A lag period of 48 to 63 days was observed before the onset of benz[a]anthracene, benzo[a]pyrene, and dibenz[a, h]anthracene removal. However, significant decreases in the concentration of these compounds (32.6, 25.2, and 18.5%, respectively) were observed after 91 days. No significant decrease in the mutagenic potential of organic soil extracts (as measured by the Ames Test) was observed after incubation of the soil with the indigenous microflora; however, the Microtox toxicity of aqueous soil extracts was reduced sevenfold. In contrast, extracts from contaminated soil inoculated with community five underwent a 43% decrease in mutagenic potential and the toxicity was reduced 170-fold after 91 days incubation. These observations suggest that community five could be utilized for the detoxification of PAH-contaminated soil.

Andrew J. Ellis, Stephen G. Hales, Naheed G. A. Ur-Rehman, Graham F. White. (School of Biosciences, Cardiff University, Cardiff CF10 3US, SEAC Environment Centre, Unilever Research Port Sunlight, Bebington, Wirral, Merseyside L63 3JW, United Kingdom). **Novel Alkylsulfatases Required for Biodegradation of the Branched Primary Alkyl Sulfate Surfactant 2-Butyloctyl Sulfate.** *Applied and Environmental Microbiology*, 68(1) (2002), 31-36.

Recent reports show that contrary to common perception, branched alkyl sulfate surfactants are readily biodegradable in standard biodegradability tests. We report here the isolation of bacteria capable of biodegrading 2-butyloctyl sulfate and the identification of novel enzymes that initiate the process. Enrichment culturing from activated sewage sludge yielded several strains capable of growth on 2-butyloctyl sulfate. Of these, two were selected for further study and identified as members of the genus *Pseudomonas*. Strain AE-A was able to utilize either sodium dodecyl sulfate (SDS) or 2-butyloctyl sulfate as a carbon and energy source for growth, but strain AE-D utilized only the latter. Depending on growth conditions, strain AE-A produced up to three alkylsulfatases, as shown by polyacrylamide gel electrophoresis zymography. Growth on

either SDS or 2-butyloctyl sulfate or in nutrient broth produced an apparently constitutive, nonspecific primary alkylsulfatase, AP1, weakly active on SDS and on 2-butyloctyl sulfate. Growth on 2-butyloctyl sulfate produced a second enzyme, AP2, active on 2-butyloctyl sulfate but not on SDS, and growth on SDS produced a third enzyme, AP3, active on SDS but not on 2-butyloctyl sulfate. In contrast, strain AE-D, when grown on 2-butyloctyl sulfate (no growth on SDS), produced a single enzyme, DP1, active on 2-butyloctyl sulfate but not on SDS. DP1 was not produced in broth cultures. DP1 was induced when residual 2-butyloctyl sulfate was present in the growth medium, but the enzyme disappeared when the substrate was exhausted. Gas chromatographic analysis of products of incubating 2-butyloctyl sulfate with DP1 in gels revealed the formation of 2-butyloctanol, showing the enzyme to be a true sulfatase. In contrast, *Pseudomonas* sp. strain C12B, well known for its ability to degrade linear SDS, was unable to grow on 2-butyloctyl sulfate, and its alkylsulfatases responsible for initiating the degradation of SDS by releasing the parent alcohol exhibited no hydrolytic activity on 2-butyloctyl sulfate. DP1 and the analogous AP2 are thus new alkylsulfatase enzymes with novel specificity toward 2-butyloctyl sulfate.

Anita Singh, Om P. Sharma and Sudarshan Ojha. (Biochemistry Laboratory, Indian Veterinary Research Institute, Regional Station, Kangra Valley, Palampur 176 061, Himachal Pradesh, India. Department of Biochemistry, Punjab University, Chandigarh 160 014, India). **Biodegradation of lantadene A, the hepatotoxin of lantana plant, by *Alcaligenes odorans*. International Biodeterioration & Biodegradation** 46(2) (2000), 107-110.

A bacterial strain, *Alcaligenes odorans*, has been isolated, by enrichment from soil, using lantadene A (LA), the pentacyclic triterpenoid from lantana plant, as the sole carbon source. The strain is Gram negative, motile, catalase positive and is capable of utilizing LA. The utilization of LA was less when glucose was used as cosubstrate. The isolate did not utilize lantadene B, a congener of lantadene A.

B. Qi, W. M. Moe, K. A. Kinney. (Department of Civil and Environmental Engineering, Louisiana State University, Baton Rouge, LA 70803, USA. Department of Civil Engineering, University of Texas, Austin, TX 78712, USA). **Biodegradation of volatile organic compounds by five fungal species**. Applied Microbiology and Biotechnology, 58(5) (2002), 684-689.

Five fungal species, *Cladosporium resinae* (ATCC 34066), *Cladosporium sphaerospermum* (ATCC 200384), *Exophiala lecanii-corni* (CBS 102400), *Mucor rouxii* (ATCC 44260), and *Phanerochaete chrysosporium* (ATCC 24725), were tested for their ability to degrade nine compounds commonly found in industrial off-gas emissions. Fungal cultures inoculated on ceramic support media were provided with volatile organic compounds (VOCs) via the vapor phase as their sole carbon and energy sources. Compounds tested included aromatic hydrocarbons (benzene, ethylbenzene, toluene, and styrene), ketones (methyl ethyl ketone, methyl isobutyl ketone, and methyl propyl ketone), and organic acids (*n*-butyl acetate, ethyl 3-ethoxypropionate). Experiments were conducted using three pH values ranging from 3.5 to 6.5. Fungal ability to degrade each VOC was determined by observing the presence or absence of visible growth on the ceramic support medium during a 30-day test period. Results indicate that *E. lecanii-corni* and *C. sphaerospermum* can readily utilize each of the nine VOCs as a sole carbon and energy source. *P. chrysosporium* was able to degrade all VOCs tested except for styrene under the conditions imposed. *C. resinae* was able to degrade both organic acids, all of the ketones, and some of the aromatic compounds (ethylbenzene and toluene); however, it was not able to grow utilizing benzene or styrene under the conditions tested. With the VOCs tested, *M. rouxii* produced visible growth only when supplied with *n*-butyl acetate or ethyl 3-ethoxypropionate. Maximum growth for most

fungi was observed at a pH of approximately 5.0. The experimental protocol utilized in these studies is a useful tool for assessing the ability of different fungal species to degrade gas-phase VOCs under conditions expected in a biofilter application.

Ch V Ramana, K Arunasri, Ch. Sasikala. (Department of Plant Science, School of Life Science, University of Hyderabad, P.O. Central University, Hyderabad 500046, India. Environmental Microbial Biotechnology Laboratory, Center for Environment, IPGS&R. J. N. T. University, Mahaveer Marg, Hyderabad 500028, India). **Photobiodegradation of pyridine by Rhodospseudomonas palustris JA1**. Indian Journal of Experiment Biology, 40 (2002), 967-970.

A purple non-sulfur bacterium isolated from dairy effluent was identified as *Rps. palustris* JA1. This organism was able to grow on pyridine as sole source of carbone in a light dependent anaerobic process with a doubling time of 30h. Intermediates of pyridine photodegradation were identified as glycine and malonate, produced in stoichiometric molar ratios with simultaneous utilization, yielding biomass.

Chaudhuri S, Gongopadhyay A, Mishra AK (Dept Civil Engng, Jadavpur Univ, Calcutta). **Bacterial degradation of indole**. *J Indian Assoc Environ Manag*, 27(3) (2000), 279-283.

Studies on aerobic degradation of indole revealed that indole can be utilized as a sole source of carbon and nitrogen by a number of microorganisms and can be transformed to its metabolites. Experiments showed that further growth of micro-orgaisms could not be made possible because of the presence of these metabolites. Attempts are being made to degrade these derivatives further to have complete oxidation of indole.

Chetan T. Goudar, Timothy G. Ellis. (School of Civil Engineering and Environmental Science, University of Oklahoma, Norman, OK 73019 USA. Department of Civil and Construction Engineering, Iowa State University, Ames, Iowa 50011 USA). **Explicit oxygen concentration expression for estimating extant biodegradation kinetics from respirometric experiments**. *Biotechnology and Bioengineering*, 75(1) (2001), 74-81.

We present a simple method for estimating extant biodegradation kinetic parameters from oxygen uptake data obtained during respirometric experiments. Specifically, a novel closed-form solution based on the Lambert W function is presented for the differential equation describing substrate biodegradation based on the Monod equation. Unlike the existing implicit solution, this novel solution is explicit with respect to the substrate concentration and, when coupled with the oxygen uptake equation, results in a simple algebraic expression for dissolved oxygen concentration in respirometric experiments. This new solution provided highly accurate estimates of dissolved oxygen concentrations with accuracy on the order of 10^{-15} for calculations performed using double precision arithmetic. The applicability of this approach for estimating extant biodegradation kinetic parameters was verified using synthetic dissolved oxygen concentration data that incorporated normally distributed noise to mimic experimental data. A combination of the W function description of oxygen concentration and a nonlinear optimization routine resulted in estimates of the Monod kinetic parameters, μ_m and K_s , that were close to the actual values, indicating the suitability of this approach for extant kinetic parameter estimation. This approach was subsequently tested on experimental oxygen concentration data obtained during ethylene-glycol biodegradation in respirometric experiments. The availability of simple algorithms for evaluating the W function makes the new solution easier to compute than current methods that rely on numerical solution of differential or nonlinear equations. The simplicity and accuracy

associated with use of the W function to describe oxygen concentration data should make it an attractive approach for estimating extant Monod biodegradation kinetic parameters from respirometric experiments.

Christopher Juneson, Owen P. Ward, Ajay Singh. (Microbial Biotechnology Laboratory, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1). **Biodegradation of dimethyl phthalate with high removal rates in a packed-bed reactor.** World Journal of Microbiology and Biotechnology, 18(1) (2002), 7-10.

Biological treatment of a dimethyl phthalate (DMP)-containing waste stream was evaluated in packed-bed bioreactors using an acclimated mixed bacterial culture. The passive immobilization start-up strategy was successful in the development of a stable biofilm on the packing material in the reactor. Nutrient supplementation significantly improved the removal efficiency. High removal rates with 100% efficiencies of DMP removal were achieved up to the phthalate-loading rate of 560 g/m³ h.

Chun F. Shen, Jalal Hawari, Guy Ampleman, Sonia Thiboutot and Serge R. Guiot. (Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec, H4P 2R2, Canada. Defense Research Establishment Valcartier, 2459 Pie XI Blvd-North, Val-Belair, Quebec G3J 1X5, Canada). **Enhanced Biodegradation and Fate of Hexahydro 1,3,5-Trinitro-1,3,5-Triazine (RDX) and Octahydro 1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX) in Anaerobic Soil Slurry Bioprocess.** Bioremediation Journal, 4(1) (2000), 27-39.

Native soil microbial populations and unadapted municipal anaerobic sludges were compared for nitramine explosive degradation in microcosm assays under various conditions. Microbial populations from an explosive-contaminated soil were only able to mineralize 12% hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (at a concentration of 800 mg/kg slurry) or 4% octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (at a concentration of 267 mg/kg slurry). In contrast, municipal anaerobic sludges were able to mineralize them to carbondioxide, with efficiencies of up to 65%. Reduction of RDX and HMX into their corresponding nitroso-derivatives was notably faster than their mineralization. The biodegradation of HMX was typically delayed by the presence of RDX in the microcosm, confirming RDX is used as an electron acceptor preferentially to HMX. The laboratory-scale bioslurry reactor reproduced the results of the microcosm assays, yet with much higher RDX and HMX degradation rates. A radiolabel-based mass balance in the soil slurry indicated that, besides a significant mineralization to carbon dioxide, 25% and 31% of RDX and HMX, respectively, appeared as acetonitrile-extractable metabolites, while the remaining part was incorporated into biomass and irreversibly bound to the soil matrix. About 10% of the HMX derivatives were estimated to be chemically bound to the soil matrix, while for RDX the estimation was nil.

Cihat Tascioglu, Barry Goodell, Roberto Lopez-Anido, Michael Peterson, William Halteman and Jody Jellison. (Wood Science and Technology, 5755 Nutting Hall, University of Maine, Orono, ME 04469-5755, USA. Department of Civil and Environmental Engineering, Advanced Engineered Wood Composite Center, Advanced Structures and Composites Laboratory, University of Maine, Orono, ME 04469-5793, USA. Department of Mechanical Engineering, Advanced Engineering Wood Composites Center, Advanced Structures and Composites Laboratory, University of Maine, Orono, ME 04469-5793, USA. Department of Mathematics, USA. Department of Molecular Plant Pathology, USA). **Monitoring fungal degradation of E-glass/phenolic fiber reinforced polymer (FRP) composites used in wood reinforcement.** International Biodeterioration & Biodegradation, 51(3) (2003), 157-165.

The susceptibility of E-glass fiber reinforced polymer (FRP)/phenolic pultruded composite plates to fungal degradation was examined. Interlaminar shear strength (ILSS) by short-beam testing and ultrasonic non-destructive evaluation (NDE) techniques were applied to monitor fungal degradation of E-glass fiber reinforced polymer composites. Since the FRP material was designed for use as reinforcement with wood, the FRP material was exposed to two common wood decay fungi, a brown rot fungus and a white rot fungus. Light and scanning electron microscopy indicated that both wood decay fungi actively grew and penetrated into the FRP material, especially in high-void content areas. The reduction in apparent ILSS of the brown rot-exposed FRP material was not statistically significant at a 95% confident level. A weak relationship between decay exposure and ILSS strength loss, however, was observed. The experimental results indicate that both mechanical property evaluation techniques (ILSS and NDE) may be sensitive enough to detect the effects of fungal degradation in FRP (5K) Fig. 1. Cutting schematic of FRP specimens: (A) 25.4 mm (1") square coupons for soil block and ultrasonic NDE tests, thickness 3.18 mm (0.125"), and (B) short beams were cut oriented with the unidirectional core fiber direction for interlaminar shear testingly exposed for 24 weeks (<1K).

D M Reynolds. (Centre for Research in Environmental Systems, Pollution and Remediation, Faculty of Applied Sciences, University of the West of England, Coldharbour Lane, Bristol BS16 1QY, UK). **The differentiation of biodegradable and non-biodegradable dissolved organic matter in wastewaters using fluorescence spectroscopy.** Journal of Chemical Technology & Biotechnology, 77(8) (2002), 965-972.

The chemical and biochemical oxygen demand values of a number of synthetic and wastewater samples were determined using fluorescence spectroscopy. Treated and untreated sewage samples were obtained from a local sewage treatment works while synthetic samples were analysed before, during, and after treatment via a rotating biodisc contactor. Fluorescence intensities were normalised using the water Raman signal as an internal standard and corrections applied to take into account the attenuation effects caused by the sample matrix. The fluorescence emission spectra ($\text{exc} = 280 \text{ nm}$) of synthetic and sewage samples were very similar in that two main fluorescence bands centred around 350 nm and 440 nm were observed in all samples. Normalised fluorescence data, centred at 350 nm, correlate well with corresponding BOD, COD and TOC values (R^2 values ranging between 0.93 and 0.98). Using BOD, COD and TOC data the fluorescence at 350 nm and 440 nm can be apportioned to biodegradable and non-biodegradable dissolved organic matter respectively. The findings of this research show that fluorescence data can be used to quantify oxygen demand values (chemical and biochemical) and total organic carbon values. Furthermore, the fluorescence spectral response can be apportioned to biodegradable (BOD) and non-biodegradable (COD - BOD) dissolved organic matter. The potential of using fluorescence spectroscopy as a possible tool for real-time monitoring of sewage wastes is discussed.

Darrell A Patterson, Ian S Metcalfe, Feng Xiong, Andrew G Livingston. (Bioseparations and Environmental Applications Group, Department of Chemical Engineering and Chemical Technology, Imperial College of Science, Technology & Medicine, London SW7 2BY, UK. Department of Chemical Engineering, UMIST, PO Box 88, Manchester M60 1QD, UK. Air Products PLC, European Technology Group, Basingstoke RG24 8FE, UK). **Biodegradability of linear alkylbenzene sulfonates subjected to wet air oxidation.** Journal of Chemical Technology & Biotechnology, 77(9) (2002), 1039-1049.

Shake flask experiments were conducted to determine the biodegradability of aqueous linear alkylbenzene sulfonate (LAS) and LAS (1600 mg dm^{-3}) subjected to wet air oxidation (WAO), to assess the suitability of WAO as a pre-treatment for biological

degradation. The effects of WAO temperature (180-240 °C) and the concentration of the orthophosphoric acid catalyst (0-1.0 mol dm⁻³) were investigated. Results showed that a higher WAO temperature increased the biodegradability of the WAO effluent. This was due to a greater removal of both recalcitrant sulfonated organics and organic concentration (TOC and COD). Conversely, greater orthophosphoric acid concentrations decreased the biodegradability of the WAO effluents. This was because the higher acid concentration increased the ionic strength and changed the WAO intermediate and product distribution, inhibiting microbial action. Nevertheless, the effluents from both variations of WAO were still more biodegradable than LAS at equivalent concentrations. However, since higher WAO temperatures can substantially increase capital costs, future work should focus on developing a WAO catalyst that both desulfonates and mildly oxidises LAS at moderate temperatures (200 °C).

Deka s (Inst Adv Std Sci Techno, Kanapara, Guwahati 781022, Assam). **Bacterial strains, degrading crude oil from petroleum polluted soil of Assam.** *Polln Res*, 20(4) (2001), 517-521.

A total of four bacterial strains have been isolated from petroleum polluted soil of oil field situated at Moran (Assam). Strains were identified as *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Bacillus aneurinolyticus* and *Serratia marcescens*. In a laboratory conducted experiment it was observed that *Pseudomonas stutzeri* was the most efficient hydrocarbon degraders amongst the bacterial strains isolated from petroleum polluted soil of Assam.

Diane Fournier, Annamaria Halasz, Jim Spain, Petr Fiuřasek, and Jalal Hawari. (Biotechnology Research Institute, National Research Council of Canada, Montreal (Quebec) H4P 2R2, Canada, United States Air Force Research Laboratory, Tyndall Air Force Base, Panama City, Florida 32403). **Determination of Key Metabolites during Biodegradation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine with *Rhodococcus* sp. Strain DN22.** *Applied and Environmental Microbiology*, 68(1) (2002), 166-172.

Rhodococcus sp. strain DN22 can convert hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to nitrite, but information on degradation products or the fate of carbon is not known. The present study describes aerobic biodegradation of RDX (175 µM) when used as an N source for strain DN22. RDX was converted to nitrite (NO₂⁻) (30%), nitrous oxide (N₂O) (3.2%), ammonia (10%), and formaldehyde (HCHO) (27%), which later converted to carbon dioxide. In experiments with ring-labeled [¹⁵N]-RDX, gas chromatographic/mass spectrophotometric (GC/MS) analysis revealed N₂O with two molecular mass ions: one at 44 Da, corresponding to ¹⁴N¹⁴NO, and the second at 45 Da, corresponding to ¹⁵N¹⁴NO. The nonlabeled N₂O could be formed only from -NO₂, whereas the ¹⁵N-labeled one was presumed to originate from a nitramine group (¹⁵N-¹⁴NO₂) in RDX. Liquid chromatographic (LC)-MS electrospray analyses indicated the formation of a dead end product with a deprotonated molecular mass ion [M-H] at 118 Da. High-resolution MS indicated a molecular formula of C₂H₅N₃O₃. When the experiment was repeated with ring-labeled [¹⁵N]-RDX, the [M-H] appeared at 120 Da, indicating that two of the three N atoms in the metabolite originated from the ring in RDX. When [U-¹⁴C]-RDX was used in the experiment, 64% of the original radioactivity in RDX incorporated into the metabolite with a molecular weight (MW) of 119 (high-pressure LC/radioactivity) and 30% in ¹⁴CO₂ (mineralization) after 4 days of incubation, suggesting that one of the carbon atoms in RDX was converted to CO₂ and the other two were incorporated in the ring cleavage product with an MW of 119. Based on the above stoichiometry, we propose a degradation pathway for RDX based on initial denitration followed by ring cleavage to formaldehyde and the dead end product with an MW of 119.

F. X. Prenafeta-Boldú, J. Vervoort, J. T. C. Grotenhuis, and J. W. van Groenestijn. (Division of Industrial Microbiology, Subdepartment of Environmental Technology, Wageningen University, 6700 EV Wageningen, Laboratory of Biochemistry, Wageningen University, 6700 HB Wageningen, Department of Environmental Biotechnology, TNO Environment, Energy and Process Innovation, Apeldoorn, The Netherlands). **Substrate Interactions during the Biodegradation of Benzene, Toluene, Ethylbenzene, and Xylene (BTEX) Hydrocarbons by the Fungus *Cladophialophora* sp. Strain T1.** Applied and Environmental Microbiology, 68(6) (2002), 2660-2665.

The soil fungus *Cladophialophora* sp. strain T1 (= ATCC MYA-2335) was capable of growth on a model water-soluble fraction of gasoline that contained all six BTEX components (benzene, toluene, ethylbenzene, and the xylene isomers). Benzene was not metabolized, but the alkylated benzenes (toluene, ethylbenzene, and xylenes) were degraded by a combination of assimilation and cometabolism. Toluene and ethylbenzene were used as sources of carbon and energy, whereas the xylenes were cometabolized to different extents. *o*-Xylene and *m*-xylene were converted to phthalates as end metabolites; *p*-xylene was not degraded in complex BTEX mixtures but, in combination with toluene, appeared to be mineralized. The metabolic profiles and the inhibitory nature of the substrate interactions indicated that toluene, ethylbenzene, and xylene were degraded at the side chain by the same monooxygenase enzyme. Our findings suggest that soil fungi could contribute significantly to bioremediation of BTEX pollution.

Fabio Fava, Lorenzo Bertin, Stefano Fedi, Davide Zannoni. (DICASM, Faculty of Engineering, University of Bologna, Viale Risorgimento 2, I-40136 Bologna, Italy. Department of Biology, University of Bologna, Bologna, Italy). **Methyl- β -cyclodextrin-enhanced solubilization and aerobic biodegradation of polychlorinated biphenyls in two aged-contaminated soils.** Biotechnology and Bioengineering, 81(4) (2003), 381-390.

The bioremediation of aged polychlorinated biphenyl (PCB)-contaminated soils is adversely affected by the low bioavailability of the pollutants. Randomly methylated- β -cyclodextrins (RAMEB) were tested as a potential PCB-bioavailability-enhancing agent in the aerobic treatment of two aged-contaminated soils. The soils, contaminated by about 890 and 8500 mg/kg of Aroclor 1260 PCBs, were amended with biphenyl (4 g/kg), inorganic nutrients (to adjust their C:N ratio to 20:1), and variable amounts of RAMEB (0%, 0.5%, or 1.0% [w/w]) and treated in both aerobic 3-L solid-phase reactors and 1.5-L packed-bed loop reactors for 6 months. Notably, significant enhancement of the PCB biodegradation and dechlorination, along with a detectable depletion of the initial soil ecotoxicity, were generally observed in the RAMEB-treated reactors of both soils. RAMEB effects were different in the two soils, depending upon the treatment conditions employed, and generally increased proportionally with the concentration at which RAMEB was applied. RAMEB, which was slowly metabolized by the soil's aerobic microorganisms, was found to markedly enhance the occurrence of the indigenous aerobic, cultivable biphenyl-growing bacteria harboring genes homologous to those of two highly specialized PCB degraders (i.e., *bphABC* genes of *Pseudomonas pseudoalcaligenes* KF707 and *bphA1A2A3A4BC1* genes of *Rhodococcus globerulus* P6) and chlorobenzoic acid-degrading bacteria as well as the occurrence of PCBs in the water phase of the soil reactors. These findings indicate that RAMEB enhanced the aerobic bioremediation of the two soils by increasing the bioavailability of PCBs and the occurrence of specialized bacteria in the soil reactors.

G. T. Taylor. (AWE, Aldermaston, Reading RG7 4PR, UK). **Evaluation of the potential of biodegradation for the disposal of cutting fluid from the machining of uranium.** International Biodeterioration & Biodegradation, 47(4) (2001), 215-224.

The disposal of used cutting fluid from the machining of uranium is problematical. Biodegradation offers the potential to convert this material into forms amenable to disposal as low level radioactive waste. The real bonus of biodegradation for radioactive applications crucially depends on the degree of mineralisation achieved. In non-radioactive trials using a consortium of bacteria selected from used cutting fluid, only 33% of the organic carbon was converted to carbon dioxide, even though 90% of the principal component (hydrocarbons) was biodegraded. Intermediate degradation products (identified as naphthenic acids) accumulated. Downstream processing of the biotreated fluid by ultrafiltration and adsorption onto activated charcoal produced a waste stream that would qualify as aqueous radioactive waste. Separated biomass was immobilised in a cement matrix that would qualify as solid radioactive waste, albeit in a volume which would make the overall process inefficient. Future work to optimize the process is proposed.

G.Emtiaz. (Biology Department, University of Esfahan, Iran, P. O. Box 81745-117). **Decolorization and biodegradation of dyes by *Aspergillus terreus* grown on wheat straw with Mn peroxidase activity.** Poll Res, 19(1) (2000), 31-35.

Aspergillus terreus isolated from rotten wood decolorized several dyes used in textile industries, including Solamine blue, Solamine yellow, Solamine red, Solamine scarlet, Terter direct orange, Terter direct Hue and Anilin blue. Decolorization was favoured when the *Aspergillus terreus* was grown on wheat straw with cellulase and Mn peroxidase activities. This fungus could biodegrade these dyes and utilize them as the only source of carbon, nitrogen and energy. The COD removal and production of ammonia during biodegradation of dyes by the isolated fungi were investigated.

Gabriele Pinto, Antonino Pollio, Lucio Previtera, Fabio Temussi. (Dipartimento di Biologia Vegetale, Università Federico II, Via Foria 223, I 80139 Napoli, Italy. Dipartimento di Chimica Organica e Biochimica, Università Federico II, Via Cynthia 4, I-80126 Napoli, Italy). **Biodegradation of phenols by microalgae.** Biotechnology Letters, 24(24) (2002), 2047-2051.

Two green microalgae, *Ankistrodesmus braunii* and *Scenedesmus quadricauda*, degraded phenols (each tested at 400 mg ml⁻¹) selected from olive-oil mill wastewaters, within 5 days, with a removal greater than 70%. Green algae may, therefore, represent an alternative to other biological treatment used for the biodegradation of phenol-containing wastewaters.

Gary T. Howard. (Department of Biological Sciences, Southeastern Louisiana University, SLU 10736, Hammond, LA 70402, USA). **Biodegradation of polyurethane: a review. International Biodeterioration & Biodegradation,** 49(4) (2002), 245-252.

Lack of degradability and the closing of landfill sites as well as growing water and land pollution problems have led to concern about plastics. Increasingly, raw materials such as crude oil are in short supply for the synthesis of plastics, and the recycling of waste plastics is becoming more important. As the importance of recycling increases, so do studies on elucidation of the biodegradability of polyurethanes. Polyurethanes are an important and versatile class of man-made polymers used in a wide variety of products in the medical, automotive and industrial fields. Polyurethane is a general term used for a class of polymers derived from the condensation of polyisocyanates and polyalcohols. Despite its xenobiotic origins, polyurethane has been found to be susceptible to biodegradation by naturally occurring microorganisms. Microbial degradation of polyurethanes is dependent on the many properties of the polymer such as molecular orientation, crystallinity, cross-linking and chemical groups present in the molecular

chains which determine the accessibility to degrading-enzyme systems. Esterase activity (both membrane-bound and extracellular) has been noted in microbes which allow them to utilize polyurethane. Microbial degradation of polyester polyurethane is hypothesized to be mainly due to the hydrolysis of ester bonds by these esterase enzymes.

Gerald E. Speitel, Jr., Trina L. Engels and Daene C. McKinney. (University of Texas at Austin, Department of Civil Engineering, Austin, TX 78712, USA. ExxonMobil Production Co., Houston, TX 77002, USA). **Biodegradation of RDX in Unsaturated Soil.** *Bioremediation Journal*, 5(1) (2001), 1-11.

Hexahydro-1,3,5-trimtro-1,3,5-triazme (RDX) is a military explosive that is a common soil and groundwater contaminant at facilities that manufacture, handle, and dispose of munitions. One such facility is the U.S. Department of Energy Pantex Plant, the focus of this research in which the feasibility of in situ bioremediation of contaminated soil in the vadose zone was assessed. A batch technique using ^{14}C -RDX was developed to investigate the degradation of RDX under aerobic, microaerobic, and anaerobic conditions. In addition, the effect of nutrients (organic carbon and phosphorus) on biodegradation rates was studied. The extent of mineralization was quantified by monitoring the production of $^{14}\text{CO}_2$, and RDX biodegradation rates were estimated for each environmental condition. The results showed that RDX degraders were indigenous to the contaminated soil and degraded RDX to a significant extent under anaerobic conditions. Little biotransformation was observed under aerobic conditions. The addition of a biodegradable organic carbon source significantly increased the RDX biodegradation rate. Under appropriate environmental conditions, significant mineralization of RDX also was observed. The half-lives for the degradation of RDX under anaerobic conditions were approximately 60 days and decreased to approximately 40 days with nutrient addition. In contrast, the half-life for aerobic degradation was on the order of 1000 days, with an upper 95% confidence interval approaching infinity.

Giovanni Vallini, Stefania Frassinetti, Felicia D'Andrea, Giorgio Catelani and Monica Agnolucci. (Department of Science and Technology, Laboratories of Microbial Biotechnology and Environmental Microbiology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy. CNR, National Research Council, Soil Microbiology Center--Via del Borghetto 80, 56124 Pisa, Italy. Department of Bioorganic Chemistry and Biopharmacy, University of Pisa--Via Bonanno 33, 56126 Pisa, Italy). **Biodegradation of 4-(1-nonyl)phenol by axenic cultures of the yeast *Candida aquatextoris*: identification of microbial breakdown products and proposal of a possible metabolic pathway.** *International Biodeterioration & Biodegradation*, 47(3) (2001), 133-140.

Candida aquatextoris, a yeast recently described for its ability to use 4-(1-nonyl)phenol (*p*NP) as the sole carbon and energy source in aerobic conditions, has been studied in order to determine the degradation products deriving from the growth on such a compound which is of environmental concern because of its proved toxicity to several organisms. Two main metabolites, namely *trans*-4-hydroxy-cinnamic acid and 4-hydroxy-acetophenone (4-acetylphenol), have been identified through either TLC and NMR spectrometry analyses of liquid substrate from cultures of *C. aquatextoris* grown on *p*NP, with 4-acetylphenol that accumulates without any further degradation. These findings suggest that *C. aquatextoris* might metabolise *p*NP via terminal oxidation of the alkyl chain, followed by a β -oxidation pathway. On the basis of this evidence, a novel metabolic route for the microbial degradation of 4-(1-nonyl)phenol, at least in certain yeasts, is proposed.

Hans-Holger Liste, Antonio Quinones-Rivera, Jixin Tang. (Institute of Comparative and

Environmental Toxicology and Department of Crop and Soil Sciences. Cornell University, Ithaca, New York 14853, U.S.A. Institute of Comparative and Environmental Toxicology and Department of Crop and Soil Sciences, Cornell University, Ithaca, New York 14853, U.S.A). **Availability of Aged Toluene and Decane for Biodegradation in Soil.** Water, Air, and Soil Pollution, 133 (1-4) (2002), 227-234.

Toluene and decane which are common volatile organic compounds (VOCs) found in natural gas spills, have been tested for their availability to biodegradation in soil after various times of aging. For this purpose, single compounds were added to sterile soil in three concentrations of up to 1000 ppm and aged for 0, 5, and 70 days. After aging, soils were vented, inoculated and then incubated for 31 days. Both compounds were rapidly biodegraded reaching final soil concentrations below 1 ppm, regardless of the aging time. However, some sequestration of small amounts of aged chemical in soil was evident for decane (up to 0.7 ppm), especially when the compound was added in larger amounts of 1000 ppm.

Hong-Gyu Song. (Division of Biological Sciences, Kangwon National University Hyoja-dong 192-1, Chuncheon 200-701, South Korea). **Degradation of humus-bound metabolites generated from toluene and o-xylene in soil.** International Biodeterioration & Biodegradation, 51(2) (2003), 129-132.

We investigated the fate of ¹⁴C-labeled toluene and o-xylene in soil. Some of the metabolites of toluene and o-xylene were bound to the humic matrix rather than mineralized or incorporated into biomass. The distribution of the bound radioactive metabolites from toluene and o-xylene were respectively, 33.4% and 32.1% of original radioactivity. Most of the humus-bound metabolites (83.5–85.4%) from toluene and o-xylene were found in the humin fraction and less than 10% were incorporated to fulvic and humic acid, respectively. The bound metabolites from radioactive toluene and o-xylene were not extracted with various solvents, and they showed slow biodegradation. The mineralization rates of bound metabolites generated from toluene and o-xylene did not differ significantly, and the turnover times ranged between 3.9 and 4.6 years.

Hubert H. Attaway, Michael G. Schmidt. **Tandem Biodegradation of BTEX Components by Two Pseudomonas sp.** Curr Microbiol, 45 (2002), 30-36.

A co-culture of two *Pseudomonas putida* isolates was enriched from sediment on a mixture of benzene, toluene, ethylbenzene, *m*-xylene, *p*-xylene, and *o*-xylene. The co-culture readily degraded each of the compounds present. Benzene, toluene, and ethylbenzene were used as growth substrates by one isolate, while toluene, *m*-xylene, and *p*-xylene were used as growth substrates by the other. Neither isolate could grow on *o*-xylene, but it was removed in the presence of the other compounds presumably by co-metabolism. The findings presented here support other reports in which constructed communities were effectively used to degrade blends of between two and four of the components of BTEX. However, here the co-culture of two *P. putida* isolates effectively degraded a complete BTEX stream containing all six of the components.

J. Bradley Dickerson, Jonathan E. Blackwell, Jao J. Ou, Vivek R. Shinde Patil, Douglas J. Goetz. (Department of Biomedical Engineering, University of Memphis, Memphis, Tennessee, USA. Department of Biomedical Engineering, Duke University, Durham, North Carolina, USA. Department of Chemical Engineering, Ohio University, 172 Stocker Center, Athens, Ohio 45701, USA). **Limited adhesion of biodegradable microspheres to E- and P-selectin under flow.** Biotechnology and Bioengineering, 73(6) (2001), 500-509.

In a variety of disease settings the expression of the endothelial selectins E- and P-selectin appears to be increased. This feature makes these molecules attractive targets around which to design directed drug-delivery schemes. One possible approach for achieving such delivery is to use polymeric biodegradable microspheres bearing a humanized monoclonal antibody (MAb) for E- and P-selectin, MAb HuEP5C7.g2. Perhaps the simplest technique for "coupling" HuEP5C7.g2 to the microspheres is via nonspecific adsorption. Previous studies suggest, however, that the adsorption of proteins onto microspheres fabricated in the presence of a stabilizer such as poly(vinyl alcohol) (PVA) is limited. It is unclear to what extent this limited level of adsorbed HuEP5C7.g2 would be able to support adhesion to E- and P-selectin under flow conditions. To explore this issue, we prepared microspheres from the biodegradable polymer, poly(ϵ -caprolactone) (PCL), using a single emulsion process and PVA as a stabilizer. We then incubated the PCL microspheres with HuEP5C7.g2 and studied the adhesion of the resulting HuEP5C7.g2 microspheres to E- and P-selectin under in vitro flow conditions. We found that the HuEP5C7.g2 PCL microspheres exhibit specific adhesion to Chinese hamster ovary cells stably expressing P-selectin (CHO-P) and 4-h IL-1 β -activated human umbilical vein endothelial cells (HUVEC). In contrast, HuEP5C7.g2 PCL microspheres exhibit little adhesion to parental CHO cells or unactivated HUVEC. The attachment efficiency to the selectin substrates was quite low, with appreciable attachment occurring only at low shear (0.3 dyn/cm²). Other supporting data strongly suggest that the limited attachment efficiency is due to a low level of HuEP5C7.g2 adsorbed to the PCL microspheres. Although the attachment was limited, a significant percentage of the HuEP5C7.g2 PCL microspheres were able to remain adherent at relatively high shear (8 dyn/cm²). Combined, our data suggest that HuEP5C7.g2 PCL microspheres exhibit selective limited adhesion to cellular substrate expressing E- and P-selectin.

J. Steven Braunera, Mark A. *Widdowson*, John T. Novak and Nancy G. (Parsons, 30 Dan Road, Canton, MA 02120-2809 USA. 200 Patton Hall, Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0105 USA). **Biodegradation of a PAH Mixture by Native Subsurface Microbiota**. *Bioremediation Journal*, 6(1) (2002), 9-24.

Laboratory microcosm studies were conducted to estimate biodegradation rates for a mixture of five polycyclic aromatic hydrocarbon compounds (PAHs). Static microcosms were assembled using soil samples from two locations collected at a No. 2 fuel oil-contaminated site in the Atlantic Coastal Plain of Virginia. In microcosms from one location, five PAHs (acenaphthene, fluorene, phenanthrene, pyrene, and benzo(b)fluoranthene) biodegraded at net first-order rates of 1.08, 1.45, 1.13, 1.11, and 1.12 yr⁻¹ respectively. No observable lag period was noted and degradation in live microcosms ceased with the depletion of oxygen and sulfate after 125 days. In microcosms from a second location, net first-order biodegradation rates after an approximately 2-month lag period were 2.41, 3.28, and 2.98 yr⁻¹ for fluorene, phenanthrene, and pyrene, respectively. Acenaphthene and benzo(b)fluoranthene mass loss rates in the live microcosms were not statistically different from mass loss rates in control microcosms. Stoichiometric mass balance calculations indicate that the dominant PAH mass loss mechanism was aerobic biodegradation, while abiotic losses (attributed to micropore diffusion and oxidative coupling) ranged from 15 to 33% and biotic losses from sulfate-reduction accounted for 7 to 10% of PAH mass loss. Stoichiometric equations that include biomass yield are presented for PAH oxidation under aerobic and sulfate-reducing conditions.

James D. Stahl, Benoit Van Aken, Michael D. Cameron and Steven D. Aust. (Biotechnology Center, Utah State University, Logan UT 84322-4705, USA. Unit of Bioengineering, Catholic University of Louvain, Place Croix du Sud 2/19, 1348 Louvain-la-Neuve, Belgium). **Hexahydro-1,3,5-trimetro-1,3,5-triazme (RDX) Biodegradation in**

Liquid and Solid-State Matrices by *Phanerochaete chrysosporium*. Bioremediation Journal, 5(1) (2001), 13-25.

Extensive biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by the white-rot fungus *Phanerochaete chrysosporium* in liquid and solid matrices was observed. Some degradation in liquid occurred under nonligninolytic conditions, but was approximately 10 times higher under ligninolytic conditions. Moreover, elimination was accounted for almost completely as carbon dioxide. No RDX metabolites were detected. The degradation rates in liquid appeared to be limited to RDX concentration in solution (approximately 80 mg/L), but degradation rates in soil were nonsaturable to 250 mg/kg. Manganese-dependent peroxidase (MnP) and cellobiose dehydrogenase (CDH) from *P. chrysosporium*, but not lignin peroxidase, were able to degrade RDX. MnP degradation of RDX required addition of manganese, but CDH degraded RDX anaerobically without addition of mediators. Attempts to improve biodegradation by supplementing cultures with micronutrients showed that addition of manganese and oxalate stimulated degradation rates in liquid, sawdust, and sand by the fungus, but not in loam soil. RDX degradation by *P. chrysosporium* in sawdust and sand was better than observed in liquid. However, degradation in solid matrices by the fungus only began after a lag period of 2 to 3 weeks, during which time extractable metabolites from wood were degraded.

Jeyaramraja PR, Anthony R, Rajendran A, Rajakumar K, (Res Cent Bot, VHN Senth Kumara Nadar Coil, Virudhunagar 26001). **Decolourization of paper mill effluent by *Aspergillus fumigatus* in bioreactor.** Polln Res,20(3) (2001), 309-312.

Decolourization and phenol reduction of paper mill effluent by the fungus, *Aspergillus fumigatus* isolated from paper mill effluent were studied. Optimum condition with regard to carbon sources, nitrogen sources and the addition of surfactant were worked out. Repeated batch experiments in an aerated bioreactor were performed with Ca-alginate immobilized fungus. The immobilized fungal beads were found to be effective for eight batches of effluent treatment.

Jian-Shen Zhao, Annamaria Halasz, Louise Paquet, Chantale Beaulieu, Jalal Hawari. (Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada). **Biodegradation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine and Its Mononitroso Derivative Hexahydro-1-Nitroso-3,5-Dinitro-1,3,5-Triazine by *Klebsiella pneumoniae* Strain SCZ-1 Isolated from an Anaerobic Sludge.** Applied and Environmental Microbiology, 68(11) (2002), 5336-5341.

In previous work, we found that an anaerobic sludge efficiently degraded hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), but the role of isolates in the degradation process was unknown. Recently, we isolated a facultatively anaerobic bacterium, identified as *Klebsiella pneumoniae* strain SCZ-1, using MIDI and the 16S rRNA method from this sludge and employed it to degrade RDX. Strain SCZ-1 degraded RDX to formaldehyde (HCHO), methanol (CH₃OH) (12% of total C), carbon dioxide (CO₂) (72% of total C), and nitrous oxide (N₂O) (60% of total N) through intermediary formation of methylenedinitramine (O₂NNHCH₂NHNO₂). Likewise, hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) was degraded to HCHO, CH₃OH, and N₂O (16.5%) with a removal rate (0.39 μmol · h⁻¹ · g [dry weight] of cells⁻¹) similar to that of RDX (0.41 μmol · h⁻¹ · g [dry weight] of cells⁻¹) (biomass, 0.91 g [dry weight] of cells · liter⁻¹). These findings suggested the possible involvement of a common initial reaction, possibly denitration, followed by ring cleavage and decomposition in water. The trace amounts of MNX detected during RDX degradation and the trace amounts of hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine detected during MNX degradation suggested that another minor

degradation pathway was also present that reduced NO₂ groups to the corresponding NO groups.

Johannes A. C. Barth, Greg Slater, Christoph Schüth, Markus Bill, Angela Downey, Mike Larkin, and Robert M. Kalin. (Scottish Universities Environmental Research Centre, East Kilbride, Glasgow G75 0QF, Scotland, Questor Centre, The Queen's University of Belfast, Belfast BT9 5AG, Northern Ireland, United Kingdom, Department of Geology, University of Toronto, Toronto, Canada, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, Applied Geology Group, Geological Institute, University of Tübingen, D-72076 Tübingen, Germany, Environmental Science, University of California, Berkeley, California). **Carbon Isotope Fractionation during Aerobic Biodegradation of Trichloroethene by *Burkholderia cepacia* G4: a Tool To Map Degradation Mechanisms.** Applied and Environmental Microbiology, 68(4) (2002), 1728-1734.

The strain *Burkholderia cepacia* G4 aerobically mineralized trichloroethene (TCE) to CO₂ over a time period of ~20 h. Three biodegradation experiments were conducted with different bacterial optical densities at 540 nm (OD_{540S}) in order to test whether isotope fractionation was consistent. The resulting TCE degradation was 93, 83.8, and 57.2% (i.e., 7.0, 16.2, and 42.8% TCE remaining) at OD_{540S} of 2.0, 1.1, and 0.6, respectively. ODs also correlated linearly with zero-order degradation rates (1.99, 1.11, and 0.64 μmol h⁻¹). While initial nonequilibrium mass losses of TCE produced only minor carbon isotope shifts (expressed in per mille δ¹³C_{VPDB}), they were 57.2, 39.6, and 17.0% between the initial and final TCE levels for the three experiments, in decreasing order of their OD_{540S}. Despite these strong isotope shifts, we found a largely uniform isotope fractionation. The latter is expressed with a Rayleigh enrichment factor, δ, and was -18.2 when all experiments were grouped to a common point of 42.8% TCE remaining. Although, decreases of δ to -20.7 were observed near complete degradation, our enrichment factors were significantly more negative than those reported for anaerobic dehalogenation of TCE. This indicates typical isotope fractionation for specific enzymatic mechanisms that can help to differentiate between degradation pathways.

Jose R Marengo, Rebecca A Kok and Lisa A Burrows Battelle, Ranga R Velagaleti*, John M Stamm. (505 King Avenue, Columbus, OH 43201, USA, BASF Corporation. 8800 Line Avenue, Shreveport, LA 71106, USA, Abbott Laboratories, 1401 Sheridan Road, North Chicago;-IL 0064). **Biodegradation of ¹⁴C-Sarafloxacin Hydrochloride, A Fluoroquinolone Antimicrobial by *Phanerochaete Chrysosporium*.** Journal of Scientific & Industrial Research, 60 (2001), 121-130.

Four soil fungi, *Aspergillus*, *Penicillium*, and *Chaetomium*, and *Phanerochaete*, were screened in static liquid cultures for their ability to mineralize, ¹⁴C-labeled sarafloxacin hydrochloride, a fluoroquinolone antimicrobial agent registered for use against poultry disease. *Phanerochaete* showed the highest amount of mineralization among these four fungi in the screening test during 7 d of incubation. This was selected for evaluating the biotransformation (formation of metabolite components) and mineralization in a definitive test where ¹⁴C-sarafloxacin hydrochloride was exposed to *Phanerochaete* grown in liquid (potato dextrose broth) shake cultures and incubated in dark at 22 C for 49 d. The liquid medium and hyphal extracts were examined for the biotransformed products using high performance liquid chromatography (HPLC). Liquid Scintillation Counting (LSC) of broth and ¹⁴C-volatile trap solutions were used to count the radioactivity in respective matrices. Combustion and LSC analysis was used to determine the radioactivity bound to or assimilated into hyphal mass. During the definitive test, - 90 per cent of ¹⁴C-sarafloxacin hydrochloride was biotransformed by *Phanerochaete* in 7 d. Biotransformation was non-linear with very little degradation of sarafloxacin from day 7 to day 49 and <3 per cent change of concentration of sarafloxacin from day 7 to day 49. Biotransformation half-life was estimated to be 5 d. Sarafloxacin degraded into at

least six quantifiable biotransformation products (components A to F), four of which exceeded 10 per cent, and two of which exceeded 5 per cent of applied dose at least at one sampling time. Sarafloxacin was mineralized to CO₂ with maximum cumulative ¹⁴CO₂ production observed being 17.3 per cent of applied dose. The mineralization half-life was estimated to be 139 d. Radioactivity mass balance at all sampling times exceeded 96 per cent.

Jun Jin Yoon, Yoon Sung Nam, Jung Hoe Kim, Tae Gwan Park. (Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Yusong-gu, Kusong-dong, Taejon, 305-701, Korea). **Surface immobilization of galactose onto aliphatic biodegradable polymers for hepatocyte culture.** *Biotechnology and Bioengineering*, 78(1) (2002), 1-10.

A novel surface modification method of biodegradable polymers was investigated for inducing the attachment of specific cells onto the polymer surface via ligand-receptor interactions. Galactose, a targeting ligand specific to asialoglycoprotein receptors present on cell membrane of hepatocytes, was introduced on the surface of poly(D,L-lactic-co-glycolic acid) (PLGA) films. A terminal end group of carboxylic acid in PLGA was activated by dicyclohexylcarbodiimide and *N*-hydroxysuccinimide for the direct conjugation of lactose by reductive amination reaction. Di-block copolymers of PLGA-b-poly(ethylene glycol) (PEG) having a free terminal amine group were also synthesized and used for the conjugation of galactose for the introduction of a PEG spacer between PLGA and galactose. The presence of galactose moieties on the blend film surface was characterized by measuring water contact angle and X-ray photon spectroscopy, and the amount of galactose was indirectly determined by a specific lectin-binding assay. With increasing the galactose concentration on the blend film surface, the initial attachment as well as the cell viability of hepatocytes concomitantly increased. The introduction of PEG spacer reduced the cell attachment and viability. Albumin secretion rate from hepatocytes was enhanced for galactose modified surfaces, whereas it was reduced for the surfaces not having galactose moieties.

K. Fadil, A. Chahlaoui, A. Ouahbi, A. Zaid and R. Borja. (Laboratoire de Biochimie et Pharmacognosie, Département de Biologie, Faculté des Sciences de Meknès, BP 4010, Beni Mhamed, Meknès, Maroc. Laboratoire de Biotechnologie Animale Appliquée, Département de Biologie, Faculté des Sciences de Meknès, BP 4010, Beni Mhamed, Meknès, Maroc. Instituto de la Grasa (CSIC), Avenida Padre García Tejero 4, 41012-, Sevilla, Spain). **Aerobic biodegradation and detoxification of wastewaters from the olive oil industry.** *International Biodeterioration & Biodegradation*, 51(1) (2003), 37-41.

Growth and polyphenol biodegradation by three microorganisms namely *Geotrichum* sp., *Aspergillus* sp. and *Candida tropicalis* were studied on olive mill wastewater (OMW). These three microorganisms were selected for their tolerance to the polyphenols. The biodegradation process of OMW was investigated in batch regime by conducting experiments where the initial concentration of chemical oxygen demand (COD) was varied. Furthermore, some tests were performed to determine the most important nutrients necessary for aerobic degradation of OMW. Average COD removals were 55.0%, 52.5% and 62.8% in wastewaters fermented with *Geotrichum* sp., *Aspergillus* sp. and *C. tropicalis*, respectively. The maximum removal of polyphenols was 46.6% (*Geotrichum* sp.), 44.3% (*Aspergillus* sp.) and 51.7% (*C. tropicalis*). In addition, significant decolorization was evident.

K. Kovar, V. Chaloupka, Th. Egli. **A Threshold Substrate Concentration Is Required to Initiate the Degradation of 3-Phenylpropionic Acid in *Escherichia coli***. *Acta Biotechnol*, 22(3-4) (2002), 285-298.

A central question concerning the fate of chemicals (or pollutants) in the environment is that of their degradation at low concentrations. This problem was tackled in well-defined experiments using the bacterium *Escherichia coli* growing with two compounds of quite different chemical structure that are degraded via different catabolic pathways, namely glucose and 3-phenylpropionic acid (3-PPA). The study was performed in carbon-limited continuous cultures supplied with mixtures of glucose (always 100 mg/l) and 3-PPA (varied from 0.25 to 25 mg/l) in the inflowing medium. Cells grown only with glucose exhibited no 3-PPA-degrading activity. When such a glucose culture was exposed to low 3-PPA concentrations (by adding <3 mg/l 3-PPA to the feed), the catabolic pathway was not induced and 3-PPA accumulated unutilized in the culture. When higher concentrations of 3-PPA were supplied in the feed (5.1, 7.7, 25.2 and 50 mg/l) 3-PPA was always utilized. Hence, an apparent threshold concentration existed (~3 mg/l) below which the degradation of 3-PPA was not initiated. The experimental data suggest that neither a certain defined proportion of 3-PPA to glucose in the feed mixture, nor a particular initial specific flux of 3-PPA, but a certain extracellular concentration of 3-PPA is needed to initiate 3-PPA utilization. Once this threshold concentration is exceeded, the specific flux of 3-PPA affects both the time required until 3-PPA degradation become detectable and the steady-state residual 3-PPA concentration. When induced, the cells are able to utilize 3-PPA down to concentrations (<2 mg/l) lower than the required induction threshold. Also the utilization of 3-PPA is initiated faster at lower specific growth rates.

K. N. Duddleston, D. J. Arp, P. J. Bottomley. (Department of Microbiology, Room 220, Nash Hall, Oregon State University, Corvallis, OR 97331-3804, USA. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-3804, USA. Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331-3804, USA. Department of Biological Sciences, University of Alaska Anchorage, Anchorage, AK 99508, USA). **Biodegradation of monohalogenated alkanes by soil NH₃-oxidizing bacteria**. *Applied Microbiology and Biotechnology*, 59(4-5) (2002), 535-539.

Although cooxidative biodegradation of monohalogenated hydrocarbons has been well studied in the model NH₃-oxidizing bacterium, *Nitrosomonas europaea*, virtually no information exists about cooxidation of these compounds by native populations of NH₃-oxidizing bacteria. To address this subject, nitrifying activity was stimulated to 125-400 nmol NO₃⁻ produced g⁻¹ soil h⁻¹ by first incubating a Ca(OH)₂-amended, silt loam soil (pH 7.0±0.2) at field capacity (270 g H₂O kg⁻¹ soil) with 10 μmol NH₄⁺ g⁻¹ soil for 14 days, followed by another 10 days of incubation in a shaken slurry (2:1 water:soil, v/w) with periodic pH adjustment and maintenance of 10 mM NH₄⁺. These slurries actively degraded both methyl bromide (MeBr) and ethyl chloride (EtCl) at maximum rates of 20-30 nmol ml⁻¹ h⁻¹ that could be sustained for approximately 12 h. Although the MeBr degradation rates were linear for the first 10-12 h of incubation, they could not be sustained regardless of NH₄⁺ level and declined to zero over 20 h of incubation. The transformation capacity of the slurry enrichments (~1 μmol MeBr ml⁻¹ soil slurry) was similar to the value measured previously in cell suspensions of *N. europaea* with similar NH₃-oxidizing activity. Several MeBr-degrading characteristics of the nitrifying enrichments were found to be similar to those documented in the literature for MeBr-degrading methanotrophs and facultatively methylotrophic bacteria.

K. Nishimura, M. Yamamoto, T. Nakagomi, Y. Takiguchi, T. Naganuma, Y. Uzuka. (Department of Applied Chemistry and Biotechnology, Faculty of Engineering, Yamanashi

University, Kofu, Yamanashi 400-8511, Japan). **Biodegradation of triazine herbicides on polyvinylalcohol gel plates by the soil yeast *Lipomyces starkeyi***. Applied Microbiology and Biotechnology, 58(6) (2002), 848-852.

The soil yeast *Lipomyces starkeyi* was tested for its ability to degrade triazine herbicides. Polyvinylalcohol (PVA) was employed as a solid medium in culture plates instead of agar. The cell sizes of the control (without nitrogen source) on the PVA gel plate were much smaller than those on the agar gel plate. The difference between the diameters of the sample and control colonies on the PVA gel plate were almost twice those of the colonies on the agar gel plate (1.9 and 1.0 mm, respectively). Thus, the PVA gel plate is much better than the agar plate for evaluating the degree of utilization of a sole nitrogen source. The yeast grew well (more than 4 mm in diameter) with 1,3,5-triazine or cyanuric acid as nitrogen source. In addition, melamine and thiocyanuric acid inhibited growth of the yeast, and the sizes of colonies were smaller than those of the control. All triazine herbicides tested (simazine, atrazine, cyanazine, ametryn, and prometryn) could be degraded and assimilated by *L. starkeyi*.

K.A. Billingsley, S.M. Backus, S. Wilson, A. Sing, O.P. Ward. (Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada. National Laboratory for Environmental Testing, National Water Research Institute, 867 Lakeshore Road, Burlington, Ontario L7R 4A6, Canada). **Remediation of PCBs in soil by surfactant washing and biodegradation in the wash by *Pseudomonas* sp. LB400**. Biotechnology Letters, 24(21) (2002), 1827-1832.

Solutions from the washing of polychlorinated biphenyl (PCB)-contaminated soil with a variety of commercial nonionic or anionic surfactants were incubated with *Pseudomonas* sp. LB400 in an attempt to remediate the soil and destroy the PCBs. Nonionic surfactants washed more PCBs from the soil (up to 89%) but inhibited their biodegradation. Anionic surfactants washed less PCBs from the soil but were more effective in biodegradation tests, removing up to 67% of total PCBs.

Kazunori Ikeda, Masahiro Ono, Hideo Akiyama, Takuo Onizuka, Satoshi Tanaka, Hitoshi Miyasaka. (Kansai Environmental Engineering Center Co. Ltd., 3-5 Adzuchimachi 1-Chome Chuo-ku, Osaka, Osaka 541-0052, Japan. Biological Science Department, Toray Research Center, Inc. Teburo III, Kamakura, Kanagawa 248-8555, Japan. The Kansai Electric Power Co., Technical Research Center, 11-20 Nakoji 3-Chome, Amagasaki, Hyogo 661-0974, Japan). **Transformation of the fresh water cyanobacterium *Synechococcus* PCC7942 with the shuttle-vector pAQ-EX1 developed for the marine cyanobacterium *Synechococcus* PCC7002**. World Journal of Microbiology and Biotechnology, 18(1) (2002), 55-56.

The transformation of the fresh water cyanobacterium *Synechococcus* PCC7942 with the shuttle-vector pAQ-EX1 developed for the marine cyanobacterium *S. PCC7002* was examined. The *S. PCC7942* cells were successfully transformed with the pAQ-EX1 vector, and the vector was stably maintained in the transformant cells.

Kazuo Sugaya, Osamu Nakayama, Naoyuki Hinata, Koichi Kamekura, Akira Ito, Kazuaki Yamagiwa, Akira Ohkawa. (Graduate School of Science & Technology, Niigata University, Ikarashi 2, Niigata 950-2181, Japan. Niigata Engineering Co Ltd, 9-3 Kamatahoncho 1-Chome, Ohta-ku, Tokyo 144-8640, Japan. Japan Bioindustry Association, 2 6-9 Hacchobori 2-Chome, Chuo-ku, Tokyo 104 -0032, Japan. Japan National oil Corporation, 2-2, Uchisaiwaicho 2-Chome, Chiyoda-ku, Tokyo 100-851 I, Japan). **Biodegradation of quinoline in crude oil**. Journal of Chemical Technology & Biotechnology, 76(6) (2001), 603-611.

Removal of quinoline, which is typical of nitrogen-containing compounds in crude oil, was achieved by a biodegradation reaction by *Comamonas* sp TKV3-2-1. The aerobic strain, *Comamonas* sp TKV3-2-1, which can grow utilizing quinoline as the sources of both carbon and nitrogen, degraded quinoline to 2-hydroxyquinoline, finally to water soluble substances. The degradation reaction of 2-hydroxyquinoline was revealed to be regarded as a rate-limiting step controlling the over all reaction of biodegradation process of quinoline in crude oil. The degradation rate of 2- hydroxyquinoline in a stirred fermenter had a mixture of 211 mg 2- hydroxyquinoline g-cell⁻¹ h⁻¹ when the portion of crude oil in the reaction mixture, the cell concentration and the rotational speed of agitation impeller were 83.3% (v/v), 28.5 gdm⁻³ and 11.7 s⁻¹, respectively. After the reaction was completed, the crude oil and the cell suspension could be separated efficiently by centrifugation. The possibility of constructing a bioprocess for removing quinoline in crude oil under storage is also discussed.

Keith Strevett, Irene Davidova, Joseph M. Suflita. (School of Civil Engineering and Environmental Science, The University of Oklahoma, Norman, Oklahoma 73019, USA. Department of Botany and Microbiology; Institute for Energy and the Environment, The University of Oklahoma, Norman, Oklahoma 73019, USA). **A comprehensive review of the screening methodology for anaerobic biodegradability of surfactants.** Reviews in Environmental Science and Biotechnology, 1(2) (2002), 143-167.

A biodegradability assay should mimic *in situ* conditions as closely as possible. If this is not entirely possible, the assay should at least include inoculum from the site. This review attempts to condense current literature on anaerobic biodegradability assay and propose a clear assessment methodology to determine the *fate* surfactants in anaerobic environments. It has *been* well documented that surfactant concentration toxic to the microflora can lead to unwarranted failure of biodegradability assay. Thus an important recommendation is to first perform a toxicity evaluation with relevant controls. Based on the results of this evaluation, a Tier 1 biodegradability assay that assesses the rate of formation of reduced endproducts or the consumption of a particular terminal electron acceptor is recommended and supported by current literature. Balanced chemical equations for the complete mineralization of the substrate are then used to compare the amount of transformation that actually occurred with that theoretically expected. When required, result should be confirmed by Tier 2 testing, which includes monitoring of substrate disappearance over time using a variety of analytical tools. These recommended procedures are scientifically defensible and have the potential of providing environmentally relevant information on the fate of surfactant materials in the environment.

Ken Rainwater, Caryl Heintz, Tony Mollhagen and Lance Hansen. (Texas Tech University Water Resources Center, Lubbock, TX 79409-1022, USA. U.S. Army Corps of Engineers Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, USA). ***In Situ* Biodegradation of High Explosives in Soils: Field Demonstration.** Bioremediation Journal, 6(4) (2002), 351-371.

The first field pilot-scale demonstration of a technology for *in situ* remediation of vadose zone soils contaminated with high explosives (HEs) has been performed at the Department of Energy's Pantex Plant. The HEs of concern at the demonstration site were hexahydro-1,3,5-trinitro-1,3,5-triazine(RDX) and the 2,4,6-trinitrotoluene(TNT) metabolite 1,3,5-trinitrobenzene (TNB). Concentrations ranged from 70 ppm, above the (prior to 1999) risk reduction clean-up criteria of 2.6 and 0.51 ppm, respectively. The shallow (<10 in depth) soils at the site could not be excavated due to the presence of buried utilities. Based on previous laboratory studies, it was found that the contaminated soils had indigenous microbial populations that could be stimulated to degrade the RDX and TNB anaerobically. A 5-spot well pattern with injection at the central well and

extraction at the four outer wells (each 4.6 m from the injection well) was used to flood the target vadose zone soils with nitrogen gas with the intent of stimulating the activity of the HE degraders. The system was monitored periodically for gas composition as well as HE concentrations and microbial activity in retrievable soil samples. After 295 days of *in situ* treatment, the average target HE concentrations were approximately one-third lower than the initial site averages. Operation of the pilot-scale treatment system continues.

Kousar Nikhath, Singara Charya MA (Dept Bot, Environ Microbio, Kakatiya Univ, Kowshik Meenal, Nazareth Santa (Dept Microbio, Goa Univ, Taleigao Plateau 403206, Goa). **Bio sedimentation of mine tailings by *Fusarium solani***. *JIndIPolln Contl*, 17(2) (2001), 341-346.

Mine tailings have a high concentration of suspended material and metal complexes. It is routinely treated for sedimentation with various chemicals that in themselves are pollutants. The mycelial mass of *Fusarium solani* added to mine tailings, greatly increases the natural rate of sedimentation. Homogenization of the biomass increases its capacity for sedimentation. Storage of the mycelium up to a week, does not affect the sedimentation rate.

L. P. Wackett, M. J. Sadowsky, B. Martinez, N. Shapir. (Department of Biochemistry, Molecular Biology and Biophysics and Biotechnology Institute, University of Minnesota, 1479 Gortner Avenue, St. Paul, MN 55108, USA. Department of Soil, water and Climate, University of Minnesota, St. Paul, MN 55108, USA). **Biodegradation of atrazine and related s-triazine compounds: from enzymes to field studies**. *Applied Microbiology and Biotechnology*, 58(1) (2002), 39-45.

s-Triazine ring compounds are common industrial chemicals: pesticides, resin intermediates, dyes, and explosives. The fate of these compounds in the environment is directly correlated with the ability of microbes to metabolize them. Microbes metabolize melamine and the triazine herbicides such as atrazine via enzyme-catalyzed hydrolysis reactions. Hydrolytic removal of substituents on the s-triazine ring is catalyzed by enzymes from the amidohydrolase superfamily and yields cyanuric acid as an intermediate. Cyanuric acid is hydrolytically processed to yield 3 mol each of ammonia and carbon dioxide. In those cases studied, the genes underlying the hydrolytic reactions are localized to large catabolic plasmids. One such plasmid, pADP-1 from *Pseudomonas* sp. ADP, has been completely sequenced and contains the genes for atrazine catabolism. Insertion sequence elements play a role in constructing different atrazine catabolic plasmids in different bacteria. Atrazine chlorohydrolase has been purified to homogeneity from two sources. Recombinant *Escherichia coli* strains expressing atrazine chlorohydrolase have been constructed and chemically cross-linked to generate catalytic particles used for atrazine remediation in soil. The method was used for cleaning up a spill of 1,000 pounds of atrazine to attain a level of herbicide acceptable to regulatory agencies.

Lakshmi Tewari and Piyush Malviya. (Department of Microbiology, College of Basic Sciences & Humanities. G. B. Pant University of Agriculture & Technology. Pantnagar 263145. India). **Biodegradation of Catechol by Fluorescent *Pseudomonas* for Sustainable Environment**. *Journal of Scientific & Industrial Research*, 61 (2002), 70-74.

A bioremediation study was undertaken to elucidate the role of a soil bacterial isolate fluorescent *Pseudomonas* sp. in biodegradation and removal of organic pollutant, catechol, from the environment. Free and immobilized cells of fluorescent *Pseudomonas*,

a plant growth promoting rhizobacteria, isolated from wheat rhizosphere and entrapped in calcium alginate beads were screened for their in vitro catechol (1, 2 dihydroxy benzene) degrading efficiency. The bacterium degraded catechol under all the four physiological states tested. Agitation enhanced its rapid degradation. Shake cultures of both immobilized and free cells degraded 83.2 per cent and 82.2 per cent catechol, respectively in 72 h indicating high efficiency of immobilized cells for bioremedial purposes. Thus versatility of fluorescent *Pseudomonas* to degrade phenolic pollutants as well as to enhance plant growth can be exploited for biotechnological applications.

Laleh Yerushalmi and Serge R. Guiot. (Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montreal, Canada H4P 2R2). **Biodegradation of Benzene in a Laboratory-Scale Biobarrier at Low Dissolved Oxygen Concentrations.** *Bioremediation Journal*, 5(1) (2001), 63-77.

A laboratory-scale permeable biobarrier exhibited high removal efficiencies of benzene at inlet concentrations of 0.4 to 35.1 mg/L and with a limited supply of dissolved oxygen. The supplied oxygen was less than the demand for a complete aerobic oxidation of benzene. Stainless steel pieces or granulated peat moss were used as packing material for microbial support in the biobarrier. Removal efficiencies ranged from 63.9% to 99.9% in the stainless steel-packed biobarrier and from 70.4% to 97.2% in the peat moss-packed biobarrier, while benzene elimination rate changed from 0.2 to 10.4 mg/L-d and from 0.1 to 3.7 mg/L-d in the two biobarriers, respectively. The consumption of sulfate and the presence of sulfate-reducing bacteria suggested the contribution of anaerobic metabolism in the biodegradation of benzene. The biodegradation of benzene under microaerophilic conditions (defined as dissolved oxygen concentrations <2 mg/L) was demonstrated during independent batch experiments. The maximum specific rate of benzene biodegradation with concentrations of 22.0 to 65.9 mg/L under microaerophilic conditions was 2.6 mg/mg biomass-d.

Luis A. Rios-Hernandez, Lisa M. Gieg, Joseph M. Suflita. (Institute for Energy and the Environment and Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019). **Biodegradation of an Alicyclic Hydrocarbon by a Sulfate-Reducing Enrichment from a Gas Condensate-Contaminated Aquifer.** *Applied and Environmental Microbiology*, 69(1) (2003), 434-443.

We used ethylcyclopentane (ECP) as a model alicyclic hydrocarbon and investigated its metabolism by a sulfate-reducing bacterial enrichment obtained from a gas condensate-contaminated aquifer. The enrichment coupled the consumption of ECP with the stoichiometrically expected amount of sulfate reduced. During ECP biodegradation, we observed the transient accumulation of metabolite peaks by gas chromatography-mass spectrometry, three of which had identical mass spectrometry profiles. Mass-spectral similarities to analogous authentic standards allowed us to identify these metabolites as ethylcyclopentylsuccinic acids, ethylcyclopentylpropionic acid, ethylcyclopentylcarboxylic acid, and ethylsuccinic acid. Based on these findings, we propose a pathway for the degradation of this alicyclic hydrocarbon. Furthermore, a putative metabolite similar to ethylcyclopentylsuccinic acid was also found in samples of contaminated groundwater from the aquifer. However, no such finding was evident for samples collected from wells located upgradient of the gas condensate spill. Microbial community analysis of the ECP-degrading enrichment by denaturing gradient gel electrophoresis revealed the presence of at least three different organisms using universal eubacterial primers targeting 550 bp of the 16S rRNA gene. Based on sequence analysis, these organisms are phylogenetically related to the genera *Syntrophobacter* and *Desulfotomaculum* as well as a member of the *Cytophaga-Flexibacter-Bacteroides* group. The evidence suggests that alicyclic hydrocarbons such as ECP can be anaerobically activated by the addition to the double bond of fumarate to form alkylsuccinate derivatives under sulfate-reducing conditions

and that the reaction occurs in the laboratory and in hydrocarbon-impacted environments.

M. B. Prieto, A. Hidalgo, C. Rodríguez-Fernández, J. L. Serra, M. J. Llama. (Enzyme and Cell Technology Group, Department of Biochemistry and Molecular Biology, Faculty of Sciences, University of the Basque Country, P.O. Box 644, 48080 Bilbao, Spain). **Biodegradation of phenol in synthetic and industrial wastewater by *Rhodococcus erythropolis* UPV-1 immobilized in an air-stirred reactor with clarifier.** Applied Microbiology and Biotechnology, 58(6) (2002), 853-860.

Phenol biodegradation by suspended and immobilized cells of *Rhodococcus erythropolis* UPV-1 was studied in discontinuous and continuous mode under optimum culture conditions. Phenol-acclimated cells were adsorbed on diatomaceous earth, where they grew actively forming a biofilm of short filaments. Immobilization protected cells against phenol and resulted in a remarkable enhancement of their respiratory activity and a shorter lag phase preceding active phenol degradation. Under optimum operation conditions in a laboratory-scale air-stirred reactor, the immobilized cells were able to completely degrade phenol in synthetic wastewater at a volumetric productivity of 11.5 kg phenol m⁻³ day⁻¹. Phenol biodegradation was also tested in two different industrial wastewaters (WW1 and WW2) obtained from local resin manufacturing companies, which contained both phenols and formaldehyde. In this case, after wastewater conditioning (i.e., dilution, pH, nitrogen and phosphorous sources and micronutrient amendments) the immobilized cells were able to completely remove the formaldehyde present in both waters. Moreover, they biodegraded phenols completely at a rate of 0.5 kg phenol m⁻³ day⁻¹ in the case of WW1 and partially (but at concentrations lower than 50 mg l⁻¹) at 0.1 and 1.0 kg phenol m⁻³ day⁻¹ in the cases of WW2 and WW1, respectively.

M. J. Hernández, B. Floriano, J. J. Ríos, and E. Santero. (Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Instituto de la Grasa, CSIC, Seville, Spain). **Identification of a Hydratase and a Class II Aldolase Involved in Biodegradation of the Organic Solvent Tetralin.** Applied and Environmental Microbiology, 68(10) (2002), 4841-4846.

Two new genes whose products are involved in biodegradation of the organic solvent tetralin were identified. These genes, designated *thnE* and *thnF*, are located downstream of the previously identified *thnD* gene and code for a hydratase and an aldolase, respectively. A sequence comparison of enzymes similar to ThnE showed the significant similarity of hydratases involved in biodegradation pathways to 4-oxalocrotonate decarboxylases and established four separate groups of related enzymes. Consistent with the sequence information, characterization of the reaction catalyzed by ThnE showed that it hydrated a 10-carbon dicarboxylic acid. The only reaction product detected was the enol tautomer, 2,4-dihydroxydec-2-ene-1,10-dioic acid. The aldolase ThnF showed significant similarity to aldolases involved in different catabolic pathways whose substrates are dihydroxylated dicarboxylic acids and which yield pyruvate and a semialdehyde. The reaction products of the aldol cleavage reaction catalyzed by ThnF were identified as pyruvate and the seven-carbon acid pimelic semialdehyde. ThnF and similar aldolases showed conservation of the active site residues identified by the crystal structure of 2-dehydro-3-deoxy-galactarate aldolase, a class II aldolase with a novel reaction mechanism, suggesting that these similar enzymes are class II aldolases. In contrast, ThnF did not show similarity to 4-hydroxy-2-oxovalerate aldolases of other biodegradation pathways, which are significantly larger and apparently are class I aldolases.

Maria De Lourdes Bellinaso, João Antônio Pêgas Henriques, Christine Claire Gaylarde. (Depto. Biologia e Química, UNIJUÍ, Ijuí-RS, Brazil; Depto. Bioquímica, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, CEP 91501-970, Porto Alegre, RS, Brazil. Depto. Bioquímica, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, CEP 91501-970, Porto Alegre, RS, Brazil; Depto. Biofísica, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, CEP 91501-970, Porto Alegre, RS, Brazil. Depto. Biofísica, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, CEP 91501-970, Porto Alegre, RS, Brazil; MIRCEN, Fac. Agronomia/UFRGS, C.P. 776, Porto Alegre, RS CEP 90001-970, Brazil). **Biodegradation as a biotechnological model for the teaching of biochemistry.** World Journal of Microbiology and Biotechnology, 18(5) (2002), 385-390.

Biodegradation as a biotechnological model for the teaching of biochemistry. A knowledge of waste treatment and the biodegradation processes involved is necessary for undergraduates in agriculture, chemistry, biology, food technology, etc. Courses in these subjects must make adequate provision for such instruction. In this article, we suggest a theoretical and practical study of composting, which stimulates the interest of the students in metabolic pathways involved in this, and other, biotechnological processes.

María Piedad Díaz, Kenneth G. Boyd, Steve J. W. Grigson, J. Grant Burgess. (Department of Biological Sciences, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, United Kingdom. Department of Civil and Offshore Engineering, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, United Kingdom. ECOPETROL-Instituto Colombiano del Petróleo, A.A 4185, Bucaramanga, Colombia). **Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers.** Biotechnology and Bioengineering, 79(2) (2002), 145-153.

The bacterial consortium MPD-M, isolated from sediment associated with Colombian mangrove roots, was effective in the treatment of hydrocarbons in water with salinities varying from 0 to 180 g L⁻¹. Where the salinity of the culture medium surpassed 20 g L⁻¹, its effectiveness increased when the cells were immobilized on polypropylene fibers. Over the range of salinity evaluated, the immobilized cells significantly enhanced the biodegradation rate of crude oil compared with free-living cells, especially with increasing salinity in the culture medium. Contrary to that observed in free cell systems, the bacterial consortium MPD-M was highly stable in immobilized systems and it was not greatly affected by increments in salinity. Biodegradation was evident even at the highest salinity evaluated (180 g L⁻¹), where biodegradation was between 4 and 7 times higher with immobilized cells compared to free cells. The biodegradation of pristane (PR) and phytane (PH) and of the aromatic fraction was also increased using cells immobilized on polypropylene fibers.

Marja Tuomela, Annele Hatakka, Sanni Raiskila, Minna Vikman, Merja Itävaara. (Department of Applied Chemistry and Microbiology, University of Helsinki, PO Box 56, Biocenter 1, 00014 University of Helsinki, Finland. Department of Chemistry, University of Helsinki, PO Box 55, 00014 University of Helsinki, Finland. VTT Biotechnology, PO Box 1501, 02044 VTT, Finland). **Biodegradation of radiolabelled synthetic lignin (14C-DHP) and mechanical pulp in a compost environment.** Applied Microbiology and Biotechnology, 57(3) (2001), 441-442.

Mineralization of radioactive synthetic lignin (14C-DHP) was studied in a compost environment at 35, 50 and 58°C. Compost samples were successively extracted with water, dioxane and alkali, and the molecular weight distribution of some extracts was determined by gel permeation chromatography (GPC). Biodegradation of lignin-

containing spruce groundwood (SGW) and pine sawdust was concurrently determined in controlled composting tests by measuring evolved CO₂. The temperatures were the same as in the 14C-DHP mineralization experiment and bleached kraft paper, with a lignin content of 0.2%, was used as a reference. The mineralization of 14C-DHP was relatively high (23-24%) at 35°C and 50°C, although the mixed population of compost obviously lacks the most effective lignin degraders. At 58°C the mineralization of 14C-DHP, as well as the biodegradation of SGW and sawdust, was very low, indicating that the lignin-degrading organisms of compost were inactivated at this temperature. SGW was poorly biodegradable (<40%) in controlled composting tests compared with kraft paper (77-86%) at all temperatures, which means that lignin inhibits the degradation of carbohydrates. During the incubation, water-soluble degradation products, mainly monomers and dimers, and the original 14C-DHP were either mineralized or bound to humic substances. A substantial fraction of 14C-DHP was incorporated into humin or other insolubles.

Mark T. Bustard, Vissanu Meeyoo, Phillip C. Wright. (Biochemical Engineering and Environmental Technologies Group, Department of Mechanical and Chemical Engineering, Heriot-Watt University, Edinburgh EH14 4AS, UK. Centre for Advanced Materials and Environmental Research, Mahanakorn University of Technology, Bangkok, Thailand). **Kinetic analysis of high-concentration isopropanol biodegradation by a solvent-tolerant mixed microbial culture.** *Biotechnology and Bioengineering*, 78(6) (2002), 708-713.

The ability of a previously enriched microbial population to utilize isopropanol (IPA) as the sole carbon source within a minimal salts medium is studied. The advantage of prior enrichment procedures for the improvement of IPA biodegradation performance is demonstrated for an IPA concentration of up to 24 g L⁻¹. Results showing the interrelationship between temperature and substrate utilization and inhibition levels at temperatures of between 2°C and 45°C are examined. Models of inhibition based on enzyme kinetics are assessed via nonlinear analysis, in order to accurately represent the growth kinetics of this solvent-tolerant mixed culture. The model that best describes the data is the Levenspiel substrate inhibition model, which can predict the maximum substrate level above which growth is completely limited. This is the first report of IPA treatment of up to 24 g L⁻¹ by an aerobic solvent-tolerant population.

Masud Hossain SK, Das Manas, Ibrahim SH (Dept Chemi Engng, Mohmed Sathak Engng Coil, Kilakarai 623806, T.N.). **Aerobic studies on pollution - abatement of sulfite pulp bleaching effluent using *Phanerochaete chrysosporium*** (MTCC-787). *J Indl Polln Contl*, 17(2) (2001), 191-200.

The white-rot fungus *Phanerochaete chrysosporium* decomposes chlorinated organic compounds in sulfite bleaching effluents which are considered to be resistant to bacterial treatment. The optimum digestion time is eight days (HRT). Seven days old aged with 15 percent (V/V) inoculum concentration is the optimum to bleach the sulfite bleaching effluent to a maximum pollution- abatement. 1.5 percent (w/v) glucose and 0.15 percent (w/v) nitrogen concentrations can degrade the maximum (78.97) percent COD and maximum (80.88) percent BOD.

Matthew F. Paige, Alvin C. Lin and M. Cynthia Goh. (Department of Chemistry, University of Toronto, 80 St. George street, Toronto, Ontario, Canada M5S 3H6). **Real-time enzymatic biodegradation of collagen fibrils monitored by atomic force microscopy.** *International Biodeterioration & Biodegradation*, 50(1) (2002), 1-10.

The enzymatic degradation of type I collagen fibrils by the collagenase from *Clostridium histolyticum* has been investigated using real-time atomic force microscope (AFM) imaging under liquid. Using a commercial AFM with fluid cell attachment, we have observed the degradation of fibrils from their mature form down to fragments whose size reaches the limits of resolution of the instrument. Upon exposure to collagenase solutions, collagen fibrils become both shorter and thinner as a function of the enzymatic incubation time, suggesting that collagenase degrades the entire fibrillar structure in a non-specific manner. The results are discussed in terms of the postulated mechanism of *C. histolyticum* collagenase activity, along with the possibility of determining enzymatic rate constants for biodegradation processes using the AFM. Experimental advantages and shortcomings of this technique are compared with other methods of following enzymatic degradation and related biological processes, and suggestions are made in order to overcome some of the technical difficulties encountered with these studies.

Michael H. Huesemnn, Tom S. Hausmann and Tim J. Fortman. (Pacific Northwest National Laboratories, Marine Sciences Laboratory, Sequim, WA 98382, USA). **Microbial Factors Rather Than Bioavailability Limit the Rate and Extent of PAH Biodegradation in Aged Crude Oil Contaminated Model Soils.** Bioremediation Journal, 6(4) (2002), 321-336.

The rate and extent of polynuclear aromatic hydrocarbons (PAH) biodegradation in a set of aged model soils that had been contaminated with crude oil at the high concentrations (i.e., >20,000 mg/kg) normally found in the environment were measured in 90-week slurry bioremediation experiments. Soil properties such as organic matter content, mineral type, particle diameter, surface area, and porosity did not significantly influence the PAH biodegradation kinetics among the 10 different model soils. A comparison of aged and freshly spiked soils indicates that aging affects the biodegradation rate and extent only for higher-molecular-weight PAHs, while the effects of aging are insignificant for 3-ring PAHs and total PAHs. In all model soils with the exception of kaolinite clay, the rate of abiotic desorption was faster than the rate of biodegradation during the initial phase of bioremediation treatment, indicating that PAH biodegradation was limited by microbial factors. Similarly, any of the higher-molecular-weight PAHs that were still present after 90 weeks of treatment were released rapidly during abiotic desorption tests, which demonstrates that bioavailability limitations were not responsible for the recalcitrance of these hydrocarbons. Indeed, an analysis of microbial counts indicates that a severe reduction in hydrocarbon degrader populations may be responsible for the observed incomplete PAH biodegradation.

Michelle M. Lora, Lisa D. Olsen, Douglas G. Capone and Joel E. Baker. (U.S. Geological Survey, 8987 Yellow Brick Road, Baltimore, Maryland 21237, USA. Chesapeake Biological Laboratory, University of Maryland, Solomons, Maryland 20688, USA). **Biodegradation of Trichloroethylene and Its Anaerobic Daughter Products in Freshwater Wetland Sediments.** Bioremediation Journal, 5(2) (2001), 101-118.

The wide range of redox conditions and diversity of microbial populations in organic-rich wetland sediments could enhance biodegradation of chlorinated solvents. To evaluate potential biodegradation rates of trichloroethylene (TCE) and its anaerobic daughter products (*cis*-1,2-dichloroethylene; *trans*-1,2-dichloroethylene; and vinyl chloride), laboratory microcosms were prepared under methanogenic, sulfate-reducing, and aerobic conditions using sediment and groundwater from a freshwater wetland that is a discharge area for a TCE contaminant plume. Under methanogenic conditions, biodegradation rates of TCE were extremely rapid at 0.30 to 0.37 d⁻¹ (half-life of about 2 days). Although the TCE biodegradation rate was slower under sulfate-reducing conditions (0.032 d⁻¹) than under methanogenic conditions, the rate was still two orders of magnitude higher than those reported in the literature for microcosms constructed

with sandy aquifer sediments. In the aerobic microcosm experiments, biodegradation occurred only if methane consumption occurred, indicating that methanotrophs were involved. Comparison of laboratory-measured rates indicates that production of the 1,2-dichloroethylene isomers and vinyl chloride by anaerobic TCE biodegradation could be balanced by their consumption through aerobic degradation where methanotrophs are active in wetland sediment. TCE degradation rates estimated using field data (0.009 to 0.016 61) agree with the laboratory-measured rates within a factor of 3 to 22, supporting the feasibility of natural attenuation as a remediation method for contaminated groundwater discharging in this wetland and other similar environments.

Murali S, Narayan Arun E, Vidyavati, Sriniketan G (Dept Chemi Engng, KRE Coil, Surathkal 574157, Karnataka). **Studies on colour removal by microbial means.** J Indl Polln Contl, 16(2) (2000), 211-215.

A laboratory scale study on decolourizing dyes was made. Since biological methods of decolourization are inherently advantageous to physical and chemical methods, they were used in this study. In the present study *Aspergillus niger* was used to decolorize different concentrations of methyl red

Nadege Etienne, David L. Butler, Alan E. Fryar and Mark S. Coyne. (Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091, USA. Current address: Department of Animal Sciences, University of Kentucky, Lexington, KY 40526, USA. Department of Geological Sciences, University of Kentucky, Lexington, KY 40506-0053, USA. Current address: Smoothstone Systems Inc., 801 S. Limestone, Ste. E, Lexington, KY 40508, USA). **Trichloroethene Biodegradation Potential in Wetland Soils and Paleowetland Sediments.** Bioremediation Journal, 5(1) (2001), 27-50.

Trichloroethene (TCE) plumes extend north-northeast toward the Ohio River from the Paducah Gaseous Diffusion Plant (PGDP), a Superfund site in the Gulf Coastal Plain of western Kentucky. Wetlands in the floodplain are in the paths of these plumes, and on-site contamination has migrated downward from the Regional Gravel Aquifer (RGA) into the upper McNairy Formation, which overlies a bedrock aquifer. Intrinsic biodegradation in these two environments at the margins of the RGA could limit further contaminant migration and ecosystem or water-quality degradation. To assess cometabolic biodegradation potential in these uncontaminated environments, we attempted to culture and enumerate methanogens, sulfate- and Fe(III)-reducers, and methanotrophs, which have been implicated elsewhere as TCE degraders. Soil samples were collected at three wetland sites in the floodplain. McNairy sediments were collected beneath one of the suspected source areas at PGDP. Methanogens, sulfate reducers, and methanotrophs were abundant in wetland soils, with populations generally decreasing with depth. Methanogens were the only group cultured from McNairy sediments, and they showed little activity compared with wetland methanogen cultures. TCE loss in methanogenic batch cultures by chemoautotrophic and acetoclastic methanogens was monitored, but no significant degradation was observed.

Nicholas V. Coleman, Timothy E. Mattes, James M. Gossett, and Jim C. Spain. (Air Force Research Laboratory-MLQL, Tyndall AFB, Florida 32403, School of Civil and Environmental Engineering, Cornell University, Ithaca, New York 14853). **Biodegradation of *cis*-Dichloroethene as the Sole Carbon Source by a β -Proteobacterium.** Applied and Environmental Microbiology, 68(6) (2002), 2726-2730.

An aerobic bacterium capable of growth on *cis*-dichloroethene (cDCE) as a sole carbon and energy source was isolated by enrichment culture. The 16S ribosomal DNA sequence of the isolate (strain JS666) had 97.9% identity to the sequence from *Polaromonas*

vacuolata, indicating that the isolate was a β -proteobacterium. At 20°C, strain JS666 grew on cDCE with a minimum doubling time of 73 ± 7 h and a growth yield of 6.1 g of protein/mol of cDCE. Chloride analysis indicated that complete dechlorination of cDCE occurred during growth. The half-velocity constant for cDCE transformation was 1.6 ± 0.2 μ M, and the maximum specific substrate utilization rate ranged from 12.6 to 16.8 nmol/min/mg of protein. Resting cells grown on cDCE could transform cDCE, ethene, vinyl chloride, *trans*-dichloroethene, trichloroethene, and 1,2-dichloroethane. Epoxyethane was produced from ethene by cDCE-grown cells, suggesting that an epoxidation reaction is the first step in cDCE degradation.

Nicolas Haroune, Bruno Combourieu, Pascale Besse, Martine Sancelme, Thorsten Reemtsma, Achim Kloepper, Amer Diab, Jeremy S. Knapp, Simon Baumberg, and Anne-Marie Delort. (Laboratoire de Synthèse et Etude de Systèmes à Intérêt Biologique, UMR 6504 CNRS-Université Blaise Pascal, 63177 Aubière Cedex, France, Department of Water Quality Control, Technical University of Berlin, Sekr KF 4, 10623 Berlin, Germany Division of Microbiology, School of Biochemistry and Molecular Biology School of Biology, University of Leeds, Leeds, LS2 9JT, United Kingdom). **Benzothiazole Degradation by *Rhodococcus pyridinovorans* Strain PA: Evidence of a Catechol 1,2-Dioxygenase Activity.** Applied and Environmental Microbiology, 68(12) (2002), 6114-6120.

The pathway for biodegradation of benzothiazole (BT) and 2-hydroxybenzothiazole (OBT) by *Rhodococcus pyridinovorans* strain PA was studied in detail. The kinetics of biodegradation were monitored by in situ ^1H nuclear magnetic resonance (NMR) in parallel with reversed-phase high-performance liquid chromatography (HPLC). Successive oxidations from BT to OBT and then from OBT to dihydroxybenzothiazole were observed. Further insight was obtained by using a mutant strain with impaired ability to grow on BT and OBT. The precise structure of another intermediate was determined by in situ two-dimensional ^1H - ^{13}C NMR and HPLC-electrospray ionization mass spectrometry; this intermediate was found to be a ring-opening product (a diacid structure). Detection of this metabolite, together with the results obtained by ^1H and ^{19}F NMR when cells were incubated with 3-fluorocatechol, demonstrated that a catechol 1,2-dioxygenase is involved in a pathway for biodegradation of BTs in this *Rhodococcus* strain. Our results show that catechol 1,2-dioxygenase and catechol 2,3-dioxygenase activities may both be involved in the biodegradation of BTs depending on the culture conditions.

P. A. Holden, M. G. LaMontagne, A. K. Bruce, W. G. Miller, and S. E. Lindow. (Donald Bren School of Environmental Science & Management, University of California, Santa Barbara, USDA Research Agricultural Service, Albany, Department of Plant and Microbial Biology, University of California, Berkeley, California). **Assessing the Role of *Pseudomonas aeruginosa* Surface-Active Gene Expression in Hexadecane Biodegradation in Sand.** Applied and Environmental Microbiology, 68(5) (2002), 2509-2518.

Low pollutant substrate bioavailability limits hydrocarbon biodegradation in soils. Bacterially produced surface-active compounds, such as rhamnolipid biosurfactant and the PA bioemulsifying protein produced by *Pseudomonas aeruginosa*, can improve bioavailability and biodegradation in liquid culture, but their production and roles in soils are unknown. In this study, we asked if the genes for surface-active compounds are expressed in unsaturated porous media contaminated with hexadecane. Furthermore, if expression does occur, is biodegradation enhanced? To detect expression of genes for surface-active compounds, we fused the *gfp* reporter gene either to the promoter region of *pra*, which encodes for the emulsifying PA protein, or to the promoter of the transcriptional activator *rhIR*. We assessed green fluorescent protein (GFP) production conferred by these gene fusions in *P. aeruginosa* PG201. GFP was produced in sand

culture, indicating that the *rhIR* and *pra* genes are both transcribed in unsaturated porous media. Confocal laser scanning microscopy of liquid drops revealed that *gfp* expression was localized at the hexadecane-water interface. Wild-type PG201 and its mutants that are deficient in either PA protein, rhamnolipid synthesis, or both were studied to determine if the genetic potential to make surface-active compounds confers an advantage to *P. aeruginosa* biodegrading hexadecane in sand. Hexadecane depletion rates and carbon utilization efficiency in sand culture were the same for wild-type and mutant strains, i.e., whether PG201 was proficient or deficient in surfactant or emulsifier production. Environmental scanning electron microscopy revealed that colonization of sand grains was sparse, with cells in small monolayer clusters instead of multilayered biofilms. Our findings suggest that *P. aeruginosa* likely produces surface-active compounds in sand culture. However, the ability to produce surface-active compounds did not enhance biodegradation in sand culture because well-distributed cells and well-distributed hexadecane favored direct contact to hexadecane for most cells. In contrast, surface-active compounds enable bacteria in liquid culture to adhere to the hexadecane-water interface when they otherwise would not, and thus production of surface-active compounds is an advantage for hexadecane biodegradation in well-dispersed liquid systems.

Paul B. Hatzinger, Kevin McClay, Simon Vainberg, Marina Tugusheva, Charles W. Condee, Robert J. Steffan. (Envirogen, Inc., Lawrenceville, New Jersey 08648). **Biodegradation of Methyl tert-Butyl Ether by a Pure Bacterial Culture.** Applied and Environmental Microbiology, 67(12) (2001), 5601-5607.

Biodegradation of methyl *tert*-butyl ether (MTBE) by the hydrogen-oxidizing bacterium *Hydrogenophaga flava* ENV735 was evaluated. ENV735 grew slowly on MTBE or *tert*-butyl alcohol (TBA) as sole sources of carbon and energy, but growth on these substrates was greatly enhanced by the addition of a small amount of yeast extract. The addition of H₂ did not enhance or diminish MTBE degradation by the strain, and MTBE was only poorly degraded or not degraded by type strains of *Hydrogenophaga* or hydrogen-oxidizing enrichment cultures, respectively. MTBE degradation activity was constitutively expressed in ENV735 and was not greatly affected by formaldehyde, carbon monoxide, allyl thiourea, or acetylene. MTBE degradation was inhibited by 1-amino benzotriazole and butadiene monoepoxide. TBA degradation was inducible by TBA and was inhibited by formaldehyde at concentrations of >0.24 mM and by acetylene but not by the other inhibitors tested. These results demonstrate that separate, independently regulated genes encode MTBE and TBA metabolism in ENV735.

Paula M. van Schie and Lily Y. Young. (Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92623, USA. Biotechnology Center for Agriculture and the Environment, Foran Hall, Cook College, 59 Dudley Road, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901-8520, USA). **Biodegradation of Phenol: Mechanisms and Applications.** Bioremediation Journal, 4(1) (2000), 1-18.

Phenol, or hydroxybenzene, is both a synthetically and naturally produced aromatic compound. Microorganisms capable of degrading phenol are common and include both aerobes and anaerobes. Many aerobic phenol-degrading microorganisms have been isolated and the pathways for the aerobic degradation of phenol are now firmly established. The first steps include oxygenation of phenol by phenol hydroxylase enzymes to form catechol, followed by ring cleavage adjacent to or in between the two hydroxyl groups of catechol. Phenolhydroxylases ranging from simple flavoprotein monooxygenases to multicomponent hydroxylases, as well as the genes coding for these enzymes, have been described for a number of aerobic phenol-degrading microorganisms. Phenol can also be degraded in the absence of oxygen. Our knowledge of this process is less advanced than that of the aerobic process, and only a few

anaerobic phenol-degrading bacteria have been isolated to date. Convincing evidence from both pure culture studies with the denitrifying organism *Thauera aromatica* K172 and with two *Clostridium* species, as well as from mixed culture studies, indicates that the first step in anaerobic phenol degradation is carboxylation in the *para*-position to form 4-hydroxybenzoate. Following *para*-carboxylation, thioesterification of 4-hydroxybenzoate to co-enzyme A allows subsequent ring reduction, hydration, and fission. *Para*-carboxylation appears to be involved in the anaerobic degradation of a number of aromatic compounds. Numerous practical applications exist for microbial phenol degradation. These include the exploitation of indigenous anaerobic phenol-degrading bacteria in the in situ bioremediation of creosote-contaminated subsurface environments, and the use of phenol as a co-substrate for indigenous aerobic phenol-degrading bacteria to enhance in situ biodegradation of chlorinated solvents.

Puvaneswari N, J Muthukrishnani & P Gunasekaran*. (Centre for Advanced Studies in Functional Genomics, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India). **Biodegradation of benzidine based azodyes Direct red and Direct blue by the immobilized cells of *Pseudomonas fluorescens* D41**. Indian Journal of Experimental Biology, 40 (2002), 1131-1136.

Benzidine based azodyes are proven carcinogens, mutagens and have been linked to Madder cancer of human beings and laboratory animals. The textile and dyestuff manufacturing industry are the two major sources that released azodyes in their effluents. The dye, Direct blue contains two carcinogenic compounds namely benzidine (BZ), 4-amino biphenyl (4-ABP), while the dye Direct red has benzidine (BZ). Among 40 isolates 0, *Pseudomonas fluorescens* screened, one isolate designated as D41 was found to be capable of extensively degrading the dyes Direct blue and Direct red. Immobilized cells of *P. fluorescens* D41 efficiently degraded Direct red (82%) and Direct blue (71 %) in the presence of glucose.

R Y Sheeja, T Murugesan. (Department of Chemical Engineering, Alagappa College of Technology, Anna University, Chennai 600 025, India). **Studies on biodegradation of phenol using response surface methodology**. Journal of Chemical Technology & Biotechnology, 77(11) (2002), 1219-1230.

Biodegradation of phenol was studied using *Pseudomonas pictorum* (NCIM 2077) immobilized on alginate and activated carbon - alginate beads. Control experiments were also performed using free cells and non-inoculated activated carbon - alginate beads. The entrapped alginate and activated carbon - alginate beads suffer from a concentration gradient for oxygen in the interior of the beads and hence free cells showed better degradation at lower concentrations of phenol. The results on entrapped alginate beads were modeled using response surface methodology which determines the dependency of the maximum percentage of phenol degraded as a function of the independent variables, namely initial phenol concentration, initial pH, temperature, and diameter of the immobilized beads. The predicted values are in close agreement with the experimental values with the coefficient of correlation equal to 0.9999 and 0.9993 for both *P pictorum* - alginate beads and activated carbon - *P pictorum* - alginate beads respectively.

R. Jayasekara, S. Sheridan, E. Loubakos, H. Beh, G. B. Y. Christie, M. Jenkins, P. B. Halley, S. McGlashan and G. T. Lonergan. (Centre for Applied Colloid and Biocolloid Science, School of Engineering and Science, Swinburne University of Technology, John Street, Hawthorn, 3122, Melbourne, Australia. CSIRO Division of Manufacturing Science and Technology, Cooperative Research Centre for International Food Manufacture and Packing Science, Clayton, Victoria 31169, Australia. Materials Characterisation and Processing Centre, The University of Queensland, Queensland 4072, Australia).

Biodegradation and ecotoxicity evaluation of a bionolle and starch blend and its degradation products in compost. International Biodeterioration & Biodegradation, 51(1) (2003), 77-81.

A polymer based on a blend of starch and "Bionolle™" has been prepared and tested for biodegradation in compost. The polymer was completely mineralised to carbon dioxide in 45 days. The potential toxicity of the polymer was tested against the earthworm *Eisenia fetida* using a modification of the American Standard for Testing Materials E1976-97. The earthworms were exposed to 30 g of the polymer for 28 days and changes in weight recorded. In addition, the polymer was firstly degraded by the compost and the worms exposed to the breakdown products for 28 days. Differences in weight were also recorded. In each case the production of juveniles was noted and all earthworms were examined for pathology. The results obtained were processed statistically using a *t*-test. The number of juveniles, produced from the breakdown products, was highly significant ($P < 0.001$) when compared to the earthworms added to the intact polymer. There was a definitely significant difference ($P < 0.01, t = 3.25$) in change in weight between the earthworms that were exposed to the polymer directly and those that were exposed to the breakdown products. There was no indication of any pathology of any earthworms. The polymer is considered safe for this species.

Rana BA, Desai PV (Dept Biosci, South Gujarat Univ, Udhana-Magdalla Rd, PB No.49, Surat). **Biodegradation of J-acid and benzediene disulfonic acid by Pseudomonads.** Indian J Environ Toxico, 11(2) (2001), 62-64.

An extensively used dye intermediate J-acid and BDSA, was studied for its degradation by *Pseudomonas* isolated from the effluent waste. Amongst the dye intermediates J-acid was degraded by the isolates when added at 0.5g% in synthetic medium, where as BDSA was less acted upon by the *Pseudomonas*.

Rebecca J. Melcher, Sabine E. Apitz, Barbara B. Hemmingsen. (Department of Biology, San Diego State University, San Diego, California 92182-4614, Sediment Management Laboratory, Space and Naval Warfare Systems Center San Diego D361, San Diego, California 92152-6325). **Impact of Irradiation and Polycyclic Aromatic Hydrocarbon Spiking on Microbial Populations in Marine Sediment for Future Aging and Biodegradability Studies.** Applied and Environmental Microbiology, 68(6) (2002), 2858-2868.

Experiments were carried out to develop methods to generate well-characterized, polycyclic aromatic hydrocarbon (PAH)-spiked, aged but minimally altered sediments for fate, biodegradation, and bioavailability experiments. Changes in indigenous bacterial populations were monitored in mesocosms constructed of relatively clean San Diego Bay sediments, with and without exposure to gamma radiation, and then spiked with five different PAHs and hexadecane. While phenanthrene and chrysene degraders were present in the unspiked sediments and increased during handling, PAH spiking of nonirradiated sediments led to dramatic increases in their numbers. Phenotypic characterization of isolates able to grow on phenanthrene or chrysene placed them in several genera of marine bacteria: *Vibrio*, *Marinobacter* or *Cycloclasticus*, *Pseudoalteromonas*, *Marinomonas*, and *Halomonas*. This is the first time that marine PAH degraders have been identified as the latter two genera, expanding the diversity of marine bacteria with this ability. Even at the highest irradiation dose (10 megarads), heterotrophs and endospore formers reappeared within weeks. However, while bacteria from the unirradiated sediments had the capacity to both grow on and mineralize ^{14}C -labeled phenanthrene and chrysene, irradiation prevented the reappearance of PAH degraders for up to 4 months, allowing spikes to age onto the sediments, which can be used to model biodegradation in marine sediments.

Richard Gattin, Alain Copinet, Céline Bertrand and Yves Couturier. (Materials and Packaging Research Centre (CERME), UMR INRA/URCA FARE, Ecole d'Ingenieurs en Emballage, Esplanade Roland Garros, PB 1029, 51686, Reims Cedex 2, France). **Biodegradation study of a starch and poly(lactic acid) co-extruded material in liquid, composting and inert mineral media.** International Biodeterioration & Biodegradation, 50(1) (2002), 25-31.

The biodegradation of a co-extruded starch/poly(lactic acid) polymeric film was studied in liquid, inert solid and composting media. Main mechanical properties of this film were Young's modulus: 2340 MPa, elongation at break: 50%, contact angle: 118°. Mineralization of the material's carbon content was followed using the appropriate experimental methods of the International Standard Organisation. Whatever be the biodegradation medium used, the percentage of mineralization was better than the required 60% value for the definition of a biodegradable material. Moreover, repartitioning of the material's carbon between the various degradation products produced was quantified throughout the duration of experimental runs. The presence of starch was found to facilitate biodegradation of the polylactic component, especially in liquid media.

Romas Ragauskas, Arvydas Dikčius, Tomas Vengris, Audrius Padarauskas. (Institute of Chemistry, Goštauto 9, 2600 Vilnius, Lithuania. Faculty of Chemistry, Vilnius University, Naugarduko 26, 2600 Vilnius, Lithuania). **Degradation of color photographic waste fix solution by anodic oxidation.** Journal of Chemical Technology & Biotechnology, 78(1) (2003), 81-86.

By use of a capillary electrophoretic method for anion analysis the anodic oxidation of thiosulfate, present in color photo bleach-fix solutions, was investigated. In a specially constructed electrolysis cell with a cation-exchange membrane separating the electrode chambers and with a carbon flow-through three-dimensional anode the two-stage anodic oxidation of $S_2O_3^{2-}$ anions was realized. In the first stage, $S_2O_3^{2-}$ anions were oxidized to a maximal degree to an intermediate compound - tetrathionate ($S_4O_6^{2-}$) - then $S_4O_6^{2-}$ anions were oxidized to the sulfate (SO_4^{2-}), avoiding the decomposition of intermediate compounds and the formation of harmful sulfur compounds.

S. I. Rupassara, R. A. Larson, G. K. Sims and K. A. Marley. (University of Illinois at Urbana-Champaign, IL, USA. United States Department of Agriculture — Agricultural Research Service, Urbana, IL, USA). **Degradation of Atrazine by Hornwort in Aquatic Systems.** Bioremediation Journal, 6(3) (2002), 217-224.

Hornwort (*Ceratophyllum demersum*) incubated in the presence of 1 mg L^{-1} ^{14}C atrazine in an aqueous nutrient solution became radioactive. Microscopic autoradiography was used to investigate the localization of ^{14}C atrazine in a hornwort/epiphyte system. Radioactivity was present within the plant tissue (and also in the nutrient solution), with larger quantities in mature tissue, including stems compared with young tissue. An irregular distribution of black silver granules (which indicate radioactivity) was observable on the plant surface, suggesting the possible involvement of epiphytes (plant surface microorganisms) in the degradation process. Labeled compounds in the extracted plant included atrazine and a major metabolite that may have been an atrazineglutathione conjugate. Concentrations of atrazine and the metabolite, and the fraction of the metabolite (based on total radioactivity), all in the extracted plant, were dependent on the initial atrazine concentration in the solution. The degradation process was light dependent and the analyses of the nutrient solution indicated that the first half life of atrazine in the presence of hornwort was 5 days under day/night conditions, while only about 30% of initial atrazine disappeared after 3 weeks under complete dark. The major metabolite in the solution was identified as deethylatrazine.

S. Malherbe, T.E. Cloete. (Department of Microbiology and Plant Pathology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0002, South Africa). **Lignocellulose biodegradation Fundamentals and applications.** Reviews in Environmental Science and Biotechnology, 1(2) (2002), 105-114.

Lignocelluloses are the building blocks of all plants and are ubiquitous to most regions of our planet. Their chemical properties make it a substrate of enormous biotechnological value. The basic chemistry of cellulose, hemicellulose, and lignin has a profound effect on lignocellulose tertiary architecture. These intricate associations constitute physical and chemical barriers to lignocellulose utilization and biodegradation in natural and man-made environments. Overcoming these barriers is the key to unlocking the commercial potential of lignocellulose. Understanding lignocellulose degradation under natural conditions forms the basis of any lignocellulose-based application. A variety of microorganisms and mechanisms are involved in the complete biodegradation of lignocellulose in natural environments ranging from soil and rumen ecosystems to the termite hindgut. The primary objective of lignocellulose pretreatment by the various industries is to access the potential of the cellulose and hemicellulose encrusted by lignin within the lignocellulose matrix. Current working technologies based on the principles of solid-state fermentation (SSF) are briefly reviewed. The use of unsterile lignocellulosics for bioremediation purposes holds promise for cost-effective environmental clean-up endeavors. Novel lignocellulose-based applications have found functionality in textile, biological control, and medical research fields and might be exploited there in the near future. Ultimately, lignocellulose will probably accompany man to his voyages into space for interest in this field is intensifying. Therefore, proper management of lignocellulose biodegradation and utilization can serve to improve the quality of the environment, further man's understanding of the universe, and ultimately change local economies and communities.

S. Mogensen, B. K. Ahring. (The Environmental Microbiology and Biotechnology Group, BioCentrum-DTU, Building 227, Technical University of Denmark, DK-2800 Lyngby, Denmark. Civil & Environmental Engineering Department, University of California, Los Angeles, Los Angeles, California 90095-1593, USA). **Formation of metabolites during biodegradation of linear alkylbenzene sulfonate in an up flow anaerobic sludge bed reactor under thermophilic conditions.** Biotechnology and Bioengineering, 77(5) (2002), 483-488.

Biodegradation of linear alkylbenzene sulfonate (LAS) was shown in an upflow anaerobic sludge blanket reactor under thermophilic conditions. The reactor was inoculated with granular biomass and fed with a synthetic medium and 3 μ mol/L of a mixture of LAS with alkylchain length of 10 to 13 carbon atoms. The reactor was operated with a hydraulic retention time of 12 h with effluent recirculation in an effluent to influent ratio of 5 to 1. A sterile reactor operated in parallel revealed that sorption to sludge particles initially accounted for a major LAS removal. After 8 days of reactor operation, the removal of LAS in the reactor inoculated with active granular biomass exceeded the removal in the sterile reactor inoculated with sterile granular biomass. The effect of sorption ceased after 185 to 555 h depending on the LAS homologs. 40% of the LAS was biodegraded, and the removal rate was 0.5×10^{-6} mol/h/mL granular biomass. Acidified effluent from the reactor was subjected to dichloromethane extraction followed by gas chromatography/mass spectrometry. Benzenesulfonic acid and benzaldehyde were detected in the reactor effluent from the reactor with active granular biomass but not in the sterile and unamended reactor effluent. Benzenesulfonic acid and benzaldehyde are the first identified degradation products in the anaerobic degradation of LAS.

Shachi Shah, Indu Shekhar Thakur. (Environmental Biotechnology Laboratory, Department of Environment Science College of Basic Science and Humanities, G B Pant University of Agriculture and Technology Pantnagar 263145). **Treatment of Tannery Effluent by Pentachlorophenol-Degrading Bacterial Community from the Chemostat.** The Journal of Scientific & Industrial Research, 44 (2002), 1051-1055.

A mixed microbial community enriched in a chemostat in presence of pentachlorophenol (PCP) as sole source of carbon and energy comprised four bacterial strains, identified as *Pseudomonas* sp. (one strain) and *Serratia marcescens* (three strains). The bacterial community applied for the treatment of tannery effluent in a suspended culture and sequential bioreactor indicated the reduction of Chemical Oxygen Demand (COD) (68 per cent), sulphide (50 per cent), total phenol (78 per cent), and pentachlorophenol (65 per cent) in sequential bioreactor.

Shachi Shah, Indu Shekhar Thakur. (Environmental Biotechnology Laboratory, Department of Environmental Sciences, College of Basic Sciences and Humanities, Qt. No. 1918, Sector-6, Ta Colony, G.B. Pant University of Agriculture and Technology, Pantnagar 263 145, Uttaranchal, India). **Enrichment and characterization of a microbial community from tannery effluent for degradation of pentachlorophenol.** World Journal of Microbiology and Biotechnology, 18(7) (2002), 693-698.

A bacterial community obtained by continuous enrichment from the microbial population of tannery effluent using pentachlorophenol (PCP) as sole source of carbon and energy, contained four different bacterial species including *Serratia marcescens* (three isolates, TE₁, TE₂ and TE₄) and *Pseudomonas fluorescens* (one isolate, TE₃). The members of the community grew separately on various chlorinated compounds, carbon and nitrogen sources and exhibited a remarkable ability to utilize PCP. Biodegradation studies revealed a time-dependent disappearance of PCP and its intermediary metabolites, tetrachloro-*p*-hydroquinone and chlorohydroquinone, and indicated the individual role of members of the community in the degradation of PCP.

Sharma Shalini, Verma SR, Malik Naresh (Lata Nursing Home, Meerut Rd, Muzaffamagar 251001). **Degradation of kraft mill waste lignins by two fungi *Phanerochaete chrysosporium* and *Trametes versicolor*.** *Aquacult*, 2(1) (2001), 79-83.

Paper deals with the degradation of lignin present in kraft mill waste water in the presence of PDA subculture *Phanerochaete chrysosporium* and *Trametes versicolor*. It has been observed that colour substantially degraded upto 68.9% in case of *Trametes versicolor* and 58.6% in case of *Phanerochaete chrysosporium*, both the fungi are capable for degrading the lignin from black liquor and the reactions are more efficient in high acidic pH.

Sheryl H. Streger, Simon Vainberg, Hailiang Dong, and Paul B. Hatzinger. (Envirogen, Inc., Lawrenceville, New Jersey 08648, Miami University, Oxford, Ohio 45056). **Enhancing Transport of *Hydrogenophaga flava* ENV735 for Bioaugmentation of Aquifers Contaminated with Methyl *tert*-Butyl Ether.** *Applied and Environmental Microbiology*, 68(11) (2002), 5571-5579.

The gasoline oxygenate methyl *tert*-butyl ether (MTBE) has become a widespread contaminant in groundwater throughout the United States. Bioaugmentation of aquifers with MTBE-degrading cultures may be necessary to enhance degradation of the oxygenate in some locations. However, poor cell transport has sometimes limited bioaugmentation efforts in the past. The objective of this study was to evaluate the

transport characteristics of *Hydrogenophaga flava* ENV735, a pure culture capable of growth on MTBE, and to improve movement of the strain through aquifer solids. The wild-type culture moved only a few centimeters in columns of aquifer sediment. An adhesion-deficient variant (*H. flava* ENV735:24) of the wild-type strain that moved more readily through sediments was obtained by sequential passage of cells through columns of sterile sediment. Hydrophobic and electrostatic interaction chromatography revealed that the wild-type strain is much more hydrophobic than the adhesion-deficient variant. Electrophoretic mobility assays and transmission electron microscopy showed that the wild-type bacterium contains two distinct subpopulations, whereas the adhesion-deficient strain has only a single, homogeneous population. Both the wild-type strain and adhesion-deficient variant degraded MTBE, and both were identified by 16S rRNA analysis as pure cultures of *H. flava*. The effectiveness of surfactants for enhancing transport of the wild-type strain was also evaluated. Many of the surfactants tested were toxic to ENV735; however, one nonionic surfactant, Tween 20, enhanced cell transport in sand columns. Improving microbial transport may lead to a more effective bioaugmentation strategy for MTBE-contaminated sites where indigenous oxygenate degraders are absent.

Silvia A. Mancini, Ania C. Ulrich, Georges Lacrampe-Couloume, Brent Sleep, Elizabeth A. Edwards, and Barbara Sherwood Lollar. (Stable Isotope Laboratory, Department of Geology, Department of Chemical Engineering and Applied Chemistry, Department of Civil Engineering, University of Toronto, Toronto, Canada). **Carbon and Hydrogen Isotopic Fractionation during Anaerobic Biodegradation of Benzene**. Applied and Environmental Microbiology, 69(1) (2003), 191-1981.

Compound-specific isotope analysis has the potential to distinguish physical from biological attenuation processes in the subsurface. In this study, carbon and hydrogen isotopic fractionation effects during biodegradation of benzene under anaerobic conditions with different terminal-electron-accepting processes are reported for the first time. Different enrichment factors (δ) for carbon (range of -1.9 to -3.6%) and hydrogen (range of -29 to -79%) fractionation were observed during biodegradation of benzene under nitrate-reducing, sulfate-reducing, and methanogenic conditions. These differences are not related to differences in initial biomass or in rates of biodegradation. Carbon isotopic enrichment factors for anaerobic benzene biodegradation in this study are comparable to those previously published for aerobic benzene biodegradation. In contrast, hydrogen enrichment factors determined for anaerobic benzene biodegradation are significantly larger than those previously published for benzene biodegradation under aerobic conditions. A fundamental difference in the previously proposed initial step of aerobic versus proposed anaerobic biodegradation pathways may account for these differences in hydrogen isotopic fractionation. Potentially, C-H bond breakage in the initial step of the anaerobic benzene biodegradation pathway may account for the large fractionation observed compared to that in aerobic benzene biodegradation. Despite some differences in reported enrichment factors between cultures with different terminal-electron-accepting processes, carbon and hydrogen isotope analysis has the potential to provide direct evidence of anaerobic biodegradation of benzene in the field solution, which gave encouraging results.

Srinivasan SV, Murthy DVS (Environ Engng Lab, Dept Chemi Engng, Indian Inst Techno, Chennai 600036). **Removal of colour from wastewater using *Trametes versicolor***. J Indian Assoc Environ Manag, 27(3) (2000), 260-263.

The white rot fungi, which are initially identified to have the ability to degrade lignin, can be used for the treatment of effluent, generated from pulp plant and dyeing industries. It is observed that the white rot fungi have a non-specific enzyme system, which oxidises the recalcitrant compounds present in the effluent. Application of this fungal treatment for removal of colour of wastewater from industries have been attempted using the white

rot fungus *Trametes versicolor*.

Subarna Roy, Dipak Hens, Debabrata Biswas, Dipa Biswas, Ranajit Kumar. (Microbial Genetics Division, National Institute of Cholera & Enteric Diseases, P-33, C IT Road, Scheme XM, Beliaghata, Calcutta 700 010, India. Regional Medical Research Centre, Port Blair, Andaman and Nicobar Islands, India. Department of Chemical Technology, Petrochemicals & Petroleum Refinery Engg. Division, University of Calcutta, 92, A.P.C Road, Calcutta 700009, India). **Survey of petroleum-degrading bacteria in coastal waters of Sunderban Biosphere Reserve.** World Journal of Microbiology and Biotechnology, 18(6) (2002), 575-581.

A survey of petroleum-degrading bacteria was carried out in the Indian part of deltaic Sunderban to evaluate the distribution of the naturally occurring petroleum-degrading aerobic bacteria. Bacteriological analysis of surface water samples collected from five different locations in the Hoogly-Matla river mouth showed that, depending on the location, 0.08-2.0% of the heterotrophic bacteria culturable in marine agar medium could degrade erode petroleum hydrocarbons as the sole source of carbon. In the entire study area, the number of heterotrophic bacteria ranged from 1×10^3 to 3.8×10^5 e.f.u/ml, amongst which 2.7×10^1 to 6×10^3 e.f.u/ml were petroleum degraders. There was a maximum number of petroleum-degrading bacteria in the waters of Haldia Port and its surrounding areas, where the water is highly polluted by hydrocarbon discharges from a nearby oil refinery and from the ships docking at the port. Among the isolates, identified on the basis of their Gram reaction, morphological and biochemical tests including the use of AP120E strips, *Pseudomonas*, *Mycobacterium*, *Klebsiella*, *Acinetobacter*, *Micrococcus*, and *Nocardia* were the most common petroleum degraders. Other heterotrophic bacteria included several species of *Escherichia*, *Klebsiella*, non-oil-degrading *Pseudomonas*, *Vibrio*, *Streptococcus*, *Staphylococcus* and *Bacillus*. Following preliminary selection, five strains, showing best growth in medium with oil fraction as sole carbon source, were chosen for estimation of the efficiency of crude oil biodegradation. The elected strain's belonged to *Pseudomonas* (two strains), *Mycobacterium* (two stains), and *Nocardia* (one strain). These strains degraded 47-78% of Arab-Mix crude oil over a period of 20 days. The best oil-degrading isolate, a strain of *Pseudomonas aeruginosa*, (BBWI), was found to degrade and multiply more rapidly in crude oil than the rest. BBWI showed profuse growth in Bushnell Haas medium containing crude oil (as sole source of carbon) at high concentrations ranging from 0.2 to 20% (v/v), with optimum at 10%. As much as 75% of the oil was degraded within 72 h of incubation with the bacteria. Physicochemical analysis showed considerable decrease in initial boiling point and carbon residue of the degraded oil. The ability to degrade erode oil was found to be associated with a single 70-kb plasmid, pBN70. Resistance to the metals Mn^{2+} (50 mM), Mg^{2+} (200 mM), Zn^{2+} (6 mM), Ni^{2+} (10 mM) and antibiotics like ampicillin (10: g/ml), cephalixin (30: g/ml), nitrofurantoin (300: g/ml) and penicillin (10 U/ml) were plasmid-mediated.

Sue Hyung Choi, Seung-Hyeon Moon, Man Bock Gu. (National Research Laboratory on Environmental Biotechnology and Department of Environmental Science and Engineering, Kwangju Institute of Science and Technology (K-JIST), 1 Oryong-dong, Puk-gu, Kwangju 500-712, S Korea). **Biodegradation of chlorophenols using the cell-free culture broth of *Phanerochaete chrysosporium* immobilized in polyurethane foam.** Journal of Chemical Technology & Biotechnology, 77(9) (2002), 999-1004.

A cell-free culture broth of *Phanerochaete chrysosporium* immobilized in polyurethane foam has been evaluated for the biodegradation of chlorophenols. Lignin peroxidase, manganese peroxidase, and oxalate concentrations in cell-free culture broth were measured and compared to find the optimum combination of secondary metabolites for the highest biodegradation of chlorophenols. The isozyme distributions and their

expression levels were significantly different and changed with increases in the culture time. The oxalate concentration was also found to vary, depending on culture time. Cell-free broth containing an optimal combination of secondary metabolites showed the greatest biodegradation of 2,4,5-chlorophenol in the presence of veratryl alcohol and H₂O₂. Phenols with greater numbers of chlorines were degraded more efficiently by this cell-free culture broth according to the results of biodegradation experiments for five chlorophenols, including 2-, 4-, 2,4-, 2,4,5-, and pentachlorophenol, as well as phenol. This degradation efficiency correlated well with remaining lignin peroxidase activity during degradation. Cell-free culture broths may readily be used for biodegradation of highly recalcitrant chemicals since the system would not be affected by the toxicity of the chemicals nor would the adsorption characteristics of the cells be of concern.

Swapna Thomas, Sami Sarfaraz, L.C. Mishra, Leela Iyengar. (Biotechnology Laboratory, Department of Chemistry, Indian Institute of Technology, Kanpur 208016, India. Department of Life Sciences, C.S.J.M University, Kanpur 208016, India). **Degradation of phenol and phenolic compounds by a defined denitrifying bacterial culture.** World Journal of Microbiology and Biotechnology, 18(1) (2002), 57-63.

Phenol, a major pollutant in several industrial waste waters is often used as a model compound for studies on biodegradation. This study investigated the anoxic degradation of phenol and other phenolic compounds by a defined mixed culture of *Alcaligenes faecalis* and *Enterobacter* species. The culture was capable of degrading high concentrations of phenol (up to 600 mg/l) under anoxic conditions in a simple minimal mineral medium at an initial cell mass of 8 mg/l. However, the lag phase in growth and phenol removal increased with increase in phenol concentration. Dissolved CO₂ was an absolute requirement for phenol degradation. In addition to nitrate, nitrite and oxygen could be used as electron acceptors. The kinetic constants, maximum specific growth rate μ_{max} ; inhibition constant, K_i and saturation constant, K_s were determined to be 0.206 h⁻¹, 113 and 15 mg phenol/l respectively. *p*-Hydroxybenzoic acid was identified as an intermediate during phenol degradation. Apart from phenol, the culture utilized few other monocyclic aromatic compounds as growth substrates. The defined culture has remained stable with consistent phenol-degrading ability for more than 3 years and thus shows promise for its application in anoxic treatment of industrial waste waters containing phenolic compounds.

Tewes Tralau, Jorg Mampel, S Alasdair M. Cook, Jürgen Ruff. (Department of Biology, The University of Konstanz, D-78457 Konstanz, Germany). **Characterization of TsaR, an Oxygen-Sensitive LysR-Type Regulator for the Degradation of p-Toluenesulfonate in Comamonas testosteroni T-2.** Applied and Environmental Microbiology, 69(4) (2003), 2298-2305

TsaR is the putative LysR-type regulator of the *tsa* operon (*tsaMBCD*) which encodes the first steps in the degradation of *p*-toluenesulfonate (TSA) in *Comamonas testosteroni* T-2. Transposon mutagenesis was used to knock out *tsaR*. The resulting mutant lacked the ability to grow with TSA and *p*-toluenecarboxylate (TCA). Reintroduction of *tsaR* *in trans* on an expression vector reconstituted growth with TSA and TCA. The *tsaR* gene was cloned into *Escherichia coli* with a C-terminal His tag and overexpressed as TsaR^{His}. TsaR^{His} was subject to reversible inactivation by oxygen, which markedly influenced the experimental approaches used. Gel filtration showed TsaR^{His} to be a monomer in solution. Overexpressed TsaR^{His} bound specifically to three regions within the promoter between the divergently transcribed *tsaR* and *tsaMBCD*. The dissociation constant (K_D) for the whole promoter region was about 0.9 μ -M, and the interaction was a function of the concentration of the ligand TSA. A regulatory model for this LysR-type regulator is proposed on the basis of these data.

Tha Thayumanavan, Rahman KSM, Lakshmanaperumalsamy P (Dept Environ Sci, Bharthiar Univ, Coimbatore 641046, Tamil Nadu). **Biodegradation of petroleum refinery waste oil sludge.** *Polln Res*, 20(2) (2001), 155-161.

Bacterial consortium prepared with *Pseudomonas* sp., *Corynebacterium* sp., *Flavobacterium* sp., *Bacillus* sp. and *Micrococcus* sp. was tested for the degradation of petroleum refinery effluent treatment plant sludge applied to sterile and non-sterile red soil. The maximum rate of oil degradation (71.23%) was observed in treatment which is amended with non-sterile red soil, non-sterile sludge and mixed consortium. It is concluded that the mixed consortium can be applied in large-scale sludge degradation, as this process is an economically feasible.

Thomas J. Boyd, Michael T. Montgomery, Barry J. Spargo, David C. Smith Richard B. Coffin, Cheryl A. Kelley and James G. Mueller. (Naval Research Laboratory, Code 6115,4555 Overlook Avenue, Washington, DC 20375, USA. b Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA. c URS/Dames & Moore, 1701 GolfRd, Tower One, Suite 1000, Rolling Meadows, IL 60008, USA). **Effects of Oxygenation on Hydrocarbon Biodegradation in a Hypoxic Environment.** *Bioremediation Journal*, 5(2) (2001), 145-157.

In 1984, an underground storage tank leaked approximately 41,000 L of gasoline into the ground water at the Naval Construction Battalion Command in Port Hueneme, CA (USA). Benzene, toluene, ethylbenzene, and xylenes (BTEX) contamination stimulated remedial action. In 1995, a ground water circulation well (GCW) and network of surrounding monitoring wells were installed. After year of operation, dissolved oxygen and nitrate concentrations remained low in all monitoring wells. Benzene utilization (the sum of respiration, uptake, and conversion to polar compounds) ranged from 0.03 to 4.6 $\mu\text{g L}^{-1} \text{h}^{-1}$ and toluene utilization ranged from 0.01 to 5.2 $\mu\text{g L}^{-1} \text{h}^{-1}$. Heterotrophic bacterial productivity (total carbon assimilation) increased dramatically in the GCW, although benzene and toluene utilization decreased markedly relative to surrounding wells. Benzene and toluene uptake accounted for a significant proportion (mean = 22%) of the heterotrophic bacterial productivity except within the GCW, indicating other fuel contaminant or indigenous organic carbon and not BTEX compounds served as primary carbon source. The GCW effectively air stripped BTEX compounds but failed to stimulate benzene and toluene biodegradation and thus would not be effective for stimulating BTEX bioremediation under current deployment parameters. Air stripping was three orders of magnitude more effective than biodegradation for removing benzene and toluene in the GCW.

Thomas K. Mitchell, William Scott Chilton, Margaret E. Daub. (Departments of Plant Pathology, Botany, North Carolina State University, Raleigh, North Carolina 27695-7616). **Biodegradation of the Polyketide Toxin Cercosporin.** *Applied and Environmental Microbiology*, 68(9) (2002), 4173-4181.

Cercosporin is a non-host-specific polyketide toxin produced by many species of plant pathogens belonging to the genus *Cercospora*. This red-pigmented, light-activated toxin is an important pathogenicity determinant for *Cercospora* species. In this study, we screened 244 bacterial isolates representing 12 different genera for the ability to degrade cercosporin. Cercosporin degradation was determined by screening for the presence of cleared zones surrounding colonies on cercosporin-containing culture medium and was confirmed by assaying the kinetics of degradation in liquid medium. Bacteria belonging to four different genera exhibited the cercosporin-degrading phenotype. The isolates with the greatest cercosporin-degrading activity belonged to *Xanthomonas campestris* pv. zinniae and *X. campestris* pv. pruni. Isolates of these pathovars removed over 90% of

the cercosporin from culture medium within 48 h. Bacterial degradation of red cercosporin was accompanied by a shift in the color of the growth medium to brown and then green. The disappearance of cercosporin was accompanied by the appearance of a transient green product, designated xanosporic acid. Xanosporic acid and its more stable lactone derivative, xanosporolactone, are nontoxic to cercosporin-sensitive fungi and to plant tissue and are labile in the presence of light. Detailed spectroscopic analysis (to be reported in a separate publication) of xanosporolactone revealed that cercosporin loses one methoxyl group and gains one oxygen atom in the bacterial conversion. The resulting chromophore (4,9-dihydroxy-3-oxaperlylen-10H-10-one) has never been reported before but is biosynthetically plausible via oxygen insertion by a cytochrome P-450 enzyme.

Todd L. Cort, Angela R. Bielefeldt. (Cameron-Cole, LLC, Boulder, Colorado, USA. Department of Civil, Environmental, and Architectural Engineering, University of Colorado, Campus Box 428, Boulder, Colorado 80309-0428, USA). **A kinetic model for surfactant inhibition of pentachlorophenol biodegradation.** *Biotechnology and Bioengineering*, 78(6) (2002), 606-616.

A kinetic model is used to describe the effect of the nonionic surfactant Tergitol NP-10 (TNP10) on pentachlorophenol (PCP) biodegradation by *Sphingomonas chlorophenolica* sp. strain RA2. Different initial biomass to initial substrate ratios ranging from 13 to 418 were tested with 23 TNP10 concentrations ranging from 0 to 1500 mg/L. Tests were also conducted at 10°C and 20°C. No PCP biodegradation inhibition was observed at concentrations below the critical micelle concentration (CMC) of 50 mg/L. TNP10 concentrations above 100 to 200 mg/L were increasingly inhibitory to PCP biodegradation rates. This inhibition was best described by the Monod kinetic equation wherein the effect of TNP10 inhibition is reflected in the half-saturation constant (K_s). The value of the K_s increased from between 1.5 and 13.5 mg/L with no surfactant present to 44 to 131 mg/L at 1000 mg/L TNP10. Using a standard competitive inhibition approach, the inhibition constant for TNP10 was approximately 100 mg/L at both 10°C and 20°C.

Trivedi GS, Ray P, Shah BG, Adhikary SK, Mishra S, Sravan Kumar VG, Tewari A, Ghosh PK (Centl Salt Marine Chemi Res Inst, Gijubhai Badheka Marg, Bhavnagar 364002, Gujarat). **Effluent treatment of fungicide manufacturing industrial waste by combination of electro dialysis and cyanobacteria.** *Res J Chem Env*, 5(2) (2001), 17-20.

Electrodialysis and cyanobacteria in combination have proved useful in treatment of industrial waste effluent. Marine cyanobacteria are preferred because they are valuable as they grow in high salt concentrations.

W.-Q. Zhuang, J.-H. Tay, A. M. Maszenan, S. T.-L. Tay. (Environmental Engineering Research Centre, Division of Environmental and Water Resources Engineering, School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798). ***Bacillus naphthovorans* sp. nov. from oil-contaminated tropical marine sediments and its role in naphthalene biodegradation.** *Applied Microbiology and Biotechnology*, 58(4) (2002), 547-554.

A *Bacillus* sp., designated as strain MN-003, was isolated as the dominant cultivatable naphthalene-degrading organism from oil-contaminated tropical marine sediments. Strain MN-003 is strictly aerobic, rod-shaped, Gram-positive, catalase positive, oxidase negative, and forms endospores. Strain MN-003 grew at salinities ranging from 0.28 to 7.00‰ and temperatures ranging from 15 to 41°C. Phylogenetic analyses reveal that

strain MN-003 is most similar to *Bacillus* sp. VAN14, with a 16S rRNA sequence identity of 97.9%. Based on taxonomic and 16S rRNA data, strain MN-003 was named *Bacillus naphthovorans* sp. nov. When grown with naphthalene as sole carbon source, strain MN-003 had a maximal specific growth rate (μ_{max}) of $0.32 \pm 0.03 \text{ h}^{-1}$, and a half-saturation constant (K_s) of $22.3 \pm 4.2 \text{ }\mu\text{M}$. A batch study of the tropical marine sediments enriched with naphthalene showed that cells of the *Bacillus* genus grew to become dominant members of the microbial community. The bacilli comprised $39.5 \pm 6.5\%$ of the microbial fraction after 20 days of enrichment.

Warangal. **Decolourisation of textile and dye amended soils by fungi.** *Indian J Environ Hlth*, 44(1) (2002), 65-70.

Four fungi viz. *Aspergillus niger*, *Curvularia lunata*, *Fusarium oxysporum*, *Mucor mucedo* isolated from textile and dye contaminated soils were tried for their efficiency in colour removal. *A. niger* and *M. mucedo* were resistant in the soils and also efficient (92 percent) in decolourisation and in the enzyme production. *C. lunata* and *F. oxysporum* though occurred abundantly were not so successful in the process of colour removal or in enzyme secretions.

Wiyada Mongkolthanaruk, Saovanee Dharmstithi. (Centre for Biotechnology, Institute for Research and development in Science and Technology, Mahidol University, Salaya, Buddhamonthon 4 Rd, Nakornpathom 73170, Thailand. Department of Biotechnology, Faculty of Science, Mahidol University, Salaya, Buddhamonthon 4 Rd, Nakornpathom 73170, Thailand). **Biodegradation of lipid-rich wastewater by a mixed bacterial consortium.** *International Biodeterioration & Biodegradation*, 50(2) (2002), 101-105.

A mixed bacterial culture comprising *Pseudomonas aeruginosa* LP₆₀₂, *Bacillus* sp. B₃₀₄ and *Acinetobacter calcoaceticus* LP₀₀₉ for use in treatment of lipid-rich wastewater was formulated. In our tests, 1 l of wastewater was treated with a mixed inoculum of 6.1×10^8 CFU of LP₆₀₂, 4.8×10^8 CFU B₃₀₄ and 8.9×10^7 CFU of LP₀₀₉ in 20 ml of basal salt solution (1.5 mg/ml Na₂HPO₄, 0.6 mg/ml KH₂PO₄, 40 $\mu\text{g/ml}$ MgSO₄ and 4 $\mu\text{g/ml}$ FeSO₄) supplemented with 1% (w/v) CaCl₂. The intended role of B₃₀₄ was that of a protease and amylase producer while those of LP₆₀₂ and LP₀₀₉ were those of lipase producers. The BOD value and lipid content were reduced from ~ 3500 and 20,000 mg/l, respectively, to < 20 mg/l within 12 days under aerobic conditions.

Yoshitoshi Nakamura, Tatsuro Sawada. (Department of Chemistry and Chemical Engineering, Faculty of Engineering, Kanazawa University, 2-40-20 Kodatsuno, Kanazawa 920-8667, Japan). **Biodegradation of phenol in the presence of heavy metals.** *Journal of Chemical Technoloav & Biotechnology*, 78(2) (2000), 137-142.

A microbial growth model was presented for estimating the dynamic behavior of cell growth and substrate consumption in the biodegradation of phenol containing a heavy metal, such as zinc or copper ions. The application of the model for experiments in a hatch culture and a continuous culture was examined. The values calculated according to the model corresponded satisfactorily with experimental data, such as the optical density of cells and the concentration of phenol. Using the model, the stability of steady states in a continuous culture was analytically evaluated based on the eigenvalues. The steady states were separated into three categories: (i) a stable steady state where phenol was consumed, (ii) an unstable steady state where phenol was consumed, and (iii) a washout state where phenol was not consumed. The number, kind, and stability of steady states varied significantly with operational conditions such as dilution rate, feed concentration of phenol, and feed concentration of heavy metal ions. The operational conditions

required to obtain the stable steady state where phenol was consumed are shown graphically.

Yüksel Orhan and Hanife Büyükgüngör. (Environmental Engineering Department, Ondokuz Mayıs University, Kurupelit-55139, Samsun, Turkey). **Enhancement of biodegradability of disposable polyethylene in controlled biological soil.** International Biodeterioration & Biodegradation, 45(1-2) (2000), 49-55.

Plastics as polyethylene are widely used in packaging and other agricultural applications. They accumulate in the environment at a rate of 25 million tons per year. Thus, the development and use of degradable plastics was proposed as a solution for plastic waste problem. Because of the ever-increasing use of plastic films, nowadays, biodegradability has become a useful characteristic for plastics. Conversely, the introduction of biodegradable plastics has generated a need for methods to evaluate the biodegradation of these polymers in landfills and solid waste treatment systems such as composting or anaerobic digestion treatment plants. The purpose of this study was to investigate the biodegradation of disposable low-density polyethylene bags containing starch (12%), autoxidizable fatty acid ester and catalytic agents in soil. Structurally this work intended to evaluate the capacity of *Phanerochaete chrysosporium* (ATCC 34541) to enhance polyethylene film biodegradation in soil microcosms. Soil samples inoculated with *P. chrysosporium* were mixed with LDPE/starch blend films and biological changes of the films and soil were monitored for 6 months. The biodegradation of polyethylene starch blend film has been determined by the physical, chemical and biological properties of the samples such as pH, biomass, CO₂ formation, percentage elongation, relative viscosity and FTIR spectrum.

Z. Zheng, G. Breedveld, P. Aagaard. (Department of Geology, University of Oslo, PB 1047 Blindern, 0316 Oslo, Norway. Norwegian Geotechnical Institute, PB 3930 Ullevaal Stadion, 0806 Oslo, Norway). **Biodegradation of soluble aromatic compounds of jet fuel under anaerobic conditions: laboratory batch experiments.** Applied Microbiology and Biotechnology, 57(4) (2001), 572-578.

Laboratory batch experiments were performed with contaminated aquifer sediments and four soluble aromatic components of jet fuel to assess their biodegradation under anaerobic conditions. The biodegradation of four aromatic compounds, toluene, *o*-xylene, 1,2,4-trimethylbenzene (TMB), and naphthalene, separately or together, was investigated under strictly anaerobic conditions in the dark for a period of 160 days. Of the aromatic compounds, toluene and *o*-xylene were degraded both as a single substrate and in a mixture with the other aromatic compounds, while TMB was not biodegraded as a single substrate, but was biodegraded in the presence of the other aromatic hydrocarbons. Substrate interaction is thus significant in the biodegradation of TMB. Biodegradation of naphthalene was not observed, either as a single substrate or in a mixture of other aromatic hydrocarbons. Although redox conditions were dominated by iron reduction, a clear relation between degradation and sulfate reduction was observed. Methanogenesis took place during the later stages of incubation. However, the large background of Fe(II) masked the increase of Fe(II) concentration due to iron reduction. Thus, although microbial reduction of Fe(III) is an important process, the evidence is not conclusive. Our results have shown that a better understanding of the degradation of complex mixtures of hydrocarbons under anaerobic conditions is important in the application of natural attenuation as a remedial method for soil and groundwater contamination.

Zeng Feng, Coi Kunyan, Fu Jiamo, Sheng Guoying, Yang Huifang. (School of Chemistry and Chemical Engineering, Zhongshan (Sun Yat-sen) University, Guangzhou, China and State Key Laboratory of Organic Geochemistry, Guangdong Key Laboratory of Environmental Protection and Resources Utilization, Guangzhou Institute of

Geochemistry, Chinese Academy of Sciences, Guangzhou, China. Analysis and Research Center, Zhongshan (Sun Yat-sien) University, Guangzhou, China. Institute of Microbiology, Chinese Academy of Science, Beijing, China). **Biodegradability of Di(2-Ethylhexyl) Phthalate by *Pseudomonas Fluorescens* FS1**. Water, Air, and Soil Pollution, 140 (1-4) (2002), 297-305.

Di(2-ethylhexyl)phthalate (DEHP), one of high-molecular weight phthalate esters (PAEs), is used in the manufacturing of polyvinylchloride (PVC) resins, polyvinyl acetate, cellulose, and polyurethanes, and contributes to environmental pollution. In this article the characteristics of DEHP biodegradation by an effective degradation bacterium, *Pseudomonas fluorescens* FS1 that isolated from the activated sludge at a petrochemical factory, was capable of using phthalate esters as the sole carbon and energy source, were investigated. Experimental result showed that the biodegradation of DEHP by *P. fluorescens* FS1 could be described by the first-order reaction model, which could be expressed as: $\ln C = -0.0688t + A$, and the half-life of DEHP biodegradation was 10.07 d when the initial concentrations of DEHP were less than 50 mg L⁻¹. The inhibition effects of DEHP as a substrate had become predominant above the concentration of 50 mg L⁻¹. The PAEs-degrading enzyme of *P. fluorescens* FS1, mainly located in the soluble part and the particle of cytoplasm, was an intracellular enzyme. The metabolites of DEHP degradation by *P. fluorescens* FS1, which monoester, phthalic acid, benzoic acid, phenol, were extracted using dichloromethane at different time intervals and identified by the GC-MS. The tentative pathway proposed for degradation of DEHP by *P. fluorescens* FS1 under aerobic condition is monoester in the beginning, further enzymatic degradation of the monoester produces phthalic acid, benzoic acid, phenol and finally CO₂ and H₂O.

Zhiwen Yuan, Jeanne M. VanBriesen. (Carnegie Mellon University, Department of Civil and Environmental Engineering, Pittsburgh, PA 15213). **Yield prediction and stoichiometry of multi-step biodegradation reactions involving oxygenation**. Biotechnology and Bioengineering, 80(1) (2002), 100-113.

Microorganisms can initiate the degradation of organic compounds by oxygenation reactions that require the investment of energy and electrons. This diversion of energy and electrons away from synthesis reactions leads to decreased overall cell yields. A thermodynamic method was developed that improves the accuracy of cell yield prediction for compounds degraded through pathways involving oxygenation reactions. This method predicts yields and stoichiometry for each step in the biodegradation pathway, thus enabling modeling a multi-step biodegradation process in which oxygenations occur and intermediates may persist. EDTA and benzene biodegradation are presented as examples. The method compares favorably with other yield prediction methods while providing additional information of yields for intermediates produced in the degradation pathway.

Bioenergy

Allen Farnswortha, Paul Summerfeltd, Daniel G. Neary, Tatersall Smithd. (San Juan Public Lands, Durango, CO 81301, USA. Flagstaff Fire Department, Flagstaff, AZ 86001, USA. Rocky Mountain Research Station, 2500 South Pine Knoll Drive, Flagstaff, AZ 86001, USA. Department of Forest Science, Texas A&M University, College Station, TX 77843, USA). **Flagstaff's wildfire fuels treatments: prescriptions for community involvement and a source of bioenergy**. Biomass and Bioenergy, 24(4-5) (2003), 277-283.

Flagstaff, Arizona, is a high elevation urban area in north-central Arizona surrounded by a dense ponderosa pine (*Pinus ponderosa*) forest. The annual wildland fire ignitions in and near to the urban area average over 200/year. Over the past 5 years, National Forest and city fire managers in the Wildland-Urban Interface (WUI) have developed a system of socially-welcomed fuel reduction treatments to reduce the wildfire threat to the community. These treatments have proven effective in reducing fire hazard, improving probability for successful initial attack on wildfires, maintaining and enhancing vegetative diversity, initiating improvement of overall forest health, and providing a local source of bioenergy. The long-term objective of the program is to facilitate socially acceptable stewardship of forested properties within the WUI. The Flagstaff WUI treatment prescription incorporates not only forestry and fire science, but also community and neighborhood input as vital components in successfully developing, implementing, and maintaining the treatments. Throughout the entire effort, project managers must maintain contact with and gather input from adjacent property owners and the community as a whole. Although current commercial markets are poor, there has been a great deal of success in utilizing bioenergy by designating free-use firewood areas. These events typically draw 200+ people who will remove 362 m³ of wood in half a day. With over 1000 ha now treated, other benefits and lessons have been noted as well.

Anju Arora, and P. K. Singh. (National Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi 110012, India). **Comparison of biomass productivity and nitrogen fixing potential of Azolla SPP.** Biomass and Bioenergy, 24(3) (2003), 175-178.

Study was conducted on six different Azolla species, available in the germplasm collection of NCCUBGA, IARI, New Delhi namely *A. filiculoides*, *A. mexicana*, *A. microphylla*, *A. pinnata*, *A. rubra* and *A. caroliniana* in a polyhouse to assess their growth potential by determining their maximal biomass productivity, doubling time and relative growth rates. Their nitrogen fixing potential was assessed by acetylene reduction assay. Among them Azolla microphylla gave highest biomass production and relative growth rate followed by Azolla caroliniana. Both these had high nitrogenase activity also. Peak nitrogenase activity of these strains was found on 14th day of growth and it declined on further incubation. Azolla microphylla and Azolla rubra were more tolerant to salinity than others. On the other hand Azolla pinnata, which is endemic species found in India, exhibited low biomass production, relative growth rate and lower nitrogenase activity compared to other species. It was unable to sustain growth in saline medium. Under polyhouse conditions, *A. microphylla* was found to perform better than other cultures in terms of biomass productivity, N fixing ability and salt tolerance. Hence it is taken up for mass production.

Bengt Hillring. (Department of Bioenergy, Swedish University of Agricultural Sciences (SLU), P.O. Box 7060, SE-750 07, Uppsala, Sweden). **Rural development and bioenergy--experiences from 20 years of development in Sweden.** Biomass and Bioenergy, 23(6) (2002), 443-451.

Activities have been going on for a number of decades in Sweden in the field of job creation, rural development and development of local economies through the use of bioenergy. This paper relates the experience of different strategies of rural development projects over a 20-year period based on the rapid development of biofuel use, especially wood fuel use in Sweden. A successful strategy for people and companies involved, has been to specify the products and services opposed to bulky raw material production and to integrate them into the companies operations. Another success factor has been size rationalisation. Systems thinking with respect to the market and in different environmental values in the environmental cycle have also been successful. In the

future, there will probably be room for different niche companies that can meet the needs of the market that the strongly rationalised companies cannot. This study calls for new studies of direct employment effects and multipliers. Continued internationalisation of the biofuel market will give greater competitiveness and press down prices among local producers. The strong competition will mean that the survivors will be those who are flexible and have activities and products integrated and apply systems thinking where contact will be with different parts of the chain and not only with the production of the raw material.

Danesh Miah, , Romel Ahmed and Mohammad Belal Uddin. (Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong 4331, Bangladesh). **Biomass fuel use by the rural households in Chittagong region, Bangladesh.** Biomass and Bioenergy, 24(6) (2003), 437-444.

An exploratory survey was carried out to assess biomass fuel use by the rural households in the Chittagong region, Bangladesh. A multistage random sampling technique was adopted to perform the study. Based on the monthly income, respondents were categorized into rich, medium and poor and a total of 45 homesteads, 15 from each category were selected randomly for the study. The study revealed that stems, branches, leaves of trees and agricultural residues were the biomass fuel used by the respondents. Market, homestead, agricultural field, secondary forests/plantation were the sources of biomass fuel identified. Male and female were identified as the major collectors of fuelwood from the nearby forests/plantations and homesteads, respectively. Six fuelwood species were identified as the most preferred in the study area. The study identified the rainy season as the woodfuel shortage period spanning between May and August.

Florian Kraxner, Sten Nilsson and Michael Obersteiner. (International Institute for Applied Systems Analysis, Schlossplatz 1, A-2361, Laxenburg, Austria). **Negative emissions from BioEnergy use, carbon capture and sequestration (BECS)--the case of biomass production by sustainable forest management from semi-natural temperate forests.** Biomass and Bioenergy, 24(4-5) (2003), 285-296.

In this paper, we show how nature oriented forestry measures in a typical temperate forest type in combination with bioenergy systems could lead to continuous and permanent removal of CO₂ from the atmosphere. We employ a forest growth model suited for modeling uneven-aged mixed temperate stands and analyze the interaction with biomass energy systems that allow for CO₂ removal and long-term sequestration in geological formations. On global scales this technological option to convert the global energy system from a CO₂ emitter to a CO₂ remover has been overlooked by both the science and policy communities. Removal of the major Greenhouse Gas (GHG) CO₂ from the atmosphere is possible using biomass energy to produce both carbon neutral energy carriers (e.g., electricity and hydrogen) and, at the same time, offer a permanent CO₂ sink by capturing carbon at the conversion facility and permanently storing it in geological formations. This technological option resolves the issues of permanence and saturation of biological sinks while at the same time this option respects the multiple benefits of sustaining diverse, healthy, and resilient forests. Our results indicate that a typical temperate forest in combination with capturing and long-term storage can permanently remove and on a continuous basis about 2.5 tCyr⁻¹ ha⁻¹ on a sustainable basis respecting the ecological integrity of the ecosystem.

G. D. P. S. Augustusa, M. Jayabalana and G. J. Seiler. (Research Centre in Botany, V.H.N.S.N College, Virudhunagar 626 001, India. USDA-ARS, Northern Crop Science Laboratory, P.O. Box 5677, Fargo, ND 58105, USA). **Alternative energy sources**

from plants of Western Ghats (Tamil Nadu, India). *Biomass and Bioenergy*, 24(6) (2003), 437-444.

Twenty-two taxa of Western Ghats plants were screened as potential alternative crops for renewable energy, oil, hydrocarbon and phytochemicals. The highest hydrocarbon yields were observed in *Carissa carandas* (1.7%), and *Jatropha gossypifolia* (1.7%). The highest polyphenol fraction was observed in *Dodonaea viscosa* (17.1%), *Carissa carandas* (7.7%), *Swietenia mahagoni* (6.6%), and *Jatropha glandulifera* (6.2%). The highest oil content was observed in *Aganosma cymosa* (10.3%), *Carissa carandas* (5.8%), and *Argemone mexicana* (5.0%). *Swietenia mahagoni* yielded the highest protein content with 8.1%. The gross heat value of 4175.0 cal/g (17.5 MJ/kg) for *Lochnera rosea* (pink flowered var.), and 4112.0 cal/g for *Dalbergia sissoo* were the highest among the species analysed. NMR spectra of the hydrocarbon fractions of *Alstonia scholaris*, *Carissa carandas*, *Ichnocarpus frutescens*, *Plumeria rubra*, *Thevetia neriifolia* (white flowered var.), *Vallaris solanacea*, *Lochnera rosea* (pink flowered var.), *Euphorbia hirta*, *E. splendens*, *Artocarpus integrifolia* and *Ficus religiosa* revealed the presence of cis-polyisoprene (natural rubber), whereas *Argemone mexicana* showed the presence of trans-polyisoprene (gutta). Several new crop species were identified with potentially useful compounds. The potential exists for growing these alternate crops in areas of underutilized lands, subsequently stimulating industrial and economic growth.

G. K. Luk. (Department of Civil Engineering, Ryerson Polytechnic University). **A Bioenergetics Approach to Modeling PCB Bioaccumulation in Lake Ontario Salmonids.** *Water Quality and Ecosystems Modeling*, 1(1-4) (2000), 223-235.

The objective of this paper is to compare the trend of polychlorinated biphenyls (PCBs) bioaccumulation in three species of Lake Ontario salmonids with similar diet patterns and habitat. The concept of bioenergetics, which relates the growth and energy expenditure of a fish directly to its food consumption, is integrated into the study of pollutant accumulation. The resulting bioaccumulation model is a comprehensive approach that combines the physiological information of the fish, such as diet, metabolism, respiration, habitat, age and species, with the environmental conditions in the lake. The three species of salmonids studied are Lake Trout (*Salvelinus namaycush*), Brown Trout (*Salmo trutta*), and Chinook Salmon (*Oncorhynchus tshawytscha*). The model, which relies on a lakewide water quality and fish sampling database from the Ontario Ministry of Environment and Energy, is applied to study the pattern of total body burden of PCBs over the life span of the fish. Results from the model compare favorably to the data, and the observations clearly demonstrate the relative effects of food and water contamination on the time-dependent accumulation of PCBs in the bodies of fish. It is observed that different species sharing the same habitat and exposed to similar water contaminant concentrations are exhibiting marked difference in the body accumulation of PCBs. This difference is successfully reproduced with the model through judicious representation of their diet preferences and generically-governed metabolisms.

Johanna Söderström, Linda Pilcher, Mats Galbe and Guido Zacchi. (Department of Chemical Engineering 1, Lund University, P.O. Box 124, SE-221 00, Lund, Sweden). **Two-step steam pretreatment of softwood by dilute H₂SO₄ impregnation for ethanol production.** *Biomass and Bioenergy*, 24(6) (2003), 475-486.

Fuel ethanol can be produced from softwood through hydrolysis in an enzymatic process. Prior to enzymatic hydrolysis of the softwood, pretreatment is necessary. In this study two-step steam pretreatment by dilute H₂SO₄ impregnation to improve the overall sugar and ethanol yield has been investigated. The first pretreatment step was performed under conditions of low severity (180°C, 10 min, 0.5% H₂SO₄) to optimise the amount of hydrolysed hemicellulose. In the second step the washed solid material from the first

pretreatment step was impregnated again with H₂SO₄ and pretreated under conditions of higher severity to hydrolyse a portion of the cellulose, and to make the cellulose more accessible to enzymatic attack. A wide range of conditions was used to determine the most favourable combination. The temperatures investigated were between 180°C and 220°C, the residence times were 2, 5 and 10 min and the concentrations of H₂SO₄ were 1% and 2%. The effects of pretreatment were assessed by both enzymatic hydrolysis of the solids and with simultaneous saccharification and fermentation (SSF) of the whole slurry, after the second pretreatment step. For each set of pretreatment conditions the liquid fraction was fermented to determine any inhibiting effects. The ethanol yield using the SSF configuration reached 65% of the theoretical value while the sugar yield using the SHF configuration reached 77%. Maximum yields were obtained when the second pretreatment step was performed at 200°C for 2 min with 2% H₂SO₄. This form of two-step steam pretreatment is a promising method of increasing the overall yield in the wood-to-ethanol process.

K. Bélafi-Bakó, F. Kovács, L. Gubicza, J. Hancsók. (Research Institute of Chemical and Process Engineering, Egyetem u. 2., Veszprém 8200, Hungary. University of Veszprém, Egyetem u. 10., Veszprém 8200, Hungary). **Enzymatic Biodiesel Production from Sunflower Oil by *Candida antarctica* Lipase in a Solvent-free System**. *Biocatalysis and Biotransformation*, 20(6) (2002), 437 - 439.

Methanolysis (transesterification with methanol) of sunflower oil by lipase from *Candida antarctica* (Novozym 435) in a solvent-free system has been studied. Stepwise as well as continuous methanol feeding was applied to avoid strong substrate inhibition. Glycerol was found to cause strong product inhibition on the enzymatic reaction, therefore glycerol removal by dialysis was investigated using a flat sheet membrane module.

Kees W. Kwant. (Novem B.V., P.O. Box 8242, NL-3503 RE, Utrecht, The Netherlands). **Renewable energy in The Netherlands: policy and instruments**. *Biomass and Bioenergy*, 24(4-5) (2003), 265-267.

To achieve a place for renewables and energy from waste in a liberalized energy market the government has to focus on a more demand-driven approach, and for specific technologies, a more supply-driven policy will be required. The available financial and fiscal instruments, regulations and voluntary agreements provide new opportunities. The Dutch government has supported renewables with fiscal instruments (green funds, tax credits and an energy tax) since 1996. As a follow-up of the green energy market and the mandated share set by the Energy Companies, the government introduced in 2001 a system for tradable green certificates. On 1 July 2001 the market for green electricity became liberalized and the consumers of green electricity were free to choose their own supplier, the number of green consumers went up to 700,000 at the end of 2001.

L. G. Giri Rao, B. Joseph, B. Sreemannarayana. (All India Co-ordinated Research Project on Agroforestry, Agricultural Research Institute. Acharya N. G. Ranga Agricultural University, Hyderabad (Andhra Pradesh)). **Growth and biomass production of some important multipurpose tree species on rainfed sandy loam soils**. *The Indian Forester*, 126(7) (2000), 772-781.

An experiment with eleven multipurpose tree species was conducted on red sandy loam soils of Agroforestry block in Acharya NG Range Agricultural University, Rajindranagar campus. Evaluation of tree species nine years after plantation revealed that *Dalbergia sissoo*, *Leucaena leucocephala*, *Acacia auriculiformis* and *Eucalyptus camaldulensis* were fast growing and suitable to southern Telangana zone of Andhra Pradesh which are dominated with red sandy loam soil. Studies on biomass production (small and log wood)

of eleven multipurpose tree species revealed that *Dalbergia sissoo* (214.6 t ha⁻¹) recorded maximum biomass followed by *Leucaena leucocephala* (187.8 t ha⁻¹) and *Acacia auriculiformis* (162.4 t ha⁻¹). Maximum Mean Annual Biomass Production (MABP) recorded was also more for *Dalbergia sissoo* (23.8 t ha⁻¹) followed by *Leucaena leucocephala* (20.9 t ha⁻¹) and *Acacia auriculiformis* (18.0 t ha⁻¹). Biomass yield component studies such as foliage yield at ninth year after planting revealed that maximum foliage production on oven dry weight basis was recorded by *Leucaena leucocephala* (16.8 t ha⁻¹) followed by *Acacia auriculiformis* (12.0 t ha⁻¹) and *Eucalyptus camaldulensis* (9.9 t ha⁻¹). Cost benefit analysis of the study showed that for every rupees spent on cultivation of these tree species, highest return was obtained from *Dalbergia sissoo* (4.4) followed by *Leucaena leucocephala* (4.0), *Acacia auriculiformis* (3.1) and *Eucalyptus* (2.9).

M. D. Summers, B. M. Jenkins, P. R. Hyde, J. F. Williams, R. G. Mutters, S. C. Scardacci and M. W. Hair. (Department of Biological and Agricultural Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA. International Agricultural Development Program, University of California, One Shields Avenue, Davis, CA 95616, USA. University of California Cooperative Extension, 142-A Garden Hwy., Yuba City, CA 95991, USA. University of California Cooperative Extension, 2279-B Del Oro Avenue, Oroville, CA 95965, USA. University of California Cooperative Extension, 100 Sunrise Blvd., Suite E, Colusa, CA 95932, USA). **Biomass production and allocation in rice with implications for straw harvesting and utilization.** Biomass and Bioenergy, 24(3) (2003), 163-173.

Variability in straw quantity and quality can have critical impacts on biomass industries. To generate better information on this variability for rice residues, trials with eight common California rice cultivars were planted at multiple sites for the 1999 and 2000 seasons. Straw yields averaged 11.2 Mgha⁻¹ in 1999 and 8.5 Mgha⁻¹ in 2000 with a consistent range of 2–3 Mgha⁻¹ between the highest and lowest yielding varieties at each site. Straw-to-grain ratios were also higher in 1999 averaging 1.50 kgkg⁻¹ with high variability while in 2000 they were a more typical 1.04 kgkg⁻¹ with little difference by site or variety. The length of the pre-heading period was the strongest indicator for straw yield. Each one day increment in the length of the time to 50% heading resulted in an additional 8.4 kWhm⁻² of solar energy and 0.2 Mgha⁻¹ of straw production at an efficiency slightly over 1%. Average stem weight ranged from 1.3 to 2.6 g and increased stem weight corresponded to higher yield but lower stand density. Harvested straw yield is also strongly affected by cutting height with a non-linear distribution resulting in nearly half of the straw biomass occurring in the lower third of the plant. Forty percent of biomass was in the internode sections of the stem, 53% in leaf and sheath, 4% in nodes and 3% in the panicle (excluding hull and seed). Stem (culm) fraction decreases and leaf fraction increases from the base of the plant to the panicle. Since many properties vary by botanical fraction, height of cut influences both the yield and composition of the straw. The ability to predict the amount and composition of the biomass material allows for greater control in the design and mobilization of the harvesting system.

Markku O. Raiko, Tom H. A. Gronfors and Pauli Haukka. (Fortum Energy Solutions, P.O.B. 10, 00048, Fortum, Finland. Tampere University of Technology, P.O.B. 589, 33101, Tampere, Finland). **Development and optimization of power plant concepts for local wet fuels.** Biomass and Bioenergy, 24(1) (2003), 27-37.

Many changes in business drivers are now affecting power-producing companies. The power market has been opened up and the number of locally operating companies has increased. At the same time the need to utilize locally produced biofuels is increasing because of environmental benefits and regulations. In this situation, power-producing

companies have on focus their in-house skills for generating a competitive edge over their rivals, such as the skills needed for developing the most economical energy investments for the best-paying customer for the local biomass producers. This paper explores the role of optimization in the development of small-sized energy investments. The paper provides an overview on a new design process for power companies for improved use of in-house technical and business expertise. As an example, illustrative design and optimization of local wet peat-based power investment is presented. Three concept alternatives are generated. Only power plant production capacity and peat moisture content are optimized for all alternatives. Long commercial experience of using peat as a power plant fuel in Finland can be transferred to bioenergy investments. In this paper, it is shown that conventional technology can be feasible for bioenergy production even in quite small size (below 10 MW). It is important to optimize simultaneously both the technology and the two businesses, power production and fuel production. Further, such high moisture content biomass as sludge, seaweed, grass, etc. can be economical fuels, if advanced drying systems are adopted in a power plant.

P. S. Thakur. (Department of Silviculture and Agroforestry, University of Horticulture and Forestry, Solan (Himachal Pradesh). **Effect of canopy management on vigour and biomass production potential in four agroforestry from temperate region.** The Indian Forester, 128(5) (2002), 493-501.

Out of the four agroforestry tree species, namely *Grewia optiva*, *Celtis australis*, *Bauhinia variegata*, *Morus alba* and two tree species (i.e *G. optiva* and *M. alba*) maintained higher growth, vigour and foliage and branchwood biomass production potential for longer period when pollarded at 1.5 or 2.0 m. *G. optiva* and *M. alba*, responded better to canopy management practices like coppicing and pollarding as compared to *C. australis* and *B.variegata*. Collar diameter increased with increase in cutting height in *G. optiva* and *M. alba*, but remained unchanged in *C. australis* and *B. variegata* up to fourth year of canopy management treatments. Significant decrease in shoot number, Leaf Area, Leaf Area Index (LAI) and foliage and branchwood biomass production occurred by fourth year of treatments although decrease was of higher magnitude at lower cutting heights in all the four tree species. *M. alba* followed by *G. optiva* produced maximum foliage and branchwood biomass at 2.0 cutting height during the entire experimental period.

R. L. Semwal, R. K. Maikhuri, K. S. Rao, K. K. Sen and K. G. Saxena. (G.B. Pant Institute of Himalayan Environment and Development, Garhwal Unit, P.B. 92, Srinagar, Garhwal 246174, India. Sustainable Development and Rural Ecosystems Programme, G.B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora 263643, India. School of Environmental Sciences, Jawaharlal Nehru University, New Delhi 110067, India). **Leaf litter decomposition and nutrient release patterns of six multipurpose tree species of central Himalaya, India.** Biomass and Bioenergy, 24(1) (2003), 3-11.

Chemical characteristics and decomposition patterns of six multipurpose tree species, viz., *Alnus nepalensis*, *Albizia lebbek*, *Boehmeria rugulosa*, *Dalbergia sissoo*, *Ficus glomerata* and *F. roxburghii* were analysed in a mixed plantation established on an abandoned agricultural land site in a village at 1200 m altitude in Central Himalaya, India. Differences in chemical quality of litter species were most marked in polyphenol and N concentrations. *A. lebbek*, *A. nepalensis* and *D. sissoo* showed higher N (2.2–2.6%) but lower polyphenol concentrations (3.2–4.7%) than *B. rugulosa*, *F. glomerata* and *F. roxburghii* (0.96–1.97% N and 5.68–11.64% polyphenol). Significant effects of species, incubation time and species×incubation time interaction on monthly mass, N, P and K release rates were observed. A linear combination of rainfall and temperature explained the variation in monthly mass loss better than rainfall and temperature

independently. Percentage mass remaining after 1 year of incubation varied from 30 to 50, N remaining from 40 to 86, P remaining from 33 to 56 and K remaining from 1 to 3. Annual decomposition constants of mass and N were positively correlated with C and N concentrations and negatively correlated with C/N, lignin/N, polyphenol/N and lignin+polyphenol/N ratios of fresh litter. As all the species studied showed the highest rates of N and P release during the rainy season, rainy season crops are not likely to be as much nutrient stressed as winter season crops if leaf litter of these species is assumed to be the sole source of nutrients to crops in tree-crop mixed agroforestry. *A. lebbek*, *A. nepalensis*, *D. sissoo* and *F. glomerata* seem to be more appropriate for rapid recovery in degraded lands as their litter decomposed faster than *B. rugulosa* and *F. roxburghii*. A diverse multipurpose tree community provides not only diverse products but may also render stable nutrient cycling.

S. Iniyan, and K. Sumathy. (Department of Mechanical Engineering, Anna University, Chennai, India. Department of Mechanical Engineering, University of Hong Kong, Hong Kong). **The application of a Delphi technique in the linear programming optimization of future renewable energy options for India.** *Biomass and Bioenergy*, 24(1) (2003), 39-50.

The role of renewable energy resources in developing countries has increased considerably over the last decade. Technological developments are so advanced that the renewables can be conveniently substituted for commercial energy sources. The extent to which renewable energy could be substituted in the commercial energy scene in respect of environment and social impact is discussed in this paper. An optimal renewable energy mathematical (OREM) model will be developed for the substitution of renewable energy sources in India over the years 2010–11, 2015–16 and 2020–21. It is a linear programming model, which allocates optimal renewable energy sources for different end-uses such as lighting, cooking, pumping, heating, cooling and transportation. The model was developed with the objective of minimizing cost/efficiency ratio based on social acceptance, reliability, demand and potential constraints. The model predicts that around 25% of the total energy consumed will be from renewable energy sources by the year 2020–21. It was found that at optimal condition, for lighting end-use, solar PV and biogas electricity conversion could be used to an extent of 520 and 750 PJ, respectively. Similarly, the optimal renewable energy sources for other end-uses were determined by running OREM model. The potential for biomass, biogas, firewood and ethanol were varied in the model and different renewable energy distribution patterns were obtained. When the potential of these resources are increased in the model, the contribution of solar energy systems would decrease as they are expensive. Sensitivity analysis was conducted to validate the OREM model. The coefficient of sensitivity has been obtained for the variation of renewable energy demand, social acceptance and potential of renewable energy sources. Sensitivity analysis revealed that the OREM model is very sensitive with regard to variation of different parameters in the model. This model can be used in the formation of energy strategies in India.

S. Kalligeros, F. Zannikos, S. Stournas, E. Lois, G. Anastopoulos, Ch. Teas and F. Sakellaropoulos. (School of Chemical Engineering, National Technical University of Athens, Iroon Polytechniou 9, Athens 157 80, Greece). **An investigation of using biodiesel/marine diesel blends on the performance of a stationary diesel engine.** *Biomass and Bioenergy*, 24(2) (2003), 141-149.

Vegetable oils are produced from numerous oil seed crops. While all vegetable oils have high-energy content, most require some processing to assure safe use in internal combustion engines. Some of these oils already have been evaluated as substitutes for diesel fuels. With the exception of rape seed oil which is the principal raw material for

biodiesel fatty acid methyl esters, sunflower oil, corn oil and olive oil, which are abundant in Southern Europe, along with some wastes, such as used frying oils, appear to be attractive candidates for biodiesel production. In this paper, fuel consumption and exhaust emissions measurements from a single cylinder, stationary diesel engine are described. The engine was fueled with pure marine diesel fuel and blends containing two types of biodiesel, at proportions up to 50%. The two types of biodiesel appeared to have equal performance, and irrespective of the raw material used for their production, their addition to the marine diesel fuel improved the particulate matter, unburned hydrocarbons, nitrogen oxide and carbon monoxide emissions.

Z. Husain, Z. A. Zainal and M. Z. Abdullah. (School of Mechanical Engineering, Engineering Campus, University Science Malaysia, 14300 Nibong Tebal Seberang Perai Seletan, Pulau Pinang, Malaysia). **Analysis of biomass-residue-based cogeneration system in palm oil mills.** *Biomass and Bioenergy*, 24(2) (2003), 117-124.

Palm oil mills in Malaysia operate on cogeneration system using biomass residue as fuel in the boiler. The boiler produces high pressure and temperature steam which expands in a backpressure steam turbine and produces enough electric power for the internal needs of the mill. The exhaust steam from the turbine goes to an accumulator which distributes the steam to various processes in the mill. The study were made on seven palm oil mills in the Perak state in Malaysia. The primary objectives of the study are to determine boiler and turbine efficiencies, energy utilization factor, oil extraction rate and heat/power ratio for various palm oil mills working under similar conditions and adopting same processes. The palm oil industry is one of those rare industries where very little attempt is made to save energy. The energy balance in a typical palm oil mill is far from optimum and there is considerable scope for improvement. Bench-marking is necessary for the components in the mill. Energy-use bench-marking can give an overview of energy performance of the mills. The calculations were done to get net gain in power when back pressure turbine is replaced by a condensing turbine. It was found that the boiler and turbine have low thermal efficiencies compared to conventional ones used in power plants due to non-homogeneity and non-uniform quality of the fuel. The extraction rate was around 0.188. The use of condensing turbine increase the power output by 60% and the utilization factor was found to be 65% for the cogeneration system.

Bioengineering

A. K. Johri, W. Blank, D. L. Kaplan. (Department of Chemical and Biological Engineering, Bioengineering and Biotechnology Center, Tufts University, 4 Colby Street, Medford, MA 02155 USA). **Bioengineered emulsans from *Acinetobacter calcoaceticus* RAG-1 transposon mutants.** *Applied Microbiology and Biotechnology*, 59(2-3) (2002), 217-223.

Transposon mutants of *Acinetobacter calcoaceticus* strain RAG-1 were studied in an effort to control fatty acid (FA) substitution patterns of emulsan, a bioemulsifier secreted by the organism. The disrupted genes, involved in the biosynthetic pathways of biotin, histidine, cysteine or purines, influenced the level and types of FAs incorporated into emulsan. The structural variants of emulsan generated by the transposon mutants were characterized for yield, FA content, molecular weight, and emulsification behavior when grown on a series of FAs of different chain lengths from C11 to C18. Yields of emulsan from the transposon mutants were found to be lower than the parent strain and depended on the type of FA used to supplement the growth medium. Mutants 13D (His-) and 52D (Cys-) grown on LB plus C16 or C14, respectively, exhibited enhanced emulsifying activity compared to *A. calcoaceticus* RAG-1. The presence and composition

of long chain FAs on the polysaccharide backbone influenced emulsification behavior: particularly a high mole percentage of C16 (48%) and C18 (42%). The results provide important insight into the bioengineering of bioemulsifier-producing microorganisms and provide a path towards highly tailored novel amphipathic structures to utilize as biodegradable in environmental, biomedical, and personal care applications.

B. Kiesel, R.H. Muller. **The *meta* Pathway as a Potential Energy-Generating Sequence and its Effects on the Growth Rate during the Utilization of Aromatics.** *Acta Biotechnol*, 22(3-4) (2002), 221-234.

Pseudomonas putida was grown on benzoate in order to determine the maximum growth rate depending on the *ortho* and/or *meta* assimilation pathway. Strain KT2440(pWWO), which expressed both the *ortho* (catechol 1,2-dioxygenase) and *meta* (catechol 2,3-dioxygenase) pathway under C-limited growth conditions, resulted in a maximum growth rate of I_{max} of 0.27 h^{-1} as determined by a transient-state cultivation technique. This rate was similar to the I_{max} of a strain in which the *meta* pathway was inactivated by Tn5 transposon mutagenesis. With strain KT2440, in which the *meta* pathway was eliminated by curing of the plasmid, a maximum growth rate of 0.44 h^{-1} was reached. By contrast, when using strain PaW94, which was deficient in a functional *ortho* pathway, the I_{max} amounted to 0.31 h^{-1} with transconjugants bearing plasmid pWWO as a harbour of the *meta* pathway. Moreover, with transconjugant strains harbouring the TOL:RP4 hybrid plasmid pWW53-4, the maximum growth rate was as high as 0.58 h^{-1} . Such a high rate was also attained (but not exceeded) by a plasmid-less strain of PaW94 in which the *meta* pathway was integrated into the chromosome. According to these results, the maximum growth rate was 1.3 times faster when the *meta* pathway was used compared to the *ortho* pathway in a chromosomal localisation of both routes. These effects are discussed in terms of an advantage attributed to the manner of energy generation by assimilating phenolic substrates via the *meta* pathway in comparison with the *ortho* pathway. The effects on the maximum growth rates may be modulated by the presence or absence of plasmids.

C. C. Giri, and G. Vijaya Laxmi. (Centre for Plant Molecular Biology, Department of Genetics, Osmania University, Hyderabad, 500007 AP, India). **Production of transgenic rice with agronomically useful genes: an assessment.** *Biotechnology Advances*, 18(8) (2000), 653-683.

Rice is the most important food crop in tropical and subtropical regions of the world. Yield enhancement to increase rice production is one of the essential strategies to meet the demand for food of the growing population. Both abiotic and biotic features limit adversely the productivity of rice growing areas. Conventional breeding has been an effective means for developing high yielding varieties, however; it is associated with its own limitations. It is envisaged that recent trends in biotechnology can contribute to the agronomic improvement of rice in terms of yield and nutritional quality as a supplement to traditional breeding methods. Genetic transformation of rice has demonstrated numerous important opportunities resulting in the genetic improvement of existing elite rice varieties and production of new plant types. Significant advances have been made in the genetic engineering of rice since the first transgenic rice plant production in the late 1980s. Several gene transfer protocols have been employed successfully for the introduction of foreign genes to rice. In more than 60 rice cultivars belonging to indica, japonica, javanica, and elite African cultivars, the protocol has been standardized for transgenic rice production. Selection and use of appropriate promoters, selectable markers, and reporter genes has been helpful for development of efficient protocols for transgenic rice in a number of rice cultivars. The present review is an attempt to assess the current state of development in transgenic rice for the transfer of agronomically

useful genes, emphasizing the application and future prospects of transgenic rice production for the genetic improvement of this food crop.

C. Lambert, D. Weuster-Botz, R. Weichenhain, E.W Kreutz, A.A de Graaf, S.M. Schoberth. **Monitoring of Inorganic Polyphosphate Dynamics in *Corynebacterium glutamicum* Using a Novel Oxygen Sparger for Real Time ^{31}P *in vivo* NMR.** Acta Biotechnol, 22(3-4) (2002), 245-260.

For the first time in intact bacterial cells, the dynamics of the build-up of soluble cytosolic inorganic polyphosphate (polyP) during aeration, and its breakdown during anaerobiosis have been observed with a time resolution of 50 s. Under conditions of 60-80% saturation with pure oxygen, the accumulation of high levels of intracellular polyP was detected when inorganic phosphate (Pi) and glucose or acetate were added to *Corynebacterium glutamicum* cell suspensions (3 ml, ~40 mg dw/ml). The maximum levels of polyP reached were estimated to 600 mM P units in the cytosol or ~3% phosphorus [w/w] in the cell dry weight. *C. glutamicum* polyP was apparently of high molecular weight (containing probably a few hundred units) as inferred from signal distribution, but a temporary average polyP chain length of about $n = 40$ could be estimated at the initial stages of polyP formation. After each addition of glucose or acetate, oxygen levels followed a steep decline to ~20% and then an increase to the previous level, in contrast, polyP levels rose after the addition of substrate, and declined again, while the oxygen level recovered. When the oxygen supply was completely switched off, the polyP signal declined immediately, with concomitant re-appearance of phosphomonoester signals (sugar phosphates and related compounds). Both processes, the increase of polyP during aeration and supply with substrate and Pi, and the decrease during anaerobiosis, occurred within minutes. Only within these relatively brief windows of time between successive feedings with substrate or between aeration and anaerobiosis, high levels of polyP could be observed. Thus, our findings indicate that polyP occurs not only as the long known granular storage material in some *Corynebacteria*, such as *C. diphtheriae* or *C. imitans*, but that formation and breakdown of soluble polyP in *C. glutamicum* is a very dynamic process that may play a decisive role in *C. glutamicum* and in other strains of this genus. These investigations were made possible by combining nuclear magnetic resonance (NMR) techniques with novel methods of oxygen sparging and online substrate distribution. The sparger was custom made from titanium to fit into 10 mm o.d. NMR tubes. Both the size and the spacing of the holes in the sparger were calculated for optimum distribution of oxygen at 30 °C through 3 ml of *C. glutamicum* cell suspensions. The experiments were carried out using *in vivo* ^{31}P NMR, and monitoring of oxygen was performed with a miniature oxygen optode in real time. Glucose or acetate and/or phosphate stock solutions could be added *in situ*. ^{31}P NMR analyses of intracellular phosphorus metabolites were sampled with a time resolution of 50 s. The sparger unit, including optode and supply lines, could be easily switched from one sample to another after completion of an experiment. It is suggested to use these analytical tools to investigate other bacteria strains and even cell extracts, shedding further light on the novel roles of polyP in living cells [Schroder, H. C., Muller, W. E. G., (eds.). Inorganic polyphosphates - biochemistry, biology, biotechnology. Prog. Mol. Subcell. Biol. 23 (1999). Springer-Verlag, Berlin].

Chia-Li Wei, Yunn-Bor Yang, Wen-Ching Wang, Wen-Chi LiU, Jyh-Shing Hsu, Ying-Chieh Tsai. (Institute of Biochemistry, National Yang-Ming University, Taipei, Synmax Biochemical Co., Ltd., Hsinchu, Department of Life Sciences, National Tsing-Hua University, Hsinchu, Taiwan). **Engineering *Streptomyces clavuligerus* Deacetoxycephalosporin C Synthase for Optimal Ring Expansion Activity toward Penicillin G.** Applied and Environmental Microbiology. 69(4) (2003), 2306-2312.

The deacetoxycephalosporin C synthase (DAOCS) from *Streptomyces clavuligerus* was

engineered with the aim of enhancing the conversion of penicillin G into phenylacetyl-7-aminodeacetoxycephalosporanic acid, a precursor of 7-aminodeacetoxycephalosporanic acid, for industrial application. A single round of random mutagenesis followed by the screening of 5,500 clones identified three mutants, G79E, V2751, and C281Y, that showed a two- to sixfold increase in the K_{cat}/K_m ratio compared to the wild-type enzyme. Site-directed mutagenesis to modify residues surrounding the substrate resulted in three mutants, N304K, 1305L, and 1305M, with 6- to 14-fold-increased K_{cat}/K_m values. When mutants containing all possible combinations of these six sites were generated to optimize the ring expansion activity for penicillin G, the double mutant, YS67 (V2751, 1305M), showed a significant 32-fold increase in the K_{cat}/K_m ratio and a 5-fold increase in relative activity for penicillin G, while the triple mutant, YS81 (V2751, C281Y, 1305M), showed an even greater 13-fold increase in relative activity toward penicillin G. Our results demonstrate that this is a robust approach to the modification of DAOCS for an optimized DAOCS-penicillin G reaction.

J. Mukherjee, M. Menge, D. Hoischen, N. Grammel, E. Winterfeldt, U. Keller, Th. Scheper. (Development of a Tryptophan Auxotrophic Mutant of *Claviceps purpurea* 1029 N5 and its Preliminary Application in the Synthesis of New Ergot Alkaloids). **Development of a Tryptophan Auxotrophic Mutant of *Claviceps purpurea* 1029 N5 and its Preliminary Application in the Synthesis of New Ergot Alkaloids.** Acta Biotechnol, 13(3-4) (2002), 411-415.

Four tryptophan dependent mutants of *Claviceps purpurea* 1029 N5 were derived by treatment with N-methyl-N'-nitro-N-nitrosoguanidine (NTG) and protoplasting of the treated fungus. The poor alkaloid production of these mutants required a systematic optimisation of the production media. Malic acid was essential for good alkaloid production. A preliminary attempt to modify the tryptophan moiety of the ergot alkaloid molecule by feeding one selected Trp-mutant with tryptophan derivatives (synthons) and preliminary analysis of the extracellular spent medium by HPLC are described. The utilisation of the synthon, 5-methoxytryptophan, from the medium by the Trp-mutant was 99%. The analysis of the spent medium extract of the flask fed with this synthon by HPLC showed the formation of a new peak in the ergot alkaloid region. This peak increased in intensity relative to the time of incubation. Its detailed analysis and further characterisation will be the focus of future studies.

K. Yamada-Onodera, H. Yamamoto, E. Emoto, N. Kawahara, Y. Tani. **Characterisation of Glycerol Dehydrogenase from a Methylophilic Yeast, *Hansenula polymorpha* DL-1, and its Gene Cloning.** Acta Biotechnol, 22(3-4) (2002), 337-353.

Glycerol dehydrogenase (EC 1.1.1.6) was purified from a methylophilic yeast, *Hansenula polymorpha* DL-1, for the characterisation and identification of its function. The yeast was grown in a glycerol medium, SDS-PAGE and gel permeation chromatography showed the enzyme to be composed of two subunits, each with a molecular mass of 36,000. The optimum pH for glycerol oxidation was 10. The optimum temperature for enzyme activity was 30 °C. CuCl_2 , HgCl_2 , and FeCl_3 were severely inhibitory. The activity decreased to 0% by 1 mM 2, 2'-dipyridyl and o-phenanthroline, which are inhibitors for the Fe^{2+} enzyme. An SH reagent, p-chloromercuribenzoate, was strongly inhibitory at 0.1 mM. The enzyme showed greater activity towards (R)-1,2-propanediol and (2R,3R)-2,3-butanediol than towards glycerol. No activity was detected towards 1,3-propanediol, ethanol, 1-propanol, 2-propanol, propionic acid, 1,4-butanediol, 1,2,3,4-butanetetraol, sorbitol, and L-iditol. The ratios of the activity for the R-form to that of the S-form were 5.0 (R):1 (S) and 38 (R):1(S) for 1,2-propanediol and 2,3-butanediol, respectively. The K_m values for glycerol and dihydroxyacetone were 118 mM and 4.87 mM, respectively. The gene encoding the enzyme was cloned from an *H. polymorpha* DL-1 genomic library. Reverse transcription PCR showed that the mRNA of

the enzyme was synthesised in cells grown on methanol as well as on glycerol. In the deduced amino acid sequence of 380 residues of the glycerol dehydrogenase of *H. polymorpha* DL-1, the NAD(H) binding pattern and the cysteine residues that correspond to the cysteine residues at the zinc atom were conserved, as they are in sorbitol dehydrogenase, L-iditol 2-dehydrogenase, and 2,3-butanediol dehydrogenase from other origins.

K. Yamada-Onodera, H. Yamamoto, N. Kawahara, Y. Tani. **Expression of the Gene of Glycerol Dehydrogenase from *Hansenula polymorpha* DL-1 in *Escherichia coli* for the Production of Chiral Compounds.** Acta Biotechnol, 22(3-4) (2002), 355-362.

For the production of chiral compounds, an *Escherichia coli* HB101 strain was transformed. A chiral column that could resolve the three 2,3-butanediol isomers and the two acetoin isomers was used to demonstrate the stereospecificity of the enzyme. The *H. polymorpha* DL-1 gene product, glycerol dehydrogenase, catalyses the NAD⁺-dependent oxidation of 2,3-butanediol to acetoin as well as the corresponding reverse reactions. The recombinant *E. coli* HB101 strain harbouring the expression plasmid produced (3R)-acetoin [99% yield, >99.9% enantiomeric excess (e.e.)] from 110 mM (2R, 3R)-2,3-butanediol and (3S)-acetoin (99% yield, >99.9% e.e.) from 110 mM meso-2,3-butanediol after 24 h of incubation, showing specificity towards the secondary alcohol in R-configuration. From a racemate of 110 mM 2,3-butanediol (the molar ratio of 17 (2R, 3R) : 15 (2S, 3S) : 76 (meso)), (2S, 3S)-2,3-butanediol (15 mM, 92% e.e.) was obtained in the resting-cell reaction without any additive to regenerate NAD⁺ from NADH.

Keisuke Ekino, Hiroyuki Hayashi, Masahiro Moriyama, Minoru Matsuda, Masatoshi Goto, Sadazo Yoshino, and Kensuke Furukawa. (Department of Applied Microbial Technology, Sojo University, Kumamoto, 860-0082, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Fukutokucho Co., Ltd., Kurume 830-0063, Japan). **Engineering of Polyploid *Saccharomyces cerevisiae* for Secretion of Large Amounts of Fungal Glucoamylase.** Applied and Environmental Microbiology, 68(11) (2002), 5693-5697.

We engineered *Saccharomyces cerevisiae* cells that produce large amounts of fungal glucoamylase (GAI) from *Aspergillus awamori* var. *kawachi*. To do this, we used the δ -sequence-mediated integration vector system and the heat-induced endomitotic diploidization method. δ -Sequence-mediated integration is known to occur mainly in a particular chromosome, and the copy number of the integration is variable. In order to construct transformants carrying the GAI gene on several chromosomes, haploid cells carrying the GAI gene on different chromosomes were crossed with each other. The cells were then allowed to form spores, which was followed by dissection. Haploid cells containing GAI genes on multiple chromosomes were obtained in this way. One such haploid cell contained the GAI gene on five chromosomes and exhibited the highest GAI activity (5.93 U/ml), which was about sixfold higher than the activity of a cell containing one gene on a single chromosome. Furthermore, we performed heat-induced endomitotic diploidization for haploid transformants to obtain polyploid mater cells carrying multiple GAI genes. The copy number of the GAI gene increased in proportion to the ploidy level, and larger amounts of GAI were secreted.

S. Underwood, A. Afoke, R. A. Brown, A. J. MacLeod, P. Ayazi Shamlou, P. Dunnill. (Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, Torrington Place, University College London, London WC1E 7JE, UK. Department of Technology and Design, University of Westminster, London, UK. Tissue Repair Unit, Department of Plastic Surgery, University College London, London, UK. Protein

Fractionation Centre, Scottish National Blood Transfusion Service, Edinburgh, UK). **Wet extrusion of fibronectin-fibrinogen cables for application in tissue engineering.** *Biotechnology and Bioengineering*, 73(4) (2001), 295-305.

A method for the wet extrusion of human plasma-derived fibronectin-fibrinogen cables is described. Solutions of fibronectin and fibrinogen with and without sodium alginate and carboxymethylcellulose (CMC) are tested. The rheological properties of the protein solutions changed from Newtonian to shear thinning non-Newtonian in the presence of small quantities of these additives, the apparent viscosity increased, and the extrusion properties of the protein solutions improved. Cables were prepared using a capillary with a diameter of 1 mm and overall length of 18 mm. Cable diameter was reduced to about 0.5 mm by drawing using a series of rollers. Cables prepared with sodium alginate were found to have suitable properties, and those made with CMC were sticky and difficult to handle. Solutions containing no sodium alginate required a minimum total protein concentration of about 70 mg/mL for extrusion. Extruded cables were prepared with solutions containing 140 mg/mL total protein with 12.9 mg/mL alginate (high protein), and 46 mg/mL total protein with 47.6 mg/mL of sodium alginate (high alginate). The mechanical strength of the extruded cables was within the range suitable for application in tissue engineering. Extrusion of the protein solutions into cables was achieved in a coagulation bath. Cables with a mechanical strength of approximately 30 N/mm², suitable for wound repair and nerve regeneration applications, were prepared with a coagulation bath containing 0.25 M HCl, 2% CaCl₂ at a pH of <0.9. These cables also had a large average elongation at break of 52%, and showed an increase in cable length after breakage (permanent set) of 20%, demonstrating the potential for drawing the cables down to a fine diameter.

Sau-Ching Wu and Sui-Lam Wong. (Department of Biological Sciences, Division of Molecular, Cellular and Microbial Biology, University of Calgary, Calgary, Alberta T2N 1N4, Canada). **Engineering of a *Bacillus subtilis* Strain with Adjustable Levels of Intracellular Biotin for Secretory Production of Functional Streptavidin.** *Applied and Environmental Microbiology*, 68(3) (2002), 1102-1108.

Streptavidin is a biotin-binding protein which has been widely used in many in vitro and in vivo applications. Because of the ease of protein recovery and availability of protease-deficient strains, the *Bacillus subtilis* expression-secretion system is an attractive system for streptavidin production. However, attempts to produce streptavidin using *B. subtilis* face the problem that cells overproducing large amounts of streptavidin suffer poor growth, presumably because of biotin deficiency. This problem cannot be solved by supplementing biotin to the culture medium, as this will saturate the biotin binding sites in streptavidin. We addressed this dilemma by engineering a *B. subtilis* strain (WB800BIO) which overproduces intracellular biotin. The strategy involves replacing the natural regulatory region of the *B. subtilis* chromosomal biotin biosynthetic operon (*bioWAFDBIorf2*) with an engineered one consisting of the *B. subtilis* *groE* promoter and gluconate operator. Biotin production in WB800BIO is induced by gluconate, and the level of biotin produced can be adjusted by varying the gluconate dosage. A level of gluconate was selected to allow enhanced intracellular production of biotin without getting it released into the culture medium. WB800BIO, when used as a host for streptavidin production, grows healthily in a biotin-limited medium and produces large amounts (35 to 50 mg/liter) of streptavidin, with over 80% of its biotin binding sites available for future applications.

Stephen Gyamfi, Ulrike Pfeifer, Michael Stierschneider, Angela Sessitsch. (ARC Seibersdorf research GmbH. Division of Environmental and Life Sciences/Biotechnology, A-2444 Seibersdorf, Austria). **Effects of transgenic glufosinate-tolerant oilseed rape *Brassica napus* and the associated herbicide application on eubacterial and**

Pseudomonas communities in the rhizosphere. FEMS Microbiology Ecology 41(2002), 181-190.

A containment experiment was carried out in order to evaluate possible shifts in eubacterial and *Pseudomonas* rhizosphere community structures due to the release of genetically modified Basta-tolerant oilseed rape and the associated herbicide application. Treatments included cultivation of the transgenic plant as well as of the wild-type cultivar in combination with mechanical removal of weeds and the application of the herbicides Basta (active ingredient: glufosinate) and Butisan S (active ingredient: metazachlor). Rhizosphere soil was sampled from early and late flowering plants as well as from senescent plants. A culture-independent approach was chosen to characterize microbial communities based on denaturing gradient gel electrophoresis of 16S rRNA gene fragments amplified from rhizosphere DNA using eubacterial and *Pseudomonas*-specific PCR primers. Dominant pseudomonads in the rhizosphere were analyzed by sequence analysis. Whole community and *Pseudomonas* electrophoresis fingerprints revealed slightly altered microbial communities in the rhizosphere of transgenic plants; however, effects were minor as compared to the plant developmental stage-dependent shifts. Both herbicides caused transient changes in the eubacterial and *Pseudomonas* population structure, whereas differences due to the genetic modification were still detected at the senescent growth stage. The observed differences between transgenic and wild-type lines were most likely due to unintentionally modified plant characteristics such as altered root exudation.

Biofertilizer

Douds D D: Gadkar, V and Adholeya A. **Mass production of VAM fungus biofertilizer.** In *Proceedings: Mycorrhizal Biology*, (2000), 197-215, edited by K G Mukerji, B P Chamola.

Vesicular: arbuscular mycorrhizal (VAM) fungi are symbiotic soil fungi which colonize the roots of approximately 80% of plant families. They impart to their hosts a variety of benefits which include increased growth and yield due to enhanced nutrient acquisition, water relations, pH tolerance, and disease and pest resistance. The most common beneficial effect of mycorrhizae is increased uptake of immobile nutrients, notably P, from soil. The extraradical mycelium of the mycorrhizal fungus acts in effect as an extension of the root system, more thoroughly exploring the soil volume. The P depletion zone around a non-mycorrhizal root extends to only 1-2mm, approximately the length of a root hair whereas extra radical hyphae of VAM fungi extends 8 cm or more beyond the root making the P in this greater volume of soil available to the host.

Mahaveer P S and Adholeya A. **Enhanced Growth and Productivity following Inoculation with Indigenous AM Fungi in Four Varieties of Onion (*Allium cepa* L.) in an Alfisol.** Biological Agriculture and Horticulture , (2000) , 1-14

A field experiment was conducted to evaluate the benefit to growth of *Allium cepa* L. of inoculation with a mixed culture of indigenous arbuscular mycorrhizal (AM) fungi. Four locally adapted onion cvs. Pusa White Flat (PWF), Pusa White Round (PWR), Early Grano (EG) and Pusa Madhvi (PM) were grown at two phosphorus levels (25 and 50 kg P ha) in a P deficient alfisol. Inoculation significantly increased mycorrhiza formation over that caused by the level of native AM fungi present at the site. At harvest, all inoculated onion varieties showed higher values of bulb diameter, fresh weight, shoot dry matter, shoot P content and bulb yield than uninoculated plants. However, the magnitude of AM response for yield in a given onion variety was found to be different at different levels of P. This holds true in all the varieties tested. Inoculated plants tended to have greater bulb yield for varieties OM and OWR grown at 25 kg P ha. On the other hand, PWF and

EG plants showed similar response at 50 kg P ha. The percent root length colonized by AM fungi between both the P levels of inoculated plants did not differ significantly. However, the extent of colonization varied among the varieties. The dependence of plants on mycorrhizal fungi for bulb production varied among the varieties grown at a particular P. EG and PWF plant showed maximum dependence on AM at 50 kg P ha, where as PM and PWR plants exhibited a maximum MD at 25 kg P ha.

R. Neelamegam and T. Govindarajalu. (Department of Botany, Annamalai University, Annamalai Nagar 608 02, Tamil Nadu, India). **Integrated application of *Trichoderma viride* Pers.: Fr. and farm yard manure to control damping-off of tomato.** J. Biol. Control, 16(1) (2002), 65-69.

In the present study, efforts were made to control the damping-off of tomato caused by *Pythium indicum* by using *Trichoderma viride* alone or in combination with farm yard manure (FYM). *T. viride* effectively controlled the disease in both sterilized and unsterilized soil than FYM when treated alone. But the combined treatment of *T. viride* and FYM showed better results in controlling the disease with concomitant increase in seedling length and biomass of tomato.

S T Zodape. (Central Salt and Marine Chemicals Research Institute, Bhavnagar 564002). **Seaweeds As A Biofertilizer.** Journal of Scientific & Industrial Research, 60 (2001), 937-382.

Seaweeds are large plants growing in the sea, especially various marine algae like the rockweeds kelps, sea lettuce and dulse. Dried or fresh seaweeds and liquid extracts have been increasingly employed by horticulturists gardeners, farmers, and orchardists as a fertilizer. Seaweed extracts are now commercially available as maxicrop, seasol, SM3, kelpak and cytokin. The effect of seaweed extract is due to the microelements and plant growth regulators such as cytokinin present in it. Seaweeds extracts is used as a foliar spray, application to soil and for soaking of seeds before sowing. It enhances the germination of seeds increases, uptake of plant nutrients, and gives resistance to frost and fungal diseases. Seaweed extract is effective for ripening of fruits, increasing shelf-life of the produce, improves the quality of produce, and serves as an excellent soil conditioner.

Sharma M P and Adholeya A. **Response of *Eucalyptus tereticornis* to inoculation with indigenous AM fungi in a semi-arid alfisol achieved with different concentrations of available soil P.** *Microbiological Research* 154(2000): (2000), 349-354.

Eucalyptus tereticornis was grown in a green house in a low phosphorus (0.67 ppm Olsen's P) soil (Typic Haplustalf) inoculated with mixed indigenous arbuscular mycorrhizal (AM) fungi. Soil was amended to achieve P levels of 10, 20, 25, 30 and 40 ppm to evaluate the growth response and dependence of *E. tereticornis* to inoculation with AM fungi. A positive response to mycorrhizal inoculation was evident at the first two levels of soil P, i.e., at 0.67 and 10 ppm but not at the higher levels of soil P. Dry matter yield of inoculated plants beyond 20 ppm soil P was similar or even less compared to their uninoculated counterparts. Inoculated plants produced maximum dry matter (root and shoot) at 10 ppm soil P, whereas uninoculated plants did not produce until the level reached 20 ppm. The percentage root length colonized by AM fungi decreased from 31% to 3 % as the concentration of P increased beyond 10 ppm soil P. Higher levels of soil P depressed the AM colonization significantly. Inoculated plants had higher shoot P and N contents compared to their uninoculated counterparts at all levels of soil P. However, at the first two lower levels of soil P, inoculated plants showed significantly higher shoot P

and N contents over their respective uninoculated counterparts. The increasing shoot P accumulation beyond 10 ppm did not enhance dry matter yields. Inoculated plants had lower values of phosphorus utilization efficiency (PUE) and nitrogen utilization efficiency (NUE) at all levels of soil P except at the unamended level (0.67 ppm) where the inoculated plants showed higher values of NLJE compared to uninoculated control plants. Taking dry matter yield into consideration, *Eucalyptus* plants were found to be highly dependent on 10 ppm of soil P for maximum dry matter production. Any further amendment of P to soil was not beneficial neither for AM symbiosis nor plant growth.

Singh R, Adholeya A, and Mukerji K G. **Mycorrhiza in control of soil-borne pathogens.** In *Mycorrhizal Biology*, (2000), 173-196, edited by K G Mukerji New York: Kluwer Academic Publishers.

Valuable data generated on induced suppression of soil-borne pathogens by mycorrhizae has no doubt proved their potentiality in controlling plant pathogens. But, so far, examples of successful practical applications are scarce (Hooker et al., 1994; Linderman, 1994) because of the complexity of this tripartite association (mycorrhiza-soil-plant) and the direct influence of prevailing environmental conditions. Experiments have only usually tested single mycorrhizal fungus and a single host genotype, diversity within mycorrhizal fungi for biocontrol of plant pathogens is unknown. There are certain pathogenic antagonists, trichoderma, Gliocladium, Pseudomonas, Bacillus and PGPR which cooperate with mycorrhizal fungi for biocontrol and the phytosanitary role of mycorrhizal fungi can be made more effective when integrated with other plant protection measures. Thus, for exploiting the prophylactic activity of mycorrhizal fungi in a best way, the right combinations of factors should be found out, the most important being the selection of appropriate/efficient mycorrhizal fungi.

Vijay Gadkar and Alok Adholeya. **Intraradical sporulation of AM *Gigaspora margarita* in long-term axenic cultivation in Ri T-DNA carrot root.** *Mycological Research* : (2000), 716-721.

Arbuscular mycorrhizal (AM) fungi are obligate biotrophic organisms. Root organ culture (ROC) can be used to grow these fungi under *in vitro* conditions. The ROC technique was used with *Gigaspora margarita* and Ri T-DNA transformed carrot roots to examine the fungal growth and physiology under long term axenic symbiosis. The fungus formed spores inside the host roots under *in vitro* conditions. Sporulation was a temporal phenomenon found in dual cultures more than 18-20 mo old. The spores formed intraradically were 10-15% of the total spores formed in a single culture. These intraradical spores were analysed for their morphology and DNA polymorphism pattern with spores which has formed conventionally in the medium. Both analyses showed no detectable variation. This is the first report of spore formation by *Gigaspora* inside the roots of a host.

Biomarker

Gail M. Teitzel and Matthew R. Parsek. (Department of Civil and Environmental Engineering, Northwestern University, Evanston, Illinois 60208). **Heavy Metal Resistance of Biofilm and Planktonic *Pseudomonas aeruginosa*.** *Applied and Environmental Microbiology*. 69(4) (2003), 2313-2320.

A study was undertaken to examine the effects of the heavy metals copper, lead, and zinc on biofilm and planktonic *Pseudomonas aeruginosa*. A rotating-disk biofilm reactor was used to generate biofilm and free-swimming cultures to test their relative levels of

resistance to heavy metals. It was determined that biofilms were anywhere from 2 to 600 times more resistant to heavy metal stress than free-swimming cells. When planktonic cells at different stages of growth were examined, it was found that logarithmically growing cells were more resistant to copper and lead stress than stationary-phase cells. However, biofilms were observed to be more resistant to heavy metals than either stationary-phase or logarithmically growing planktonic cells. Microscopy was used to evaluate the effect of copper stress on a mature *P. aeruginosa* biofilm. The exterior of the biofilm was preferentially killed after exposure to elevated concentrations of copper, and the majority of living cells were near the substratum. A potential explanation for this is that the extracellular polymeric substances that encase a biofilm may be responsible for protecting cells from heavy metal stress by binding the heavy metals and retarding their diffusion within the biofilm.

J. R. M. Swan, P. Beckett, D. Fishwick, K. Oakley, N. Raza, R. McL. Niven, A. M. Fletcher, H. Francis, C. A. C. Pickering, R. Rawbone, B. Crook and A. D. Curran. (Health and Safety Laboratory, Broad Lane, Sheffield S3 7HQ, UK. North West Lung Centre, Manchester, UK. Health and Safety Executive, Magdalen House, Bootle, Merseyside, UK). **A review of the use of CD14: a biomarker for workplace airborne endotoxin exposure?** International Biodeterioration & Biodegradation, 50(2) (2002), 127-134.

Occupational exposure to endotoxin, a component of Gram-negative bacteria, causes short-term illness and contributes to long-term illness. There are currently no recognised objective markers of endotoxin exposure. Such a biomarker could be used to distinguish between symptoms caused by inhaled endotoxin or by other contaminants of organic aerosols and to demonstrate a cause and effect relationship between endotoxin exposure and impairment of respiratory function. Flow cytometry has been used to measure CD14, an endotoxin receptor on monocytes, which may be a useful biomarker of endotoxin exposure. An in vitro model was developed, CD14 expression on monocytes was significantly upregulated in response to endotoxin. In cotton dust workers exposed to 1–400 EU/m³ air, CD14 expression significantly increased after 6 h and at 72 h levels had fallen to baseline or lower. We propose that CD14 expression on monocytes may be used to monitor workers exposure to endotoxin.

L. A. Khatib, Y. L. Tsai, B. H. Olson. (Department of Environmental Analysis and Design, 1368 Social Ecology II, University of California, Irvine, CA 92697, USA). **A biomarker for the identification of cattle fecal pollution in water using the LTIIa toxin gene from enterotoxigenic *Escherichia coli*.** Applied Microbiology and Biotechnology, 59(1) (2002), 97-104.

This research describes a method based on PCR to identify cattle fecal pollution in water using a portion of the heat labile toxin IIA (LTIIa) gene from enterotoxigenic *Escherichia coli* (ETEC). We describe the development of the primers and target. DNA extracts (221) from different animal fecal and human sewage samples were screened and showed no cross-reactivity. Minimum detection limits using centrifugation and filtration methods to concentrate *E. coli* seeded into stream, ocean, and secondary effluent waters were found to be at femtogram and attogram levels, respectively. Stability of the biomarker in stream, ocean, and secondary effluent waters was 2-4 weeks for all water types. Finally, 33 farm lagoon and waste samples were collected and 31 tested to validate the method; 93% were positive for the LTIIa trait when >1,000 *E. coli* were screened and 100% positive when >10⁵ *E. coli* were screened. Prevalence of the toxin gene in the *E. coli* population affected the outcome of the analyses. The cow biomarker can be used in watershed studies to identify cattle waste with great accuracy if the appropriate numbers of *E. coli* are screened.

Pradosh Roy and Anupama Saha. (Department of Microbiology, Bose Institute, P-1/12, C.I.T. Scheme VII-M, Kolkata 700 054, India). **Metabolism and toxicity of arsenic: A human carcinogen.** *Current Science*, 82(1) (2002), 38-45.

Inorganic arsenic is considered the most potential human carcinogen, and humans are exposed to it from soil, water, air and food. In the process of arsenic metabolism, inorganic arsenic is methylated to monomethylarsonic acid and finally to dimethylarsinic acid, followed by excretion through urine. Thus, arsenic exposure may cause DNA hypomethylation due to continuous methyl depletion, facilitating aberrant gene expression that results in carcinogenesis. Further, though arsenic is nonmutagenic, it interacts synergistically with genotoxic agents in the production of mutations, and also induces chromosome abnormalities and cell proliferation. Few epidemiological investigations in the arsenic endemic regions of West Bengal (India) have established that inorganic arsenicals have the potential to cause skin and lung cancers in humans. Studies on the genetic polymorphism in the arsenic methyltransferase(s) with the population exposed to arsenic, and characterization in the arsenic-induced mutational spectra may be useful for the development of molecular markers and therapeutics and for furthering the knowledge of arsenic-induced carcinogenesis.

Biopesticide

A Barber, C A M Campbell, H Crane, R Lilley, E Tregidga. (Horticulture Research International West Malling ME19 6BJ Kent). **Biocontrol of Two-spotted Spider Mite *Tetranychus urticae* on Dwarf Hops by the Phytoseiid Mites *Phytoseiulus persimilis* and *Neoseiulus californicus*.** *Biocontrol Science and Technology*, 13(3) (2003), 275 – 284.

Two female *Phytoseiulus persimilis* and their offspring eliminated two-spotted spider mite *Tetranychus urticae* from hop leaf discs faster than two female *Neoseiulus californicus* and their offspring at 25°C. A combination of one female of each species and their offspring eliminated spider mites faster than the *N. californicus* alone, but slower than *P. persimilis* alone. Air relative humidities of 55% and 93% had no effect on predation. Both predator species cannibalised eggs and juveniles when spider mite numbers were low. In field experiments in 1996, fewer spider mites were recorded where *P. persimilis* was released, irrespective of the presence of *N. californicus*. Pest numbers on cv. 'First Gold' were lower than on cv. 'Herald'. No differences were recorded between the numbers of spider mite eggs in predator release treatments on 'First Gold', but fewer active stages of spider mites were recorded on plots with *P. persimilis* than controls soon after the time of peak pest population densities. On 'Herald', fewer spider mite active stages and eggs were recorded where predators were released than on untreated controls.

D. C. Sharma, N. P. Kashyap. (Department of Entomology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur 176 062, H.P, India). **Impact of pesticidal spray on seasonal availability of natural predators and parasitoides in the tea ecosystem.** *J. Biol. Control*, 16(1) (2002), 31-35.

Tea plant (*Camellia sinensis* (L.) O' Kuntze) is attacked by numerous insect pests, which causes considerable damage to the quality and quantity of tea. The most important pest attacking tea bushes in H. P. are purple mite, *Calacarus carinatus* (Green), thrips, *Scirtothrips dorsalis* Hood, leafroller, *Gracilaria theivora*, Walsom, aphid, *Toxoptera aurantii* Boyer, mealybug, *Nipaecoccus* sp. And leafminer, *Tropiomyxus theae* (Cotes). The important natural enemies associated in the tea orchards are *Syrphus* sp., *Coccinella septempunctata* Linn., *Oxyopes* sp. and parasitoid, *Diaeretiella* sp. Among the

insecticides and biopesticides evaluated against natural enemies, deltamethrin, cypermethrin and ethion sprays were found highly toxic to *Syrphis* sp. and *C. septempunctuata* and their adult and larva) population was not seen even on 7th and 4th days of spray, respectively. Neemark, Achook and *B.t.* (Dipel 8L) were quite safe to natural enemies. Endosulfan was relatively safe to *Syrphis* sp. but highly toxic to *C. septempunctata*.

David G James. (Department of Entomology Washington State University, Irrigated Agriculture Research and Extension Center Washington 99350 Prosser). Pesticide Susceptibility of Two Coccinellids (*Stethorus punctum picipes* and *Harmonia axyridis*) **Important in Biological Control of Mites and Aphids in Washington Hops.** *Biocontrol Science and Technology*, 13(2) (2003), 253 – 259.

The susceptibility of *Stethorus punctum picipes* (Casey) and *Harmonia axyridis* Pallas larvae to pesticides used or with potential for use in Washington hops, was examined in laboratory bioassays. All pesticides tested except the miticide, hexythiazox, the insecticides, chlorpyrifos and pirimicarb, and the fungicide, mycobutanil, produced 100% mortality in *S. punctum picipes* at concentrations equivalent to field rates. The insecticides, pirimicarb, endosulfan, and thiamethoxam were least toxic to *H. axyridis*. Bifenthrin, diazinon, dimethoate, methomyl, carbaryl, malathion, phosmet, imidacloprid, and chlorpyrifos were highly toxic. The miticides, abamectin and fenpyroximate were highly toxic, milbemectin was moderately toxic but all other miticides tested were non-toxic. All fungicides had low toxicity. Selection and use of pesticides compatible with natural enemies and conservation biological control in Washington hop production is discussed.

G.P. Jagtap. (Anil Biotech International, Tigaon Road, Faridabad-121 002, Haryana, India). **Bioshield™ (*pseudomonas fluorescens*): more than biopesticide.** *Pestology*, 26(2) (2002), 38.

Studies on the shelf life of formulated product (Bioshield™) based on *Pseudomonas fluorescens* at different locations have shown that the product can be safely stored for 180 days. It also effectively inhibited the growth of *Pythium aphanidermatum* casual agent of damping off disease of chilli. Treating the chilli seeds with Bioshield™ (*P. fluorescens*) significantly increased the germination percent, shoot length, root length, dry matter production and vigour index of chilli compared to untreated control. Efficacy of Bioshield™ in inducing the yield contributing characters of chilli was comparable with that of the seed treatment fungicide Captan.

J. Jayakumar, S. Ramakrishnan, G. Rajendran. (Department of Nematology, Tamil Nadu Agricultural University, Coimbatore-3, India). **Bio-Control of Reniform Nematode, *Rotylenchufus Reniformh* through Fluorescent Pseudomonads.** *Pestology*, 26(10) (2002), 45.

Pseudomonas fluorescens Strain PF1 available as commercial formulation was evaluated by applying as seed treatment, soil application and split application individually for the management of *R. reniformis* in Cotton cv. MCU-5 under glasshouse conditions. Seeds were soaked in water @ 100 ml/kg seed containing the talc based product of *P. fluorescens*, strain PF1 @ 10 g/kg seed (6×10^8 cfu/g) for 12 hours. Results of the experiment revealed that maximum colonization of rhizobacterium in root was observed in plants treated with seed + split application of PF1 and followed by split application of PF1 in soil. All the methods of application of *P. fluorescens* through seed and soil as single and split application significantly reduced the soil and root population of *R. reniformis* compared to untreated control. The maximum reduction in reniform nematode

population in roots was noticed in the treatment of combining seed and split application of *P. ftuorescens* (74.2%) compared to different methods of applications evaluated.

J.C. van Lenteren, D. Babendreier, F. Bigler, G. Burgio, H.M.T. Hokkanen, S. Kuske, A.J.M. Loomans, I. Menzler-Hokkanen, P.C.J. van Rijn, M.B. Thomas, M.G. Tommasini, Q.-Q. Zeng. (Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH, Wageningen, The Netherlands. Swiss Federal Research Station for Agroecology and Agriculture, Zürich, Switzerland. Department of Agroenvironmental Sciences and Technologies, University of Bologna, Italy. Department of Applied Biology, University of Helsinki, Finland. Swiss Federal Research Station for Agroecology and Agriculture, Zürich, Switzerland. Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH, Wageningen, The Netherlands. Department of Applied Biology, University of Helsinki, Finland. NERC Centre for Population Biology and CABI Bioscience, Silwood Park, Ascot, UK. Centre for Research on Environment and Agriculture, Centrale Ortofruticola, Cesena, Italy. Department of Applied Biology, University of Helsinki, Finland). **Environmental risk assessment of exotic natural enemies used in inundative biological control.** *BioControl*, 48 (1) (2003) 3-38.

In the past 100 years many exotic natural enemies have been imported, mass reared and released as biological control agents. Negative environmental effects of these releases have rarely been reported. The current popularity of inundative biological control may, however, result in problems, as an increasing number of activities will be executed by persons not trained in identification, evaluation and release of biological control agents. Therefore, a methodology for risk assessment has been developed within the EU-financed project 'Evaluating Environmental Risks of Biological Control Introductions into Europe [ERBIC]' as a basis for regulation of import and release of exotic natural enemies used in inundative forms of biological control (i.e. not in 'classical biological control' though some of the same principles and approaches apply). This paper proposes a general framework of a risk assessment methodology for biological control agents, integrating information on the potential of an agent to establish, its abilities to disperse, its host range, and its direct and indirect effects on non-targets. Of these parameters, estimating indirect effects on non-targets will be most difficult, as myriads of indirect effects may occur when generalist natural enemies are introduced. The parameter 'host range' forms a central element in the whole risk evaluation process, because lack of host specificity might lead to unacceptable risk if the agent establishes and disperses widely, whereas, in contrast, a monophagous biological control agent is not expected to create serious risk even when it establishes and disperses well. Drawing on published information and expert opinion, the proposed risk assessment methodology is applied to a number of biological control agents currently in use. These illustrative case histories indicate that the risk assessment methodology can discriminate between agents, with some species attaining low 'risk indices' and others scoring moderate or high. Risk indices should, however, not be seen as absolute values, but as indicators to which a judgement can be connected by biological control experts for granting permission to release or not.

Poonam Jasrotia, S.M. Suri. (Department of Entomology, Himachal Pradesh Krishi Vishvavidyalaya, Palampur, H.P-176 062, India). **Bioefficacy of Certain Biopesticides against Lepidopterous Pests of offseason Cabbage.** *Pestology*, 26(7) (2002), 60.

Four biopesticides viz., Dipel, Biobit, Neemax, Neemark @ 0.3% and combinations of Dipel (0.15%) and Biobit (0.15%) with Ripcord @ 0.005% were evaluated and compared with already recommended Ripcord (0.01%) and Cythion (0.05%) against lepidopterous larvae of offseason cabbage. The combinations of Dipel or Biobit @ 0.15% with Ripcord (0.005%) were the most effective treatments against the pests whereas neem based formulations viz., Neemax and Neemark proved to be the least effective. The two *Bt*

based insecticides Dipel (0.3%) and Biobit (0.3%) alone gave reduction of less than 10 per cent after one day of spraying but proved better after 14 days of spraying.

Sharma M P and Adholeya A. **Sustainable management of arbuscular mycorrhizal fungi in the biocontrol of soil-borne plant diseases.** *Biocontrol Potential and its Exploitation in Sustainable Agriculture* (Vol. 1: Crop Diseases, Weeds and Nematodes) (2000), 117-138, edited by R K Upadhyaya, K G Mukerji, and B P Chamola New York: Kluwer Academic Publishers .

In view of the increased concern for environmental quality, sustainable technologies need to be incorporated into agricultural systems. Management of AM fungi is an important aspect of such an approach (Linderman and Bethenfalvay, 1992). However, the obligate symbiotic nature of AM fungi and limited inoculum supplies continue to impede research aimed at managing these beneficial fungi. The application of selected AM fungi will not only benefit plant growth and development, but it offers the possibility of increasing the resistance to soil-borne plant pathogens as well. Under natural conditions this can be regarded as a positive side effect of AM but not as an evidence of its potential in biological control. In most instances, AM significantly change the Physiology and chemical constituents of the host, the pattern of root exudation, and the microbial composition of the rhizosphere. Experimental studies differed in fungal symbiont, inoculum doses, and pathogen types. The combination of all factors is responsible for successful disease suppression. A low level of fungal aggressiveness and a weak plant reaction are two factors that permit establishment of a symbiotic relationship (Gianinazzi, 1984). A weak but permanent activity may explain the enhanced resistance of AM plants to certain plant pathogens (Bagyaraj, 1984). In this respect, the mechanism involved although the tendency is to implicate only one. Since AM effects on plant nutrition, especially P nutrition as a mechanism of disease control. However, many reports mention mechanisms excluding P, such as biochemical changes in the tissues/cells, as a physiological mechanism, and antagonistic microorganisms of the mycorrhizosphere. Most of the studies do not cover the mechanisms based on morphological changes and changes in the mycorrhizosphere, probably are consolidated and multiple. Since microbial composition in soil reaches a new equilibrium as a result of the selective pressure induced by mycorrhizae, it should be considered a cornerstone foundation component in rhizosphere. In managing rhizosphere populations for biological control of plant diseases, compatible endophyte and effective antagonists should be moved to the production system to ensure that their potential in the management of plant diseases is fully used. Greater attention needs to be placed on the evaluation of the level, vigour and production required for combating plant diseases. Species or isolate-specific screening of AM and of AM-plant combinations needs to be carried out for specific target pathogens.

Sushil K. Shahi, Mamta Patra, A.C. Shukla, Anupam Dikshit. (Biological Product Laboratory, Botany Department, University of Allahabad, Allahabad -211002, India). **Use of essential oil as botanical-pesticide against post harvest spoilage in *Malus pumilo* fruit.** *BioControl*, 48 (2) (2003), 223-232.

During antifungal screening of the essential oils of some angiospermic plants, oil of *Cymbopogon flexuosus* showed potent bioactivity against dominant post harvest fungal pathogens. The minimum bioactive concentrations with fungicidal action of the oil was found to be 0.2 $\mu\text{l ml}^{-1}$ for *Alternaria alternata*, 0.4 $\mu\text{l ml}^{-1}$ for *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *Cladosporium cladosporioides*, *Colletotrichum capsici*, *C. falcatum*, *Curvularia lunata*, *Fusarium cerealis*, *F. culmorum*, *F. oxysporum*, *F. udum*, *Gloeosporium fructigenum*, *Penicillium expansum*, *P. italicum*, *P. implicatum*, *P. digitatum*, *P. minio-luteum*, *P. variable*, and 0.5 $\mu\text{l ml}^{-1}$ for *Botrytis cinerea*, *Helminthosporium oryzae*, *H. maydis*, *Phoma violacea*, *Rhizopus nigricans*. The oil

exhibited potency against heavy doses (30 mycelial disc, each of 5 mm in diameter) of inoculum at $1.0 \mu\text{l ml}^{-1}$ concentrations. The bioactivity of the oil was thermostable up to 100°C and lasted up to 48 months. The oil preparation did not exhibit any phytotoxic effect on the fruit skins of *Malus pumilo* up to $50 \mu\text{l ml}^{-1}$ concentrations. In vivo trials of the oil as a fungicidal spray on *Malus pumilo* for checking the rotting of fruits, it showed that $20 \mu\text{l ml}^{-1}$ concentration controls 100% infection by pre-inoculation treatment, while in post-inoculation treatment, $30 \mu\text{l ml}^{-1}$ concentration of fungicidal spray was required for the 100% control of rotting. The fungicidal spray was found to be cost effective (INR 15/l), has long shelf life (48 month) and was devoid of any adverse effects. Therefore, it can be used as a potential source of sustainable eco-friendly botanical pesticide, after successful completion of wide range trials.

Bioremediation

A. Ganguli, A. K. Tripathi. (School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi - 221005, India). **Bioremediation of toxic chromium from electroplating effluent by chromate-reducing *Pseudomonas aeruginosa* A2Chr in two bioreactors.** Applied Microbiology and Biotechnology, 58(3) (2002), 416-420.

The chromate-reducing ability of *Pseudomonas aeruginosa* A2Chr was compared in batch culture, with cells entrapped in a dialysis sac, and with cells immobilized in an agarose-alginate film in conjunction with a rotating biological contactor. In all three systems, the maximum Cr(VI) reduction occurred at 10 mg Cr(VI)/l. Whereas at 50 mg Cr(VI)/l concentration, only 16% of the total Cr(VI) was reduced, five spikings with 10 mg chromate/l at 2-h intervals led to 96% reduction of the total input of 50 mg Cr(VI) /l. Thus maximum Cr(VI) reduction was achieved by avoiding Cr(VI) toxicity to the cells by respiking with lower Cr(VI) concentrations. At 10 mg Cr(VI)/l, the pattern of chromate reduction in dialysis-entrapped cells was almost similar to that of batch culture and 86% of the bacterially reduced chromium was retained inside the dialysis sac. In electroplating effluent containing 100 mg Cr(VI)/l, however, the amount of Cr(VI) reduced by the cells immobilized in agarose-alginate biofilm was twice and thrice the amount reduced by batch culture and cells entrapped in a dialysis sac, respectively.

A. V. Pethkar, R. P. Gaikawari, K. M. Paknikar. (Division of Microbial Sciences, Agharkar Research Institute, G.G. Agarkar Road. Pune 41 1 004. India, Hi-Tech Bio Laboratories. 36/1/1 Vadgaon Khurd. Pune 41 1 041, India). **Biosorptive removal of contaminating heavy metals from plant extracts of medicinal value.** Current Science, 80(9) (2001), 1216.

Granulated *Cladosporium cladosporioides* # 2 biosorbent removed lead and cadmium from aqueous extracts of *Nordostachys jatamansi* and *Vitis vinifera* with high efficiency. Different properties of the extracts such as pH, UV-visible spectra and total dissolved solids were unaltered after biosorption, indicating that none of the components of the extracts were removed and the biosorbent itself did not transfer its components to the extracts. These findings open up new avenues for the application of metal biosorption technology.

Albert D. Venosa, Kenneth Lee, Makram T. Suidan, Susana Garcia-Blanco, Susan Cobanli, Moustafa Moteleb, John R. Haines, Gilles Tremblay and Melynda Hazelwood. (U.S. Environmental Protection Agency, National Risk Management Research Laboratory, Cincinnati, OH 45268, USA. Bedford Institute of Oceanography, Fisheries and Oceans-Canada, Dartmouth, Nova Scotia B2Y 4A2, Canada. Department of Civil and Environmental Engineering, University of Cincinnati, Cincinnati, OH 45221, USA. BDR

Research, Halifax, NS; Statking Consulting, Fairfield, OH 45014, USA). **Bioremediation and Biore Restoration of a Crude Oil-Contaminated Freshwater Wetland on the St. Lawrence River.** *Bioremediation Journal*, 6(3) (2002), 261-281.

Biostimulation by nutrient enrichment and phytoremediation were studied for the restoration of an acutely stressed freshwater wetland experimentally exposed to crude oil. The research was carried out along the shores of the St. Lawrence River at Ste. Croix, Quebec, Canada. The research determined the effectiveness of fertilizer addition in enhancing the biodegradation rates of residual oil. It further examined the rate at which the stressed ecosystem recovered with and without the addition of inorganic fertilizers and the role of nutrients in enhancing wetland restoration in the absence of healthy wetland plants. Chemical analysis of integrated sediment core samples to the depth of oil penetration within the experimental plots indicated that addition of inorganic nutrients did not enhance the disappearance of alkanes or PAHs. In surface samples, however, hydrocarbon disappearance rates were higher when the metabolic activity of wetland plants was suppressed by the removal of emergent plant growth. These results suggest that oxygen limitation plays a major role in preventing rapid biodegradation of hydrocarbons in anoxic wetland sediment.

Albert L. Juhasz and Ravendra Naidu. (CSIRO Land and Water, PMB 2, Glen Osmond, Adelaide, SA 5064, Australia). **Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene.** *International Biodeterioration & Biodegradation*, 45(1-2) (2000), 57-88.

Over the past 30 years, research on the microbial degradation of polycyclic aromatic hydrocarbons (PAHs) has resulted in the isolation of numerous genera of bacteria, fungi and algae capable of degrading low molecular weight PAHs (compounds containing three or less fused benzene rings). High molecular weight PAHs (compounds containing four or more fused benzene rings) are generally recalcitrant to microbial attack, although some fungi and algae are capable of transforming these compounds. Until recently, only a few genera of bacteria have been isolated with the ability to utilise four-ring PAHs as sole carbon and energy sources while cometabolism of five-ring compounds has been reported. The focus of this review is on the high molecular weight PAH benzo[a]pyrene (BaP). There is concern about the presence of BaP in the environment because of its carcinogenicity, teratogenicity and toxicity. BaP has been observed to accumulate in marine organisms and plants which could indirectly cause human exposure through food consumption. This review provides an outline of the occurrence of BaP in the environment and the ability of bacteria, fungi and algae to degrade the compound, including pathways for BaP degradation by these organisms. In addition, approaches for improving microbial degradation of BaP are discussed.

Bharati J. Bhadbhade, Seema S. Sarnaik, Pradnya P. Kanekar. **Bioremediation of an Industrial Effluent Containing Monocrotophos.** *Curr Microbiol*, 45 (2002), 346-349.

Almost 30% of the precious agricultural output of India is lost owing to pest infestation. In India, pesticide consumption for protecting crops is about 3% of the total world consumption. Monocrotophos (MCP), an organophosphorus insecticide, is widely used to control insects on crops. Being readily water soluble and highly toxic, its removal from wastewater generated during manufacture becomes inevitable. Bioremediation of wastewater containing MCP by *Arthrobacter atrocyaneus*, *Bacillus megaterium*, and *Pseudomonas mendocina* was highest at pH 8.0, but maximum reduction in Chemical Oxygen Demand (COD) was at pH 7.0. Removal of MCP and reduction in COD by *B. megaterium* and *Ps. mendocina* were highest at 35°C, while with *A. atrocyaneus*, it was maximum at 30°C, under aerated culture condition and inoculum density of 10⁸ cells/ml.

Use of pure cultures for bioremediation of effluent containing MCP appears to be the first such attempt.

Bill W. Bogan, Lisa M. Lahner and J. Robert Paterek. (Gas Technology Institute, 1700 South Mount Prospect Road, Des Plaines, IL 60018, USA). **Limited Roles for Salicylate and Phthalate in Bacterial PAH Bioremediation.** *Bioremediation Journal*, 5(2) (2001), 93-100.

We have isolated and identified 12 previously unreported strains of polycyclic aromatic hydrocarbon (PAH)-degrading bacteria. Three of these isolates belong to the genus *Burkholderia*, with the remainder comprised of two *Pseudomonas* species, and seven strains from the genus *Sphingomonas*. These isolates were examined for their ability to utilize a variety of three- to five-ring PAHs as sole sources of carbon and energy. All were also checked for the ability to grow on salicylic acid and phthalic acid, two compounds that are key intermediates in almost all published PAH degradative pathways. Only 3 of the 12 strains were able to grow on both of these monoaromatic acids, while three others (all *Sphingomonas*) could not grow on either. The implications of these findings on the universal applicability of known PAH catabolic pathways, and on possible induction of PAH degradation by pathway intermediates, are discussed.

Blanca Escobar, Inés Godoy. (Centro de Hidro/ElectroMetalurgia, Departamento de Ingeniería de Minas e Ingeniería Química, Universidad de Chile, Tupper 2069, Santiago, Chile). **Enumeration of *Acidithiobacillus ferrooxidans* adhered to agglomerated ores in bioleaching processes.** *World Journal of Microbiology and Biotechnology*, 18(9) (2002), 875-879.

In bioleaching processes, *bacteria* adhered to agglomerated ores are frequently determined in the washing solution after treating the mineral with different techniques like sonification or chemical treatment with SDS, Tween 20, Triton X-100, or only basal medium to release the adhered cells. In this work we compare the efficiency of these techniques, not only by determination of the number of released cells, but also by establishing their viability. The result indicate that, in spite of the high number of bacteria that can be released from an agglomerated ore, when detergent solutions are used, bacteria are heavily damaged and lose their ferrous-iron oxidation activity. On the other hand, when hand stirring with basal medium is used to release bacteria, a method that does not produce damage to the cells, only a percentage of the total population of active ferrous-iron-oxidizing adhered bacteria is released; therefore, the enumeration or determination of bacteria in the washing solution would be inaccurate. We thus propose that agglomerated ores be monitored directly for the presence of active bacteria, by determination of the ferrous-iron oxidation ability of the attached bacteria.

Bruce E. Logan and Jun Wu. (Dept. of Civil and Environmental Engineering, Pennsylvania State University, University Park, PA, USA). **Enhanced Toluene Degradation Under Chlorate-Reducing Conditions by Bioaugmentation of Sand Columns with Chlorate-and Toluene-Degrading Enrichments.** *Bioremediation Journal*, 6(2) (2002), 87-95.

Chlorate was examined as a potential electron acceptor for enhancing toluene degradation. Most chlorate respiring bacteria (CRB) use nitrate as an electron acceptor, and toluene is known to be degraded under denitrifying conditions. Therefore, it was hypothesized that there would be bacteria that could degrade toluene using chlorate as an electron acceptor, and that chlorate could be used to stimulate toluene degradation. Repeated tests and different approaches in batch tests failed to produce an enrichment capable of toluene degradation supported by chlorate reduction. However, the addition of

chlorate increased the overall rate of toluene degradation in bioaugmented columns that were fed chlorate vs. a control column. Toluene removal at an influent toluene concentration of 11 mg/L was $93\pm 5\%$, which was larger by a factor of 1.95 than toluene removal in a nonbioaugmented control column. Following the discontinued feed of chlorate, toluene removal decreased to $69\pm 4\%$, demonstrating that chlorate could be used to produce a 1.36-fold increase in toluene removal.

C. H. Chaîneau, C. Yepremian, J. F. Vidalie, J. Ducreux, D. Ballerini. (Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie, Paris, France. TotalFinaElf, DGEP/SE/ENP, Paris la Défense, France. Institut Français du Pétrole, Rueil-Malmaison, France). **Bioremediation of a Crude Oil-Polluted Soil: Biodegradation, Leaching and Toxicity Assessments.** *Water, Air, and Soil Pollution*, 144(1) (2003), 419-440.

The combined fate and effects of hydrocarbons (HC) on a soil ecosystem affected by bioremediation were studied during 480 days in a field experiment. The HC removal rates, the HC and metabolites mobility and the potential toxicity were assessed. A clayey soil polluted by 18 000 mg HC kg⁻¹ dry soil, was treated with either static-ventilated biopile or series of five windrows periodically tilled in order to determine the relative influence of nutrients, bulking agents, aeration and soil temperature. HC concentrations were determined by infrared spectrometry, gravimetry, gas chromatography and thermodesorption. Between 70 to 81% of the initial HC were removed through biological processes in fertilized soils, whereas natural attenuation without added nutrients was 56%. When adding fertilizers, residual HC were cyclic compounds poorly biodegraded and strongly trapped on the organo-mineral matter. Leaching of HC and water-soluble metabolites was demonstrated during the first stages of biodegradation. Low levels of the HC were detected in the leachates at day 480. Maximal toxicity was highest immediately after the introduction of oil then decreased as biodegradation proceeded. No toxic effect was recorded on worms survival and on seeds germination at day 480. However growth of plants was reduced in treated soils and a potential residual toxicity was observed on the basis of photosynthesis inhibition and bacterial bioluminescence (Microtox) tests.

Carsten Vogt, Albin Alfreider, Helmut Lorbeer, Joerg Ahlheim, Bernd Feist, Olaf Boehme, Holger Weiss, Wolfgang Babel, Lothar Wuensche. (UFZ Centre for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany. UFZ Centre for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany. University of Technology Dresden, Institute of Waste Management and Contaminated Site Treatment, Pirna, Germany). **Two Pilot Plant Reactors Designed for the *In Situ* Bioremediation of Chlorobenzene-contaminated Ground Water: Hydrogeological and Chemical Characteristics and Bacterial Consortia.** *Water, Air and Soil Pollution*, 2(3) (2002), 161-170.

The SAFIRA *in situ* pilot plant in Bitterfeld, Saxonia-Anhalt, Germany, currently serves as the test site for eight different *in situ* approaches to remediate anoxic chlorobenzene (CB)-contaminated ground water. Two reactors, both filled with original lignite-containing aquifer material, are designed for the microbiological *in situ* remediation of the ground water by the indigenous microbial consortia. In this study, the hydrogeological, chemical and microbiological conditions of the inflowing ground water and reactor filling material are presented, in order to establish the scientific basis for the start of the bioremediation process itself. The reactors were put into operation in June 1999. In the following, inflow CB concentrations in the ground water varied between 22 and 33 mg L⁻¹; a chemical *steady state* for CB in both reactors was reached after 210 till 260 days operation time. The sediments were colonized by high numbers of aerobic, iron-reducing and denitrifying bacteria, as determined after 244 and 285 days of operation time. Furthermore, aerobic CB-degrading bacteria were detected in all reactor

zones. Comparative sequence analysis of 16S rDNA gene clone libraries suggest the dominance of *Proteobacteria* (*Comamonadaceae*, *Alcaligenaceae*, *Gallionella* group, *Acidithiobacillus*) and members of the class of low G+C gram-positive bacteria in the reactor sediments. In the inflowing ground water, sequences with phylogenetic affiliation to sulfate-reducing bacteria and sequences not affiliated with the known phyla of Bacteria, were found.

Chatterjee Priya, Golwalkar Sucheta, MukundaJi Usha. (Plant Biotechno Lab, RJ Coil, Ghatkopar (W), Mumbai 400086). **Removal of aromatic amines and phenols from water by peroxidase produced by cell cultures of *Momordica dioica***. Polln Res, 20(4) (2001), 523-527.

Plant cell cultures of *Momordica dioica* produce intracellular and extracellular peroxidase. These can precipitate aromatic amines and phenols with varying efficiency. When the concentration of -dianisidine was 0.1 mg/ml it was removed with a maximum efficiency of 96% when the pH of the reaction mixture was 6.0 and the H₂O₂ concentration was 4.8 mM and the amount of enzyme required for the removal was 0.061 mg/ml. The removal efficiency of Naphthyl amine was 95% while phenols were removed at comparatively lower efficiency of 65%.

Christof Bolliger, Frank Schonholzer, Martin H. Schroth, Dittmar Hahn, Stefano M. Bernasconi and Josef Zeyer. (Swiss Federal Institute of Technology (ETH) Zurich, Institute of Terrestrial Ecology, Soil Biology, CH-8952 Schlieren, Switzerland Present address: Department of Chemical Engineering, Chemistry and Environmental Science, New Jersey Institute of Technology (NJIT), and Department of Biological Sciences, Rutgers University, 101 Warren Street, Newark, NJ 07102, USA. Swiss Federal Institute of Technology (ETH) Zurich, Institute of Geology, CH-8092 Zurich, Switzerland). **Characterizing Intrinsic Bioremediation in a Petroleum Hydrocarbon-Contaminated Aquifer by Combined Chemical, Isotopic, and Biological Analyses**. Bioremediation Journal, 4(4) (2000), 359-371.

Chemical, isotopic, and biological parameters were evaluated over a 1-year period to characterize microbial processes associated with intrinsic bioremediation in a petroleum hydrocarbon-contaminated aquifer located in Studen, Switzerland. Chemical parameters measured included oxidants such as O₂, NO₃⁻, and SO₄⁻ reduced species such as Fe²⁺ and CH₄, and dissolved inorganic carbon (DIC). Stable carbon isotope analyses of DIC were used to differentiate between different processes that contribute to DIC production. Microbial populations were identified by sequence analysis of archaeal 16S rDNA and in situ hybridization using a general DNA binding dye (DAPI) and specific probes targeting the domain Archaea (Arch915) and Bacteria (Eub338), as well as the species *Methanosaeta concilii* (Rotc 11) and *Methanospirillum* sp. (Rotc 12). Groundwater exhibited reduced conditions and elevated concentrations of DIC within the contaminated zone. Spatially distinct values of ¹³C ranging from -16.51‰ to -4.44‰ were found, indicating the presence of different ongoing microbial processes. Detected microbial populations (% of DAPI-stained cells) within the contaminated zone belonged to Archaea (9 ± 2% to 31 ± 13%) and Bacteria (13 ± 3% to 32 ± 13%). In wells with methanogenic activity, *Methanosaeta concilii* accounted for up to 26% of all DAPI-detected microorganisms. These results demonstrate that this novel combination of chemical, isotopic, and biological analysis provides valuable insights that can be used for the characterization of microbial processes in contaminated aquifers.

D P, M V Kulkarni, V L Maheshwari and R M Koihari. (School of Life Science. North Maharashtra University, P.B. 80. Jalgaon 425 001, India). **A Sustainable Agro-**

biotechnology for Bioremediation of Saline Soil. Journal of Scientific & Industrial Research, 61 (2002), 517-528.

To restore fertility and productivity of saline soil through bioremediation, a farm-scale trial was undertaken by exploring the effect of three factors, viz, soil conditioner (SC, recycled agrowaste), halophiles culture, and a plant growth regulator (PGR), modified industrial byproduct. A three factor factorial design was used with each factor at two levels - the lower level indicated no treatment while the upper level indicated treatment, and there were eight experiments in all, which were replicated thrice. One out of the eight treatment combinations was without SC, PGR, or halophiles inputs served as control. The plantation and growth of *Casuarina equisetifolia*, in the treated soils as per the design, was monitored through various relevant parameters that included soil characteristics, level of (micro) nutrients, and exchangeable cations, and growth-related parameters. The analysis of data thus generated through appropriate ANOVA indicated an overwhelming role of SC in the bioremediation of soil salinity and growth parameters of the *C equisetifolia* plantation, followed by that of halophiles and then the PGR as envisaged. The role of PGR was however important in first establishing the plantation in the saline soil, when halophiles get enough time to establish favourable pattern and concentration of microbes in soil by up to more than a million times of the initial microflora level of 5.9×10^3 /g soil. Data collected up to six months clearly indicated bioremediation of the otherwise saline soil, and effectiveness of the given treatments was evident. It is proposed that such bio-measures may go a long way in converting large tracts of saline soils into fertile ones with healthy soil-microflora in an eco-friendly manner which is cost-effective as well.

Deka S. (Inst Adv Std Sci Techno, Kanapara, Guwahati 781022, Assam). **Bacterial strains, degrading crude oil from petroleum polluted soil of Assam.** Polln Res, 20(4) (2001), 517-521.

A total of four bacterial strains have been isolated from petroleum polluted soil of oil field situated at Moran (Assam). Strains were identified as *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Bacillus aneurinolyticus* and *Serratia marcescens*. In a laboratory conducted experiment it was observed that *Pseudomonas stutzeri* was the most efficient hydrocarbon degraders amongst the bacterial strains isolated from petroleum polluted soil of Assam.

E. Bozau, G. Strauch. (Department of Hydrogeology, UFZ-Centre for Environmental Research Leipzig-Halle, Halle/Saale, Germany). **Hydrogeological Basis for Biotechnological Remediation of the Acidic Mining Lake 'RL 111', Lusatia (Germany).** Water, Air and Soil Pollution, 2(3) (2002), 15-25.

The drainage basin of the acidic mining lake 'RL 111' was characterized by hydrogeological and geochemical models to assess its influence on a planned biotechnological remediation of the lake water and lake sediment. Ground, seepage and lake water, as well as the surrounding sediments, were examined to model the hydrodynamic processes and the geochemical development of the lake. The geochemical conditions seem to behave in a stable manner (steady state conditions). A reduction of the high sulphate and acid input has not been observed since the beginning of our investigations. The biotechnological remediation of the whole lake should consider a treatment of the dump sediments to improve the quality of the inflowing groundwater, as well as to reduce erosion.

E.A. Kern, R.H. Veeh, H.W. Langner, R.E. Macur and A.B. Cunningham. (Department of Microbiology, Montana State University, Bozeman, Montana 59717, USA. NSF Center for

Biofilm Engineering, Montana State University, Bozeman, Montana 59717, USA. Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, Montana 59717, USA). **Characterization of Methyl tert-Butyl Ether-Degrading Bacteria from a Gasoline-Contaminated Aquifer.** *Bioremediation Journal*, 6(2) (2002), 113-124.

Molecular microbial community analysis was combined with traditional cultivation strategies to investigate the presence of methyl *tert*-butyl ether (MTBE)-degrading bacteria in a gasoline-contaminated aquifer (Ronan, MT). A bacterial consortium, RS24, which is capable of complete mineralization of MTBE as a sole carbon and energy source was enriched from soil and aquifer materials taken from the contaminated site. The consortium was capable of degrading MTBE at rates up to 0.66 mg d⁻¹, with corresponding gross biomass yields of 0.25 ± 0.02 mg dry biomass (mg MTBE)⁻¹. Two MTBE-degrading isolates identified as *Pseudomonas* Ant9 and *Rhodococcus koreensis* were obtained from the consortium. However, both isolates required the presence of 2-propanol as a cosubstrate for MTBE degradation. Denaturing gradient gel electrophoresis (DGGE) of Polymerase Chain Reaction (PCR)-amplified 16S rDNA confirmed the presence of both isolates in the initial consortium and indicated their disappearance with transfer and subculturing. MTBE degradation and cell growth by the consortium was stimulated by the presence of spent culture medium, suggesting the production of a growth factor during MTBE degradation. These results indicate the presence of naturally occurring MTBE-degrading bacteria in a contaminated aquifer and suggest the potential for natural attenuation or enhanced aerobic oxidation.

Evguenii I. Kozliak, Tana L. Ostlie-Dunn, Michele L. Jacobson, Steven R. Mattson and Ryan T. Domack. (University of North Dakota, Dept. of Chemistry, Grand Forks, ND 58202-9024, USA). **Efficient Steady-State Volatile Organic Compound Removal from Air by Live Bacteria Immobilized on Fiber Supports.** *Bioremediation Journal*, 4(1) (2000), 81-96.

Fibers are suggested for bacterial immobilization in trickle-bed bioreactors used for the removal of volatile organic compounds (VOCs) from air. Fiber-based bioreactors retain up to 200 to 300 mg of dry biomass per 1 g of support, which is a much larger value than that of traditional, granule-based bioreactors. Air pollutant removal efficiency for fiber-based bioreactors remains high with large inlet pollutant concentrations or space velocities (lower contact times). Efficient removal is achieved not only for a water-miscible substrate (ethanol), but also for some less water-soluble compounds, such as ethyl acetate and styrene. Specific pollutant elimination capacity per unit fiber-based biocatalyst volume (up to 4000 g/m³-h) exceeds those of biological air purification methods and is comparable to chemical methods. Unlike granule-based biocatalysts, oxygen limitation for pollutant biodegradation is not observed. Evidence obtained shows that the higher air purification efficiency is due to the greater surface-to-volume ratio of fibers when compared with granules, which results in a more efficient substrate mass transfer.

Francesca Bosco, Antonio Capolongo and Bernardo Ruggeri. (Dip. Scienza dei Materiali e Ingegneria Chimica, Politecnico di Torino, C.so Duca degli Abruzzi 24, 10129 Torino, Italy). **Effect of Temperature, pH, Ionic Strength, and Sodium Nitrate on Activity of LiPs: Implications for Bioremediation.** *Bioremediation Journal*, 6(1) (2002), 65-76.

The present work is aimed to show the effects of environmental parameters such as temperature, pH, ionic strength, and sodium nitrate on enzyme activity of a LiP Isoenzymes Mixture (LIM) obtained from an immobilized culture of *Phanerochaete chrysosporium*. LIM enzyme stability was also evaluated. The results are discussed in detail and a comparison with literature data is carried out. LIM showed high activity at

pH 3.0 in the temperature range 30 to 40°C, it is able to catalyze oxidation reactions at acid pH ($2.5 < \text{pH} < 6$) and over a wide range of temperatures (25 to 60°C). Ionic strength below 0.2 M had no effect on enzyme activity at pH 4.7 and 39°C. An evaluation of the time decay constant of LIM activity under a specific combination of parameters was also conducted. Finally, an LIM activity and durability map shows the optimal working conditions that might be suitable for its practical application in waste bioremediation processes.

Gary M. Kleka, David L. Rick, Michael E. Witt, Kirsti Ritalahti and Terence L. Marsh. (Toxicology and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674, USA. Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824, USA). **Natural Biological Attenuation of Phenoxy Herbicides in Groundwater: Dow Agrosiences Paritutu Site, New Zealand.** *Bioremediation Journal*, 5(1) (2001), 79-92.

Groundwater beneath a manufacturing site previously used for herbicide production has been shown to contain low levels of chlorinated phenols and phenoxy herbicides. The importance of biological processes in the natural attenuation of the groundwater contaminants was examined as part of an ongoing investigation. Analysis of the groundwater chemistry indicated that the aquifer is essentially aerobic in the area of interest. Laboratory microcosm experiments demonstrated that the naturally occurring microorganisms rapidly degraded a mixture of the predominant organic contaminants under conditions that simulate those in the aquifer. The time required for 50% degradation ranged from 7 to 27 days for 2,4-dichlorophenoxyacetic acid (2,4-D) and 9 to 49 days for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The rapid biodegradation rates were consistent with the results of microbiological analyses, which demonstrated that a substantial proportion of the culturable bacteria were capable of growth on 2,4-D as a sole carbon source. Results of gene probe assays suggested the numbers of bacteria with the potential to degrade 2,4-D were one to two orders of magnitude higher than were detected using plate counts. Computer model simulations illustrated that biodegradation would be expected to significantly contribute to the attenuation of 2,4-D and 2,4,5-T in the aquifer. On the basis of the various lines of evidence and the distances the groundwater must travel, the groundwater contaminants would be expected to naturally biodegrade to below levels of concern before the plume reaches potential environmental receptors.

H. I. Atagana, R. J. Haynes, F. M. Wallis. (School of Earth Sciences, Mangosuthu Technikon, Jacobs 4026, Durban, South Africa and School of Applied Environmental Sciences, University of Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa. School of Applied Environmental Science, University of Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa). **The Use of Surfactants as Possible Enhancers in Bioremediation of Creosote Contaminated Soil.** *Water, Air, and Soil Pollution*, 142 (1-4) (2003), 137-149.

A study on five nonionic surfactants (Arkopal-N-060, Arkopal-N-080, Arkopal-N-100, Hosaf-541 -KS and Tween-80) commercially available in South Africa was carried out to determine their effect on the desorption and degradation of creosote in a soil contaminated with, 250000 mg kg⁻¹ creosote with a view to developing a cost effective methodology for treating creosote contaminated soils. The surfactants were studied in concentrations of 0.01, 0.1, 0.35, 0.5 and 1.0% (v/v) in liquid cultures. Results from the studies showed that all the surfactants studied were able to enhance the desorption and degradation of creosote to different extents. The enhancement ranged from as little as <10% in 0.1% surfactant to as high as 45% in 0.5% surfactant. The effect on degradation of creosote was more obvious (30-65%) in the different surfactants at different concentrations. Arkopal-N-060 was observed to be the most effective in the

desorption and degradation of creosote. The effect of Hosaf-541-KS on the degradation of creosote was found to be comparable with those of Arkopal-N-060, however, its desorption capabilities were much lower than those of Arkopa-N-060. The concentration of the surfactant was found to play a significant role in desorption of creosote. It was observed that surfactant concentrations of 0.35 and 0.5% were the most effective in the desorption of creosote. Above and below these concentrations, the effect of the surfactants was observed to decrease. All surfactants studied were not found to inhibit microbial growth at the concentrations studied.

Harald von Canstein, Ying Li, Johannes Leonhauser, Elke Haase, Andreas Felske, Wolf-Dieter Deckwer, Irene Wagner-Dobler. (Division of Microbiology and Division of Biochemical Engineering, German Research Centre for Biotechnology, 38124 Braunschweig, Germany). **Spatially Oscillating Activity and Microbial Succession of Mercury-Reducing Biofilms in a Technical-Scale Bioremediation System.** Applied and Environmental Microbiology, 68(4) (2002), 1938-1946.

Mercury-contaminated chemical wastewater of a mercury cell chloralkali plant was cleaned on site by a technical-scale bioremediation system. Microbial mercury reduction of soluble Hg(U) to precipitating Hg(O) decreased the mercury load of the wastewater during its flow through the bioremediation system by up to 99%. The system consisted of a packed-bed bioreactor, where most of the wastewater's mercury load was retained, and an activated carbon filter, where residual mercury was removed from the bioreactor effluent by both physical adsorption and biological reduction. In response to the oscillation of the mercury concentration in the bioreactor inflow, the zone of maximum mercury reduction oscillated regularly between the lower and the upper bioreactor horizons or the carbon filter. At low mercury concentrations, maximum mercury reduction occurred near the inflow at the bottom of the bioreactor. At high concentrations, the zone of maximum activity moved to the upper horizons. The composition of the bioreactor and carbon filter biofilms was investigated by 16S-23S ribosomal DNA intergenic spacer polymorphism analysis. Analysis of spatial biofilm variation showed an increasing microbial diversity along a gradient of decreasing mercury concentrations. Temporal analysis of the bioreactor community revealed a stable abundance of two prevalent strains and a succession of several invading mercury-resistant strains, which was driven by the selection pressure of high mercury concentrations. In the activated carbon filter, a lower selection pressure permitted a steady increase in diversity during 240 days of operation and the establishment of one mercury-sensitive invader.

Helmut Lorbeer, Sophie Starke, Misri Gozan, Andreas Tiehm, Peter Werner. (Dresden University of Technology, Institute of Waste Management and Contaminated Site Treatment, Pirna, Germany. Water Technology Centre Karlsruhe, Karlsruhe, Germany). **Bioremediation of Chlorobenzene-Contaminated Groundwater on Granular Activated Carbon Barriers.** Water, Air and Soil Pollution, 2(3) (2002), 183-193.

During the past decade, various promising technologies have been developed for the decontamination of groundwater *in situ* which do not require long-term pumping or high energy consumption. One approach is to use funnel and gate technology. In the case described here, the combination of adsorption of contaminants on granular activated carbon (GAC) and its biodegradation is applied to considerably extend the operating time of the filling material in the barrier system. Monochlorobenzene (MCB), a recalcitrant groundwater contaminant under anaerobic conditions, undergoes high-capacity adsorption on GAC up to about 450 mg per gram. Aerobic enrichment cultures, obtained from a contaminated aquifer, were able to mineralize initially adsorbed MCB. In respirometer experiments the rate of carbon dioxide formation was dependent on the equilibrium concentration of MCB. The oxygen consumption of activated carbon by

means of autoxidative reactions may delay aerobic biodegradation in GAC filters. The oxygen uptake of pristine activated carbon amounted to 5.6 mg per gram GAC in laboratory column experiments. When GAC was pre-loaded with MCB, autoxidation rates were considerably reduced. Hence, it is advisable not to stimulate the biodegradation of MCB by oxygen supply in GAC biobarriers until after an initial period of solely sorptive MCB removal from the groundwater flow.

Ines Pöhler, Dirk F. Wenderoth, Katrin Wendt-Potthoff, Manfred G. Höfle. (GBF-German Research Centre for Biotechnology, Dept. of Environmental Microbiology, Mascheroder Weg 1, D-38124 Braunschweig, Germany. UFZ-Centre for Environmental Research Leipzig-Halle, Dept. of Inland Water Research, Brückstr. 3a, D-39114 Magdeburg, Germany). **Bacterioplankton Community Structure and Dynamics in Enclosures During Bioremediation Experiments in an Acid Mining Lake.** *Water, Air and Soil Pollution*, 2(3) (2002), 111-121.

In an acid mining lake (pH 2.6) enclosure experiments were performed with the addition of different concentrations of organic carbon, nitrogen and phosphorus. SSCP-community fingerprints, based on 16S rRNA gene amplicons, were performed to monitor changes in the structure of the total bacterial community and the sulfate reducing bacteria (SRB) in the mesocosms. Total bacterial cell counts, as assessed by epifluorescence microscopy, were increased in the mesocosms amended with organic carbon. The addition of carbon also increased the number of abundant bacterial taxa substantially along depth. Sulfate reducing bacteria (SRB) could be detected in all enclosures and all parts of the water column. These SRB belonged to genus *Desulfobacter* as indicated by corroborating molecular data.

J. W. C. Wong, K. M. Lai, C. K. Wan, K. K. Ma, M. Fang. (Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong). **Isolation and Optimization of PAH-Degradative Bacteria from Contaminated Soil for PAHs Bioremediation.** *Water, Air, and Soil Pollution*, 139(1-4) (2002), 1-13.

The objective of the present study is to isolate PSH-degradative bacteria from petroleum-contaminated soils and to optimize their degradative conditions including pH, glucose, nitrogen and phenanthrene concentrations purposes. Several bacterial strains were isolated through enrichment and one strain, *Burkholderia cocovenenans* (BU-3) that was tentatively identified by the Biolog system, demonstrated a high removal rate of phenanthrene over other strains. More than 95% in 100 and 500mg L⁻¹ and 65% in 1000 mg L⁻¹ of phenanthrene contents was reduced in the culture media respectively. Maximum rate of phenanthrene removal up to 4.2 mg hr⁻¹ occurred in the culture containing 1000 mg L⁻¹ phenanthrene. Media at a pH between 6.5 to 7.0 were more favorable for the degradation of phenanthrene by BU-3. although increasing glucose concentration from 0.45 to 3 g L⁻¹ resulted in a better bacterial growth of the isolated bacteria, the degradation of phenanthrene was reduced significantly. Nitrogen supplement did not exert a significant effect on bacterial growth and phenanthrene degradation. The isolated *Burkholderia cocovenenans* BU-3 demonstrated to be a feasible strain for degradation of phenanthrene at a neutral pH, even up to a phenanthrene concentration of 1000 mg L⁻¹.

J. Widada, H. Nojiri, T. Omori. (Biotechnology Research Center, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Laboratory of Soil and Environmental Microbiology, Department of Soil Science, Faculty of Agriculture, Gadjah Mada University, Bulaksumur, Yogyakarta 55281, Indonesia). **Recent developments in molecular techniques for identification and monitoring of xenobiotic-degrading**

bacteria and their catabolic genes in bioremediation. Applied Microbiology and Biotechnology, 60(1-2) (2002), 45-59.

The pollution of soil and water with xenobiotics is widespread in the environment and is creating major health problems. The utilization of microorganisms to clean up xenobiotics from a polluted environment represents a potential solution to such environmental problems. Recent developments in molecular-biology-based techniques have led to rapid and accurate strategies for monitoring, discovery and identification of novel bacteria and their catabolic genes involved in the degradation of xenobiotics. Application of these techniques to bioremediation has also improved our understanding of the composition, phylogeny, and physiology of metabolically active members of the microbial community in the environment. This review provides an overview of recent developments in molecular-biology-based techniques and their application in bioremediation of xenobiotics.

James L. Brown, Royal J. Nadeau. (Lockheed Martin/REAC 2890 Woodbridge Avenue, Edison, NJ 08837, USA. The Eco-Strategies Group, P.O. Box 433, Allamuchy, NJ 07820, USA). **Restoration of Petroleum-Contaminated Soil Using Phased Bioremediation.** Bioremediation Journal, 6(4) (2002), 315-319.

A conceptual approach is presented for the restoration of petroleum-contaminated sites by combining bioremediation with revegetation using native plants. Phased bioremediation includes active and passive treatment options for soil containing greater than 1% total petroleum hydrocarbons (TPHs). Phase I is used when initial soil TPH exceeds 1% Phase I utilizes either active land treatment, with regular soil tillage, or passive bioremediation to attain a treatment endpoint of 1% soil TPH. Passive treatment utilizes static soil and TPH-tolerant plants. Phase II is utilized when soil contains 1% TPH or less. It combines passive bioremediation with revegetation using native plants to complete the site restoration process. The phased approach to bioremediation was developed from results of full-scale field bioremediation and laboratory treatability studies. This approach assumes that the kinetics of TPH biodegradation is initially rapid, followed by a much slower second stage. It provides active initial treatment, followed by lower-cost passive treatment. The selection of either active or passive treatment in Phase I depends on whether total cost or time of treatment is more important. Passive treatment, although less costly than active treatment, generally requires more time. Phased bioremediation may provide a flexible, cost-effective, and technically sound approach for restoration of petroleum-contaminated sites. Vegetation used with passive bioremediation has several benefits. Plants stabilize soil, preventing erosion and thereby minimizing exposure to soil contaminants. Phytoremediation may also occur within the rhizosphere. The use of native plants has a strong ecological basis. They provide ecological diversity, are aesthetically pleasing and beneficial to wildlife, while requiring little maintenance. Phased bioremediation can provide a flexible, cost effective, and technically sound approach for the restoration of petroleum-contaminated sites.

Jennifer L. Nyman, Frank Caccavo, Jr. Al B. Cunningham and Robin Gerlach. (Center for Biofilm Engineering, Montana State University-Bozeman, Bozeman, MT 59717 USA. Department of Biology, Whitworth College, Spokane, WA 99251 USA). **Biogeochemical Elimination of Chromium (VI) from Contaminated Water.** Bioremediation Journal, 6(1) (2002), 39-55.

Ferrous iron [Fe(II)] reductively transforms heavy metals in contaminated groundwater, and the bacterial reduction of indigenous ferric iron [Fe(III)] to Fe(II) has been proposed as a means of establishing redox reactive barriers in the subsurface. The reduction of Fe(III) to Fe(II) can be accomplished by stimulation of indigenous dissimilatory metal-reducing bacteria (DMRB) or injection of DMRB into the subsurface. The microbially

produced Fe(II) can chemically react with contaminants such as Cr(VI) to form insoluble Cr(III) precipitates. The DMRB *She-wanella algae* BrY reduced surface-associated Fe(III) to Fe(II), which in batch and column experiments chemically reduced highly soluble Cr(VI) to insoluble Cr(III). Once the chemical Cr(VI) reduction capacity of the Fe(II)/Fe(III) couple in the experimental systems was exhausted, the addition of *S. algae* BrY allowed for the repeated reduction of Fe(III) to Fe(II), which again reduced Cr(VI) to Cr(III). The research presented herein indicates that a biological process using DMRB allows the establishment of a biogeochemical cycle that facilitates chromium precipitation. Such a system could provide a means for establishing and maintaining remedial redox reactive zones in Fe(III)-bearing subsurface environments.

Jennifer L. Kirk, John N. Klironomos, Hung Lee and Jack T. Trevors. (Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Department of Botany, Guelph, Ontario, Canada N1G 2W1). **Phytotoxicity Assay to Assess Plant Species for Phytoremediation of Petroleum-Contaminated Soil.** *Bioremediation Journal*, 6(1) (2001), 57-63.

A phytotoxicity bioassay was used to select plant species for phytoremediation that were able to germinate and grow in petroleum-contaminated soil from an industrial site in Canada. Perennial ryegrass (*Lolium perenne* var. "Affinity") and alfalfa (*Medicago sativa* L.) were more successful at germination and root growth than were little bluestem (*Schizachyrium scoparium*) and crown vetch (*Coronilla varia*). The phytotoxicity assay provides a rapid, efficient mechanism of prescreening potential plant species and eliminating those not able to germinate and establish in soil conditions present at the contaminated site. This bioassay can potentially reduce the number of pot or greenhouse degradation studies that need to be conducted before plant species can be chosen for petroleum phytoremediation.

Jeyaramraja PR, Anthony R, Rajendran A, Rajakumar K. (Res Cent Bot, VHN Senthikumara Nadar Coil, Virudhunagar 626001). **Decolorization of paper mill effluent by *Aspergillus fumigatus* in bioreactor.** *Polln Res*, 20(3) (2001), 309-312.

Decolorization and phenol reduction of paper mill effluent by the fungus, *Aspergillus fumigatus* isolated from paper mill effluent were studied. Optimum condition with regard to carbon sources, nitrogen sources and the addition of surfactant were worked out. Repeated batch experiments in an aerated bioreactor were performed with Ca-alginate immobilized fungus. The immobilized fungal beads were found to be effective for eight batches of effluent treatment.

Jiasong Fang, Michael J. Barcelona and Pedro J. Alvarez. (The University of Michigan, Ann Arbor, MI 48109, USA. The University of Iowa, Iowa City, Iowa 52242, USA). **Phospholipids of Five Pseudomonad Archetypes for Different Toluene Degradation Pathways.** *Bioremediation Journal*, 4(3) (2000), 181-185.

Liquid chromatography/electrospray ionization/mass spectrometry (LC/ESI/MS) was used to determine intact phospholipid profiles for five reference pseudomonad strains harboring different (aerobic) toluene catabolic pathways: *Pseudomonasputida* mt-2, *Pseudomonasputida* F1, *Burkholderia cepacia* G4, *Burkholderiapickettii* PKO1, and *Pseudomonas mendocina* KRI. These five strains contained a predominant pool of phosphatidylethanolamines. Other phospholipids identified include phosphatidylglycerol, phosphatidylserine, phosphatidylmethylethanolamine, and phosphatidylidimethylethanolamine. There was a clear separation in phospholipid profiles that allows for the differentiation between the *Pseudomonas* and *Burkholderia* genera. Factor analysis of the phospholipid profiles showed that *B. cepacia* G4, *P. putida* mt-2,

and *B. pickettii* PKO1 were clearly separated, while *P. putida* F1 and *P. mendocina* KR1 were clustered as a group. These results suggest that intact phospholipid profiling could be used to evaluate the relative abundance of specific degraders in bioreactors or in aquifer material. Nevertheless, the usefulness of this technique for taxonomic characterization of such complex samples remains to be demonstrated because of potential confounding effects of overlapping profiles and potential changes in phospholipid composition due to different growth conditions.

Julia Foght, Trevor Aprilb, Kevin Biggar and Jackie Aislabie. (Biological Sciences, University of Alberta, Edmonton, Alberta Canada T6G 2E9. Biological Sciences, Northern Alberta Institute of Technology, Edmonton Alberta, Canada T5G 2R1. Civil and Environmental Engineering, University of Alberta, Edmonton, Alberta, Canada T6G 2G7. Landcare Research, Private Bag 3127, Hamilton, New Zealand). **Bioremediation of DDT-Contaminated Soils: A Review**. *Bioremediation Journal*, 5(3) (2002), 225-246.

The insecticide 1,1,1-trichloro-2, 2-bis-(4-chlorophenyl)ethane (DDT) has been used extensively since the 1940s for control of agricultural pests, and is still used in many tropical countries for mosquito control. Despite a ban on DDT use in most industrialized countries since 1972, DDT and its related residues (DDTr) persist in the environment and pose animal and human health risks. Abiotic processes such as volatilization, adsorption, and photolysis contribute to the dissipation of DDTr in soils, often without substantial alteration of the chemical structure. In contrast, biodegradation has the potential to degrade DDTr significantly and reduce soil concentrations in a cost-effective manner. Many bacteria and some fungi transform DDT, forming products with varying recalcitrance to further degradation. DDT biodegradation is typically co-metabolic and includes dechlorination and ring cleavage mechanisms. Factors that influence DDTr biodegradation in soil include the composition and enzymatic activity of the soil microflora, DDTr bioavailability, the presence of soil organic matter as a co-metabolic substrate and (or) inducer, and prevailing soil conditions, including aeration, pH, and temperature. Understanding how these factors affect DDTr biodegradation permits rational design of treatments and amendments to stimulate biodegradation in soils. The DDTr-degrading organisms, processes and approaches that may be useful for bioremediation of DDTr-contaminated soils are discussed, including *in situ* amendments, *ex situ* bioreactors and sequential anaerobic and aerobic treatments.

Jun Wu, Richard F. Unz, Husen Zhang and Bruce E. Logan. (Dept. of Civil and Environmental Engineering, The Pennsylvania State University, University Park, PA, USA). **Persistence of Perchlorate and the Relative Numbers of Perchlorate- and Chlorate-Respiring Microorganisms in Natural Waters, Soils, and Wastewater**. *Bioremediation Journal*, 5(2) (2001), 119-130.

Cell numbers of perchlorate (PRM)- and chlorate (CRM)-reducing microorganisms and the persistence of perchlorate were determined in samples of soils, natural waters, and wastewater incubated under laboratory conditions. Complete perchlorate reduction in raw wastewater and creek water was achieved in 4 to 7 days and 8 to 29 days, respectively, depending on the individual growth substrate (acetate, lactate, citric acid, or molasses) employed. Perchlorate persisted in most mixed cultures developed with 2 g of "pristine" soil, but declined in mixed cultures developed with 100 g of soil. Less than seven days were required to completely reduce perchlorate in cultures started with 10 g of a perchlorate-contaminated soil obtained from a site in Texas. The concentration of PRM was estimated using a 5-tube most probable number (MPN) procedure. To account for discrepancies due to differences in the total number of bacteria (per mass of sample) in the samples, difficulty in removing bacteria from soil samples, and the lack of an unequivocal method to measure total viable cells in these different systems, we normalized our MPN results on the basis of 10⁶ or 10⁹ total bacteria counted using

acridine orange direct counts (AODC). There were more PRM in wastewater samples on a per-cell basis (15 to 350 PRM/10⁶-AODC) than in water samples (0.02 to 0.4 PRM/10⁶-AODC). There were also more PRM in soils from sites exhibiting direct evidence of perchlorate contamination (100 to 200 PRM/10⁹-AODC) than from other sites (nondetectable to 0.77 PRM/10⁹-AODC). These results demonstrate that perchlorate-reducing bacteria are present at perchlorate-contaminated sites, and that perchlorate can be degraded by these microorganisms through the addition of different electron donors, such as acetate and lactate.

K. Knöller, G. Strauch. (UFZ-Center for Environmental Research Leipzig-Halle, Department of Hydrogeology, Halle/Saale, Germany). **The Application of Stable Isotopes for Assessing the Hydrological, Sulfur, and Iron Balances of Acidic Mining Lake ML 111 (Lusatia, Germany) as a Basis for Biotechnological Remediation.** Water, Air and Soil Pollution, 2(3) (2002), 3-14.

Stable isotope ($\delta^{18}\text{O-H}_2\text{O}$, $\delta^2\text{H-H}_2\text{O}$, $\delta^{34}\text{S-SO}_4^{2-}$) and hydrochemical data (SO_4^{2-} , Fe-concentrations) have been used to estimate the annual groundwater inflow and outflow of mining lake ML 111 and to calculate the total amount of dissolved sulfate and iron that is carried into the lake by groundwater. The hydrological balance suggests an annual groundwater inflow of 23 700 m³ and an annual groundwater outflow of 15 700 m³. The calculation of the sulfur and iron balances yielded an annual sulfate input of 37 800 kg and an annual iron input of 7000 kg with the groundwater inflow. Furthermore it was shown that significant fluxes of these elements go into the lake sediments which results in continuous release of acidity in the lake water.

Kowshik Meenal, Nazareth Santa. (Dept Microbio, Goa Univ, Taleigao Plateau 403206, Goa). **Bio sedimentation of mine tailings by *Fusarium solani*.** J Indl Polln Contl, 17(2) (2001), 341-346.

Mine tailings have a high concentration of suspended material and metal complexes. It is routinely treated for sedimentation with various chemicals that in themselves are pollutants. The mycelial mass of *Fusarium solani* added to mine tailings, greatly increases the natural rate of sedimentation. Homogenisation of the biomass increases its capacity of sedimentation. Storage of the mycelium upto a week, does not affect the sedimentation rate.

L. E. J. Lee, J. Stassen, A. McDonald, C. Culshaw, A. D. Venosa and K. Lee. (Department of Biology, Wilfrid Laurier University. Waterloo. Ontario. N2L 3C5, USA. National Risk Management Research Laboratory, Oil Spill Program, U.S. Environmental Protection Agency, Cincinnati, Ohio, 45268, USA. Department of Fisheries and Oceans, Centre for Offshore Oil and Gas Environment Research, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, B2Y 4A2, USA). **Snails as Biomonitors of Oil-Spill and Bioremediation Strategies.** Bioremediation Journal, 6(4) (2002), 373-386.

Aquatic and pulmonate snails were evaluated for their suitability as biomonitors of habitat recovery following an experimental oil spill in a freshwater marshland. The mystery snail, *Viviparus georgianus*, and the mimic pondsnail, *Pseudosuccinea columella*, were used as sediment quality biomonitors for a controlled oil-spill experiment at a wetland site along the St. Lawrence River (Ste. Croix, Quebec) to assess the impacts of crude oil, rates of natural recovery, and the efficacy of bioremediation treatments to enhance the bacterial degradation of residual oil in the sediments. Sediments from control sites and oiled sites with or without the application of fertilizers as bioremediation strategies, were evaluated both *in situ* and under controlled laboratory conditions at various time intervals. Snail survival, growth, and histopathological changes were

monitored. While *V. georgianus* proved to be good biomonitors, *P. columella* appeared unaffected by the treatments. The differing sensitivity may depend on the gastropods' feeding habits. *V. georgianus* being a detritivore assimilated contaminants from the sediments, while *P. columella*, being an herbivore, did not directly assimilate contaminants. Nevertheless, snails show potential as important and ideal "tools" for testing environmental conditions because of their abundance, ease of collection, wide distribution, and relatively sedentary nature.

Loren A. Launen, Vincent H. Buggs, Michael E. Eastep, Rica C. Enriquez, Joseph W. Leonardo Michael J. Blaylock, Jian-Wei Huang and Max M. Haggblom. (Dept. of Biochemistry and Microbiology and the Biotechnology Center for Agriculture and the Environment, Cook College, Rutgers, the State University of New Jersey, New Brunswick, NJ 08901-8525, USA. Present address: GeoSyntec Consultants, 1531 Dick Lonas Road, Building A, Knoxville, TN 37909, USA. Edenspace Systems Corporation, Enterprise Court, Suite 100, Dulles, VA 20151-1217, USA). **Bioremediation of Polyaromatic Hydrocarbon-Contaminated Sediments in Aerated Bioslurry Reactors.** *Bioremediation Journal*, 6(2) (2002), 125-141.

Treatment of dredged sediments contaminated by polyaromatic hydrocarbons (PAHs) is a significant problem in the New York/New Jersey (NY/NJ) Harbor. 0.5 m³ -scale slurry-phase bioreactors were used to determine whether bioaugmentation with a PAH-degradative bacterial consortium, or with the salt marsh grass *S. alterniflora*, could enhance the biodegradation of PAHs added to dredged estuarine sediments from the NY/NJ Harbor. The results were compared to biodegradation effected by the indigenous sediment microbial community. Sediments were diluted 1: 1 in tap water and spiked to a final concentration of 20 mg/kg dry weight sediment of phenanthrene, anthracene, acenaphthene, fluorene, fluoranthene, and pyrene. The sediment slurry was then continuously sparged with air over 3 months. In all bioreactors a rapid reduction of greater than 95% of the initial phenanthrene, acenaphthene, and fluorene occurred within 14 days. Pyrene and fluoranthene reductions of 70 to 90% were achieved by day 77 of treatment. Anthracene was more recalcitrant and reductions ranged from 30 to 85%. Separate experiments showed that the sediment microbial communities mineralized ¹⁴C-pyrene and ¹⁴C-phenanthrene. PAH degradation, and the number of phenanthrene-degrading bacteria, were not enhanced by microbial or plant bioaugmentation. These data demonstrate that bioaugmentation is not required to effect efficient remediation of PAH-contaminated dredged sediments in slurry-phase bioreactors.

M. E. Losi, T. Giblin, V. Hosangadi and W. T. Frankenberger, Jr. (Center for Environmental Microbiology, 1960 Chicago Ave D-15, Riverside, CA 92705, USA. Foster Wheeler Environmental Corporation, 1940 E. Deere Avenue, Santa Ana, CA 92705, USA. Department of Natural Sciences, Stephens College, Columbia, MO, USA 65215; Dept. of Environmental Science, Univ. of California, Riverside). **Bioremediation of Perchlorate-Contaminated Groundwater Using a Packed Bed Biological Reactor.** *Bioremediation Journal*, 6(2) (2002), 97-103.

The objective of this study was to assess the efficacy of a bench-scale, acetate-fed, packed bed bioreactor (PBR) to treat low concentrations (<1 mg L⁻¹) of perchlorate (C10₄⁻) in groundwater collected from an impacted site. The PBR consisted of a cylindrical plexiglass column packed with Celite, a diatomaceous earth product, as a solid support medium. The reactor was inoculated with a C10₄⁻ reducing bacterial isolate, perclace. Results showed that with influent C10₄⁻ concentrations of approximately 800 ug L⁻¹ nondetectable effluent concentrations (<4 ug L⁻¹) were achieved with the PBR/perclace system at residence time as low as 0.3 h. Influent acetate concentrations of less than 500 mg L⁻¹ yielded nondetectable effluent C10₄⁻ concentrations, and acetate

concentrations generally less than 50 mg L⁻¹ were present in the effluent. Nitrate (NO₃⁻) was also removed in this system, while sulfate (SO₄²⁻) reduction was not observed. The pH remained relatively constant during the process.

M.C. Sãágua, L. Baeta-Hall, A.M. Anselmo. (Instituto Nacional de Engenharia e Tecnologia Industrial, Estrada do Paço do Lumiar, 1649-038 Lisboa, Portugal). **Microbiological characterization of a coke oven contaminated site and evaluation of its potential for bioremediation.** World Journal of Microbiology and Biotechnology, 18(9) (2002), 841-845.

The soil microbial population of a coke oven site was investigated in order to evaluate its potential for bioremediation. The study was carried out in soil samples with distinct polynuclear aromatic hydrocarbon (PAH) contamination levels, comparing the population profiles constituted by total heterotrophic and PAH-utilizing strains. Isolation of degrading strains was performed with phenanthrene or pyrene as sole carbon sources. The ability to degrade other PAHs, such as anthracene, fluorene and fluoranthene was also investigated. The results showed a reduction of 30% in species diversity and microbial density drops one order of magnitude in contaminated samples. Furthermore, the number of PAH-utilizing colonies was higher in the contaminated area and about 20% of the isolates were able to degrade phenanthrene and pyrene, while this value decreased to 0.15% in uncontaminated samples. Three PAH-degrader strains were identified as: CDC gr. IV C-2, *Aeromonas* sp. and *Pseudomonas vesicularis*. The ability of these strains to degrade other PAHs was also investigated.

Marja A. Tirola, Minna K. Männistö, Jaakko A. Puhakka, Markku S. Kulomaa1. (Department of Biological and Environmental Science, University of Jyväskylä, 40351 Jyväskylä, Institute of Environmental Engineering and Biotechnology, Tampere University of Technology, 33101 Tampere, Finland). **Isolation and Characterization of *Novosphingobium* sp. Strain MT1, a Dominant Polychlorophenol-Degrading Strain in a Groundwater Bioremediation System.** Applied and Environmental Microbiology, 68(1) (2002), 173-180.

A high-rate fluidized-bed bioreactor has been treating polychlorophenol-contaminated groundwater in southern Finland at 5 to 8°C for over 6 years. We examined the microbial diversity of the bioreactor using three 16S ribosomal DNA (rDNA)-based methods: denaturing gradient gel electrophoresis, length heterogeneity-PCR analysis, and restriction fragment length polymorphism analysis. The molecular study revealed that the process was dependent on a stable bacterial community with low species diversity. The dominant organism, *Novosphingobium* sp. strain MT1, was isolated and characterized. *Novosphingobium* sp. strain MT1 degraded the main contaminants of the groundwater, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol, at 8°C. The strain carried a homolog of the *pcpB* gene, coding for the pentachlorophenol-4-monooxygenase in *Sphingobium chlorophenicum*. Spontaneous deletion of the *pcpB* gene homolog resulted in the loss of degradation ability. Phenotypic dimorphism (planktonic and sessile phenotypes), low growth rate (0.14 to 0.15 h⁻¹), and low-copy-number 16S rDNA genes (single copy) were characteristic of strain MT1 and other MT1-like organisms isolated from the bioreactor.

Mark J. Strynar, Jerzy Dee and Jean-Marc Bollag. (Laboratory of Soil Biochemistry, Center for Bioremediation and Detoxification, Pennsylvania State University, University Park, PA 16802, USA). **Anaerobic/Aerobic Composting of Soil contaminated with 2,4,6-Trinitrotoluene.** Bioremediation Journal, 6(2) (2002), 177-190.

A loam soil from Pennsylvania without a history of exposure to explosives was incubated

with 5 g kg⁻¹ of ¹⁵N-labeled 2,4,6-trinitrotoluene (TNT) and 200 μ Ci kg⁻¹ of ¹⁴C-TNT for 3 days and then amended with compost at a 1:2 soil to compost ratio. The compost was prepared by mixing 40% alfalfa hay, 40% grass hay, 10% spent mushroom compost, and 10% municipal biosolids. The mixture of soil and compost was inoculated with methanogens from cattle manure, amended with glucose and starch, and incubated for 37 days under anaerobic conditions. The anaerobic incubation was followed by 26 days of forced aerobic incubation. At the end of the aerobic phase, most of the radioactivity was associated with organic matter; only 8.7% could be extracted with water and methanol, but no TNT was present in the extracts as determined by high-performance liquid chromatography. The unextractable radioactivity was associated with humic acid (40.0 \pm 1.0%), fulvic acid (14.3 \pm 1.4%), and humin (28.2 \pm 0.5%). Radioactive materials associated with humic acid and humin were analyzed by solid-state ¹⁵N-nuclear magnetic resonance (NMR) spectrometry. The NMR spectra indicated that nitro groups of TNT had been reduced to amino groups that were subsequently involved in the formation of covalent bonds with soil organic matter.

Masud Hossain SK, Das Manas, Ibrahim SH. (Dept Chemi Engng, Mohmed Sathak Engng Coll, Kilakarai 623806, T.N.). **Aerobic studies on pollution - abatement of sulfite pulp bleaching effluent using *Phanerochaete chrysosporium* (MTCC-787)**. J Indl Polln Contl, 17(2) (2001), 191-200.

The white-rot fungus *Phanerochaete chrysosporium* decomposes chlorinated organic compounds in sulfite bleaching effluents which are considered to be resistant to bacterial treatment. The optimum digestion time is eight days (HRT). Seven days old aged with 15 percent (V/V) inoculum concentration is the optimum to bleach the sulfite bleaching effluent to a maximum pollution-abatement. 1.5 percent (w/v) glucose and 0.15 percent (w/v) nitrogen concentrations can degrade the maximum (78.97) percent COD and maximum (80.88) percent BOD.

P Vasudeban, V Padmavathy, N Tewari and S C Dhingra. (Centre for Rural Development & Technology, Indian Institute of Technology, Delhi 110016, India). **Biosorption of Heavy Metal Ions**. Journal of Scientific & Industrial Research, 60 (2001), 112-120.

Increasing environmental pollution by metal ions has led to the necessity of evolving efficient and cost-effective treatment technologies. Biosorptions of metal ions using microbial biomass could be useful, especially for wastewater treatment. The metal uptake capacity of various biosorbents and the mechanism of uptake are reviewed in this context.

Pandey, B.V, A. Kumar and R.S Upadhyay 2002. (Department of Botany, Banaras Hindu University, Varanasi 221005). **Impact of textile effluents on water bodies and their microbial bioremediation**. In ecology and Conservation of Lakes, Reservoirs and Rivers, 2 (ed. A. Kumar), ABD Publishers, India, 459-502.

Textile wastes are characterized by high volume and extreme variability in composition which contain non-biodegradable dyes and toxic substances. The variability arises both from the diversity in the types of industrial processes employed and the immense range of chemical and other materials involved in each industrial category. To alleviate the ill effects of dye factory effluent and textile waste water with specific role on the color reduction and its degradation, several methods have been employed. To expertise the technique attempt has been made to isolate certain biosystems (fungi and bacteria) from the dye effluent drenched soil and screen the elite ones for faster investigation. It has been found that bacteria exhibit better effect in removing toxicity where as fungi are registered with better decolorization. Thus, industrial wastes should be properly treated

before mixing in the water bodies. The chemical properties of the water bodies are improved if they are properly treated, employing microorganisms can be effective method and may substitute the conventional recovery and removed processes. Broad screening of microorganisms should be under taken for the development of need technology. Improve techniques of immobilization should be used commercially for recovery processes involving an inexpensive stripping agent which can also be recycled. These strategies can solve the problem of water pollution caused due to discharge of industrial effluent due to discharge of industrial effluents in water bodies.

Paul M. Bradley and Francis H. Chapelle. (U.S. Geological Survey, 720 Gracern Rd, Suite 129, Columbia, SC 29210-7651 USA). **Microbial Mineralization of Ethene Under Sulfate-Reducing Conditions.** Bioremediation Journal, 6(1) (2002), 1-8.

Previous investigations demonstrated that respiratory reductive dechlorination of vinyl chloride (VC) can be efficient even at H_2 concentrations (<2 nM) that are characteristic of SO_4 -reducing conditions. In the study reported here, microorganisms indigenous to a lake-bed sediment completely mineralized [$1, 2-^{14}C$] ethene to $^{14}CO_2$ when incubated under SO_4 -reducing conditions. Together, these observations argue for a novel mechanism for the net anaerobic oxidation of VC to CO_2 : reductive dechlorination of VC to ethene followed by anaerobic oxidation of ethene to CO_2 . Moreover, the results of this study suggest that reliance on ethene and/or ethane accumulation as a quantitative indicator of complete reductive dechlorination of chloroethene contaminants may not be warranted.

Peter D. Franzmann, Luke R. Zappia, A. L. Tilbury, Bradley M. Patterson, Greg B. Davis and Raphi T. Mandelbaum. (Centre for Groundwater Studies, CSIRO Land and Water, Underwood Ave. Floreat Park WA 6014, Australia. Department of Chemistry, The University of Western Australia, Australia. Institute of Soil and Environmental Sciences, Volcani Research Center, 50250 Beit Dagan, Israel). **Bioaugmentation of Atrazine and Fenamiphos Impacted Groundwater: Laboratory Evaluation.** Bioremediation Journal, 4(3) (2000), 237-248.

After the failure of a three-month pump-and-treat exercise to clean up an aquifer contaminated with the pesticides atrazine and fenamiphos, microcosm experiments using ^{14}C -labeled compounds were undertaken to determine under what conditions bioremediation would be most effective, and to investigate the prospects for the use of bioaugmentation. The calculated half-lives for atrazine and fenamiphos mineralization to carbon dioxide in unamended, anaerobic aquifer material were 730 and 1,000 years, respectively. Oxygenation, coupled with bioaugmentation with enrichments of atrazine-mineralizing bacteria obtained from the contaminated site or an imported, atrazine-mineralizing pure strain, *Pseudomonas* sp. strain ADP, decreased the half-life of atrazine mineralization, to <20 days. Although strain ADP does not use atrazine as a source of carbon and energy, amendment of the aquifer material with citrate, which strain ADP uses as a source of carbon and energy, did not appreciably stimulate the mineralization rate of atrazine in the microcosms, suggesting that the aquifer contains enough natural organic carbon for atrazine mineralization. Aerobic enrichments of fenamiphos-degrading bacteria were prepared; however, oxygenation and bioaugmentation of aquifer material with these strains did not enhance mineralization of fenamiphos within the time constraints of the experiments. The shortest calculated half-life of fenamiphos mineralization in the microcosms was 6.8 years, which is exceedingly long compared with the half-life of fenamiphos in most surface soils.

R. Boopathy. (Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310, USA). **Bioremediation of explosives contaminated soil**. International Biodeterioration & Biodegradation, 46(1) (2000), 29-36.

This research paper presents two bioremediation technologies for the treatment of explosives-contaminated soil. The technologies include soil slurry reactor, and in situ bioremediation. Both bioremediation technologies showed promising results and these treatment methods used co-metabolic process with molasses as a co-substrate for bacterial growth. The soil slurry reactor removed the explosive contaminants present in the soil within three weeks of incubation period. The in situ treatment method also removed all the explosives present in the soil, but the treatment time was approximately 12 months. Each of the bioremediation methods described in this study has advantages and disadvantages. The major advantage in the soil slurry reactor method is the short treatment time, but the disadvantage is that it is labor intensive and expensive due to the excavation of soil, operation of slurry reactors, and post-treatment costs. The in situ treatment method has the advantage of lower treatment costs, but the disadvantage is the treatment time, which is considerably longer.

R. Margesin. (Institute of Microbiology (NF), University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria). **Potential of cold-adapted microorganisms for bioremediation of oil-polluted Alpine soils**. International Biodeterioration & Biodegradation, 46(1) (2000), 3-10.

The environmental contamination by organic pollutants is a widespread problem in all climates. The most widely distributed pollution can be attributed to oil contamination. Bioremediation methods can provide efficient, inexpensive and environmentally safe cleanup tools. The role of cold-adapted microorganisms for the bioremediation of experimentally and chronically oil-contaminated Alpine soils was evaluated in the studies described. The results demonstrated that there is a considerable potential for oil bioremediation in Alpine soils. Oil biodegradation can be significantly enhanced by biostimulation (inorganic nutrient supply), but a complete oil elimination is not possible by employing biological decontamination alone.

Reetta Piskonen, Anu Kapanen, Tine Mansikka, Jorma *Rytkonen* and Merja Itavaara. (VTT Biotechnology, P.O.Box 1500,02044 VTT, Finland. VTT Chemical Technology, P.O.Box 1403, 02044 VTT, Finland. VTT Manufacturing Technology, P.O.Box 1705, 02044 VTT, Finland). **Evaluation of Bioremediation Treatments in a Shoreline-Simulating Microcosm**. Bioremediation Journal, 6(2) (2002), 143-158.

A microcosm test was designed to study the efficiency of bioremediation treatments at oil contaminated shorelines. The biodegradation in the hermetically closed microcosm was monitored by measuring the total cumulative inorganic carbon evolved during the bioremediation process. The effects of three different additives, medium-release methylene urea (MU) + apatite, fast-release MU + superphosphate, and a biosorbent, on the biodegradation of weathered crude oil (North Sea Brent) were evaluated at +10° C. All the additives significantly increased mineralization. The total amount of inorganic carbon evolved during the 10-week study was measured in the microcosm treated with oil, and with oil and medium- release MU + apatite, fast-release MU + superphosphate, and biosorbent. The amounts were 40, 670, 490, and 580 mg, respectively. The respirometric measurements were supported by microbiological determinations, ATP content in the sand, number of heterotrophic bacteria, and amount of biomass-C determined by the substrate-induced respiration method. Nutrient analysis indicated that biodegradation was nitrogen limited. The microcosm test proved to be suitable for comparing the effectiveness of different treatments in enhancing the biodegradation of crude oil-contaminated shores.

Richard T. Townsend, James S. Bonner and Robin L. Autenrieth. (North Texas Municipal Water District, 505 E. Brown Street, Wylie, TX 75098, USA. Conrad Blucher Institute for Surveying and Science, 6300 Ocean Drive, Corpus Christi, TX 78412-5503, USA. Division of Environmental and Water Resources Engineering, Department of Civil Engineering, Texas A&M University, College Station, TX 77843-3136, USA). **Microbial Dynamics during Bioremediation of a Crude Oil-Contaminated Coastal Wetland.** *Bioremediation Journal*, 4(3) (2000), 203-218.

In 1996, a controlled crude oil application was conducted at a Texas intertidal, coastal wetland to determine the effectiveness of two biostimulation treatments in these sensitive areas. An inorganic nutrient treatment and inorganic nutrient plus a potential electron acceptor (nitrate) treatment were examined. As part of this research, polycyclic aromatic hydrocarbon (PAH)-degrading, aliphatic-degrading, and total heterotrophic microbial numbers were monitored. Using a randomized, complete block design consisting of 21 plots, microbial data from biostimulation treatment plots were statistically compared to oiled control plots to assess treatment differences. Sediment samples from all plots receiving oil showed exponential increases in the numbers of aliphatic (*n*-alkane) and PAH-degrading microorganisms. This increase was observed at both 0 to 5 cm and 5 to 10 cm sample depths. Statistical analysis, however, revealed no significant differences in the numbers of aliphatic-degrading or PAH-degrading microorganisms between treatment plots and oiled control plots or between treatments on any sample day. The numbers of PAH- and aliphatic-degrading microorganisms returned to near pre-application levels by the end of the monitoring period. Ratios of hydrocarbon-degrading microbes to total heterotrophs also increased as a result of the oil application and returned to pre-application levels by the end of the monitoring period. Overall, the populations of hydrocarbon-degrading microorganisms illustrated a well-documented response to crude oil. However, the addition of the biostimulation treatments did not significantly increase the numbers of aliphatic-degrading, PAH-degrading, or total heterotrophic microorganisms over populations on control plots.

Roger Dobler, Matthias Saner and Reinhard Bachofen. (University of Zurich, Institute for Plant Biology, Zollikerstr. 107, CH-8008 Zurich, Switzerland). **Population Changes of Soil Microbial Communities Induced by Hydrocarbon and Heavy Metal Contamination.** *Bioremediation Journal*, 4(1) (2000), 41-56.

Substrate utilization tests with Biolog® plates were used to obtain information on shifts in community composition and on changes in the metabolic diversity and activity of microorganisms in soil polluted with hydrocarbons and/or heavy metals. Differences between the patterns of substrate utilization of endogenous microorganisms of pristine and contaminated soils were investigated by multivariate analysis. Population changes and shifts in metabolic diversity were observed both after hydrocarbon pollution or heavy metal contamination. The overall activity on the 95 Biolog® Gram-negative (GN) substrates correlated well with the respiration rate of the soil. Soils contaminated with hydrocarbons showed higher metabolic potentials than the corresponding controls. In contrast, heavy metal pollution caused both lower metabolic activity and a loss in diversity. The Biolog® assay was found to be suitable to describe changes in functional diversity of soils caused by hydrocarbon contamination or heavy metal stress.

Russell AP Thomas, Alan J Beswick, Gabriela Basnakova, Rachel Moller, Lynne E Macaskie. (School of Biosciences, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK). **Growth of naturally occurring microbial isolates in metal-citrate medium and bioremediation of metal-citrate wastes.** *Journal of Chemical Technology and Biotechnology*, 75(3) (2000), 187-195.

The use of citrate as a chelating agent in decontamination operations is of environmental concern as it can mobilize toxic heavy metals if discharged into the environment. Many heavy metal-citrate complexes are recalcitrant to biodegradation. Citrate-utilizing strains of *Pseudomonas aeruginosa* and *Pseudomonas putida* were isolated from a mixed culture which had been maintained with EDTA as the carbon source for 2 years. Citrate (5 mM) was used as the sole carbon source in medium supplemented with 5 mM Cd, Zn, Cu, Fe, Co, or Ni. Removal of the metals from the medium was promoted by the incorporation of inorganic phosphate as a precipitant, with formation of nickel and cobalt phosphates confirmed by X-ray powder diffraction analysis. The potential of *P. putida* to biodegrade citrate in a nickel-citrate secondary waste was illustrated using a fill-and-draw reactor supplied with effluent from a bioinorganic ion exchange column that had been used previously to concentrate nickel from aqueous solution.

S. Mana Capelli, J. P. Busalmen and S. R. de Sánchez. (División Corrosión, INTEMA-CONICET, Universidad Nacional de Mar del Plata, Juan B. Justo 4302, B7608FDQ, Mar del Plata, Argentina). **Hydrocarbon bioremediation of a mineral-base contaminated waste from crude oil extraction by indigenous bacteria.** International Biodeterioration & Biodegradation, 47(4) (2001), 233-238.

The susceptibility to bioremediation of the hydrocarbons contained in a waste from crude oil extraction was examined. Laboratory scale batch reactors were inoculated with indigenous bacteria and biodegradation was followed for 45 days. The total hydrocarbon content was reduced to ~70% of its initial value at the end of the experiments. Saturated and aromatic hydrocarbons were the most readily degraded fractions with, respectively, ~70% and ~60% of the fraction remaining at the end of the experiment. A minor degradation was observed in the resins fraction (~20%), whereas the asphaltenes fraction remained almost constant. The substrate preferences of the natural population towards various fractions of the crude oil were determined by both the length of the lag phase and the slope of the exponential growth in a mineral salt-base medium containing either of the different hydrocarbon fraction as the sole source of carbon. The highest consumption rate for every fraction during the time course experiments was in agreement with the shortest lag phase and the greatest exponential growth slope in the corresponding selective media, indicating changes in the population composition.

S. S. Radwan, R. H. Al-Hasan, S. Salamah and S. Al-Dabbous. (Department of Biological Sciences, Faculty of Science, Kuwait University, P.O. Box 5969, Safat 13060, Kuwait). **Bioremediation of oily sea water by bacteria immobilized in biofilms coating macroalgae.** International Biodeterioration & Biodegradation, 50(1) (2002), 55-59.

Using the standard plate method and a solid mineral medium containing crude oil as a sole source of carbon and energy, 10 different macroalgae from the Arabian Gulf were found associated with large numbers of oil-utilizing bacteria. Each gram fresh alga was associated with about two to about 30 million cells of bacteria predominantly belonging to the nocardioforms and the genus *Acinetobacter*. Shaking macroalgal samples in sea water batches containing known amounts of individual hydrocarbons led to considerable attenuation of these compounds as measured by GLC. Thus, bacteria associated with macroalgae consumed about 64–98% of n-octadecane and about 38–56% phenanthrene from medium aliquots containing 0.03% of the test hydrocarbon after 2 weeks. Meanwhile, the oil-utilizing bacteria, especially the nocardioforms, associated with the macroalgae increased in number by about 32–490 fold, depending on the macroalgae and hydrocarbons studied. On the other hand, relatively negligible numbers of bacteria were released into the sea water compared with the numbers immobilized on the macroalgal surfaces. Individual bacterial isolates could grow on a wide range of pure alkanes and aromatic hydrocarbons as sole sources of carbon and energy. It was

concluded that macroalgae submerged in the sea waters are coated with biofilms rich in oil-utilizing bacteria, that contribute to hydrocarbon attenuation in water. These natural biological consortia represent valuable tools that could be of high potential for phytoremediation of oily sea water.

S. Vincent, M. Mary Jee Cruz And A. Leo Thomas. (P. G. & Research Department of Zoology, Loyola College, Chennai - 600 034, India). **Bioremediation of chromium by the aquatic macrophyte *Caldesia paranassipolia* (L) parl.** Poll Res., 20(1) (2001), 75-77.

Young plants of *Caldesia paranassipolia* were selected and analyzed to study their role in bioremoval of chromium. The observations showed the uptake of chromium by the plant samples show increase in chromium as the concentration increase. It has been interpreted that due to increase in the concentration and exposure to number of days, the uptake of chromium also increases. Thus by the introduction of biological agent such as *Caldesia paranassipolia* we can reduce the contamination of natural habitat at a lower cost.

Shukia Siddhartha, Sharma Rajeev, Thakur Indu Sekhar. (VI/1918 Ta Colony, Sector 6, P.O. Pantnagar, Dist, U.S. Nagar, GBPUA&T, Pant Nagar 263145, Uttaranchal). **Enrichment and characterization of pentachlorophenol degrading microbial community for the treatment of tannery effluent.** Polln Res, 20(3) (2001), 353-363.

A mixed microbial community isolated from the sediment core of the pulp and paper mill having potentiality to degrade pentachlorophenol was enriched in a chemostat containing mineral salt medium and sodium pentachlorophenol as sole source of carbon and energy. The bacterial community obtained on day 240 after continuous enrichment comprised of three bacterial strains, identified as *Pseudomonas* sp. (two strains) and *Arthrobacter* sp. (one strain). The community produced significant reduction in COD (74.4%), chromium (75%), sulfides (88.9%), total phenol (84%) and pentachlorophenol (80.8%).

Siwei Zou, Krista M. Anders and John F. Ferguson. (Department of Civil and Environmental Engineering, University of Washington, Seattle, WA 98195, USA). **Biostimulation and Bioaugmentation of Anaerobic Pentachlorophenol Degradation in Contaminated Soils.** Bioremediation Journal, 4(1) (2000), 19-25.

The effects of bioaugmentation with a pentachlorophenol (PCP)-adapted consortium and biostimulation with glucose as a carbon source as anaerobic bioremediation of PCP-contaminated soil were investigated in terms initial PCP removal rate and the extent of PCP dechlorination and mineralization. Samples from two PCP contaminated sites were prepared, put into a series of hungate tube, inoculated, and fed under different condition. Chlorophenols in the tube were monitored over a 4-month period to measure PCP transformation in the soil. In less contaminated soil (10 mg PCP/kg soil) it was found that biostimulation were applied, but higher levels of glucose (2 g/kg soil) or inoculum (0.56 g VSS/kg soil) had little additional effect. The highest initial PCP removal rate reached 8.1 $\mu\text{mol/kg soil-d}$, which is almost 20 times greater than unamended controls. PCP mineralization approached 70% in 4 months. In highly PC-contaminated soil (90 mg PCP/kg soil), PCP degradation was partially inhibited, but the relative effects of augmentation, stimulation, and combined treatments were the same as the less contaminated soil.

Srivastava RK, Ayachi AK, Mish / Mona. (Environ Res Lab, Dept Bot Bnv Sci, Govt Autonomous Coil, Jabalpur 482001, MP). **Removal of chromium (VI) by utilization of bidi leaves.** Polln Res, 20(4) (2001), 639-643.

Conventional methods for the removal of Cr (VI) from waste water include chemical precipitation, ion exchange, electrolysis and adsorption by activated carbon. Owing to operational difficulties and the cost of the treatment, some new methods have been tried for a long time, on among them being adsorption on low cost adsorbents. In the present investigations the cut chips of bidi leaves has been used as these are produced as waste in bidi industries and doesn't find any specific uses except for dumping here and there.

Srivastava RK, Ayachi AK, Sehgal Vandana, Sen Anoop. (Environ Res Lab, Dept Bot Env Sci, Govt Sci Coil, Jabalpur MP). **Studies on the nitrate removal by water hyacinth and Ipomea leaves.** Plant Arch, 1 (1&2) (2001), 81-85.

Paper deals with the use of two Indian plants species i.e. water hyacinth and ipomea leaves for the removal of nitrate. Various parameters like contact time, temperature, pH, adsorbent dose and stirring time were taken to get maximum adsorption of nitrate on to water hyacinth and ipomea leaves. Both plants species were found to be efficient in removing nitrate from waste water and upto 98% removal was recorded.

Srivastava RK, Sehgal Vandane, Sen Anoop. (Environ Res Lab, Dept Bot Env Sci, Govt Autonomous Sci Coll, Jabalpur, MP). **Sorption studies on cadmium removal by Ipomea leaves.** Ecology & Environment Corner, 7(4) (2001), 373-377.

The removal of cadmium from waste water by adsorption on ipomea leaves was investigated to determine the effects of contact time, pH, quantity to adsorbent and temperature. The alkaline (pH 9) aqueous medium favoured the removal of cadmium by ipomea leaves. Batch adsorption experiments conducted at 30°C, 50°C, and 70°C, showed that adsorption of ipomea leaves increases when temperature is increased from 30° to 50°C and then decreases when temperature increases from 50° to 70°C. Low adsorbent dose gave better, results. The maximum removal of cadmium by ipomea leaves was upto 98%.

Steven D. Siciliano, James J. Germida, Kathy Banks, Charles W. Greer. (Environmental Microbiology Group, Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec. Department of Soil Science, University of Saskatchewan, Saskatchewan, Canada. School of Civil Engineering, Purdue University, West Lafayette, Indiana). **Changes in Microbial Community Composition and Function during a Polyaromatic Hydrocarbon Phytoremediation Field Trial.** Applied and Environmental Microbiology, 69(1) (2002), 483-489.

The purpose of this study was to investigate the mechanism by which phytoremediation systems promote hydrocarbon degradation in soil. The composition and degradation capacity of the bulk soil microbial community during the phytoremediation of soil contaminated with aged hydrocarbons was assessed. In the bulk soil, the level of catabolic genes involved in hydrocarbon degradation (*ndoB*, *alkB*, and *xylE*) as well as the mineralization of hexadecane and phenanthrene was higher in planted treatment cells than in treatment cells with no plants. There was no detectable shift in the 16S ribosomal DNA (rDNA) composition of the bulk soil community between treatments, but there were plant-specific and -selective effects on specific catabolic gene prevalence. Tail Fescue (*Festuca arundinacea*) increased the prevalence of *ndoB*, *alkB*, and *xylE* as well as naphthalene mineralization in rhizosphere soil compared to that in bulk soil. In contrast, Rose Clover (*Trifolium hirtum*) decreased catabolic gene prevalence and

naphthalene mineralization in rhizosphere soil. The results demonstrated that phytoremediation systems increase the catabolic potential of rhizosphere soil by altering the functional composition of the microbial community. This change in composition was not detectable by 16S rDNA but was linked to specific functional genotypes with relevance to petroleum hydrocarbon degradation.

T. Giblin, M. E. Losi, V. Hosangadi and W. T. Frankenberger, Jr. (Department of Natural Sciences, Stephens College, Columbia, MO, 65215, USA. Foster Wheeler Environmental Corporation, 1940 E. Deere Avenue, Santa Ana, CA 92705, USA). **Bacterial Perchlorate Reduction in Simulated Reverse Osmosis Rejectate**. *Bioremediation Journal*, 6(2) (2002), 105-111.

Reverse osmosis (RO) is capable of removing perchlorate (ClO_4^-) from contaminated groundwater and producing potable effluent; however, RO does not destroy ClO_4^- , but collects it in a concentrated waste stream (rejectate) that must be treated or disposed of appropriately. A packed bed bioreactor, inoculated with the pure culture perclace, was tested for its ability to remove ClO_4^- from a simulated RO rejectate. Perchlorate concentrations were lowered from 5 mg/L to <0.004 mg/L with a residence time of 0.8 h. In addition, this system removed 98% of ClO_4^- from a twice-concentrated rejectate with an influent ClO_4^- concentration of 8 mg/L and a residence time of 2.0 h. In both experiments, nitrate (NO_3^-) was removed simultaneously with ClO_4^- from an initial concentration as high as 900 mg/L NO_3^- to below 4 mg/L. Despite the efficiency of ClO_4^- removal, the system suffered from clogging due to the high total dissolved solids (TDS) of the twice-concentrated rejectate.

T. Macek, M. Macková and J. Káb. (Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague, Czech Republic. Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, ICT Prague, Technická 3, 166 28 Prague, Czech Republic). **Exploitation of plants for the removal of organics in environmental remediation**. *Biotechnology Advances*, 18(1) (2000), 23-34.

This review concentrates on the description of various phytoremediation technologies, paying special attention to removal of organics and the application of in vitro systems for basic research in the role of plants for the remediation of contaminated sites or flows, and in the improvement of their effectiveness. Various aspects of xenobiotic metabolism in plant cells, the role of enzymes involved, and the cooperation with rhizospheric microorganisms accelerating remediation of organics are shown. Application of this approach as well as the possibility of introduction of foreign genes into plant genome that can enhance the rate of the bioremediation are discussed.

Takao Raku, Masaru Kitagawa, Hiromi Shimakawa, Yutaka Tokiwa. (New Energy and Industrial Technology Development Organization (NEDO), Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan. Toyobo Research Center, Katata 2-1-1, Ohtsu, Shiga 520-0292, Japan. Konan Chemical Industry Co. Ltd., 5-21 Nakagawa-cho, Takatsuki, Osaka 569-0066, Japan. National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan). **Enzymatic synthesis of hydrophilic undecylenic acid sugar esters and their biodegradability**. *Biotechnology Letters*, 25(2) (2003), 161-166.

To enhance water solubility of 10-undecylenic acid, which has anti-fungus, anti-bacterial and anti-virus activity, d-glucose, trehalose and sucrose were regioselectively esterified with, vinyl 10undecylenic acid ester in dimethyl formamide by a commercial protease, Biopraser cone., from *Bacillus subtilis*. 6-O-(10-Undecylenoyl)d-glucose, 6-O-(10-

undecylenoyl) trehalose and 1'-O-(10- undecylenoyl) sucrose were obtained. The influence of structural variation by changing the sugar moiety was analyzed the surface tension and biodegradability.

Thomas D. DiStefano, Rishi Baral, Metin Duran and Richard E. Speech. (Bucknell University, Civil & Environmental Engineering Department, Lewisburg, Pennsylvania 17837, USA. Vanderbilt University, Department of Civil & Environmental Engineering, Nashville, TN 37240, USA). **A Comparison of Complex Electron Donors for Anaerobic Dechlorination of PCE.** *Bioremediation Journal*, 5(2) (2001), 131-143.

The potential of sugar, flour, corn steep liquor, molasses, non-fat milk, and whey to serve as electron donors for anaerobic dechlorination of tetrachloroethene (PCE) was examined. The electron donors were compared based on acclimation time, the extent of PCE dechlorination achieved, the minimum electron donor dose necessary to achieve PCE removal, and unit cost. The time required to achieve routine dechlorination of PCE (to any daughter product) for each donor was (in days): corn steep liquor (10), milk (10), whey (10), methanol (12), molasses (14), sugar (26), flour (30). Ethene production was achieved by milk-, whey-, and methanol-fed cultures, whereas the other donors did not facilitate ethane production over a 135-day period. Corn steep liquor-, whey-, molasses-, and sugar-fed cultures needed five times the stoichiometric amount (e.g., donor per eq PCE to ethene) to facilitate PCE conversion to dichloroethene (DCE). Cultures fed milk and flour needed 20 times the stoichiometric amount, and methanol-fed cultures required 50 times the stoichiometric amount, perhaps due to competition from methanogenic organisms. Minimum laboratory-scale electron donor costs to achieve stoichiometric conversion of PCE to DCE are (\$ per pound [lb] PCE) whey (0.04), molasses (0.07), milk (0.14), corn steep liquor (0.19), sugar (0.38), methanol (0.58), and flour (1.30).

Valeric Becaert, Maude Beaulieu, Josee Gagnon, Richard Villemur, Louise Deschenes and Rejean Samson. (NSERC Industrial Chair for Site Remediation and Management, Department of Chemical Engineering, Ecole Polytechnique, c.p. 6079, station centre-ville, Montreal, Quebec, Canada H3C 3A7. INRS, Institute Armand-Frappier-Microbiology and Biotechnology, 531 boul des Prairies, Laval, Quebec, Canada H7V 1B7). **Development of a Microbial Consortium from a Contaminated Soil That Degrades Pentachlorophenol and Wood-Preserving Oil.** *Bioremediation Journal*, 5(3) (2001), 183-192.

An indigenous microbial consortium capable of degrading pentachlorophenol (PCP) and petroleum hydrocarbons (C₁₀-C₅₀) was produced from a soil contaminated with wood-preserving oil. Two IO-L stainless steel soil slurry (10% w/v) bioreactors were operated in fed-batch mode. To verify the growth and efficiency of PCP degraders in the presence of other contaminants, one bioreactor was fed with a PCP-based wood-preserving mixture (WPM) and a second reactor was fed with technical-grade NaPCP. During the 90-day period of activation, PCP, C₁₀-C₅₀, Cl⁻, pH, and dissolved oxygen levels were monitored. The microbial community was monitored using specific most probably number (MPN) bacterial counts and mineralization tests. PCP degradation rates increased similarly in both reactors, from 19 to 132 mg/L-d in the NaPCP reactor, and from 41 to 112 mg/L-d in the WPM reactor. Contaminant loss calculations showed that 99.5% of PCP and 92.5% of C₁₀-C₅₀ added to the WPM reactor were biodegraded. It also revealed that 83% of polychlorinated dioxins and furans were removed. PCP-degrading bacteria increased from 7x10² to 1.6 x 10⁶ bacteria/mL in both reactors, and petroleum hydrocarbon degraders increased from 1.7x10² to 3.4 x 10⁸ bacteria/mL in the WPM reactor, indicating that the activity of PCP degraders was not inhibited by the presence of microorganisms growing on petroleum hydrocarbons.

Vasudevan N, Rajaram P. (Cent Environ Std, AnnaUniv, Chennai 600025). **Bioremediation of oil sludge-contaminated soil.** *Env Int*, 26(5-6) (2001), 409-411.

Experiments were undertaken for bioremediation of oil sludge- contaminated soil in the presence of a bacterial consortium, inorganic nutrients, compost and a hulking agent (wheat bran). Experiments were conducted in glass troughs for the 90-day period. Bulked soil showed more rapid degradation of oil compared to all other amendments. Addition of the bacterial consortium in different amendments significantly enhanced the removal of oil from the petroleum sludge from different treatment units.

Victor S. Magar, H. David Stensel, Jaakko Puhakka and John F. Ferguson. (Department of Civil and Environmental Engineering, 301 More Hall, University of Washington, Seattle, WA 98195-2700, USA. Institute of Water and Environmental Engineering, Tampere University of Technology, Tampere, Finland). **Characterization Studies of an Anaerobic, Pentachlorophenol-Dechlormating Enrichment Culture.** *Bioremediation Journal*, 4(4) (2000), 285-293.

Dechlorination studies were conducted using microbial cultures developed in a fluidized-bed reactor (FBR) that dechlorinates pentachlorophenol (PCP) to 3,4-dichlorophenol (3,4-DCP) and 4-monochlorophenol (4-MCP). Electron donor experiments demonstrated that lactate, propionate, and H₂ can serve as electron donors for chlorophenol (CP) dechlorination in mixed, anaerobic, PCP-enriched cultures. Dechlorination did not proceed in the absence of an electron donor. Acetate, which resulted in little H₂ production, was a poor electron donor. The results of inhibition studies using vancomycin and 2-bromoethanesulfonic acid implicate members of the domain bacteria in the dechlorination of CPs, whereas methanogens do not appear to be involved in dechlorination. Brief heat treatment (80°C for 90 min) of the FBR enrichment cultures implicated endospore formers in the dechlorination of CPs, primarily at the *ortho* position, where PCP was dechlorinated to 3,4,5-trichlorophenol (3,4,5-TCP) (the sole TCP detected) and subsequently to 3,4-DCP. Both lactate and H₂ served as electron donors in the heat-and oxygen-treated cultures. In contrast, a lactate-fed anaerobic spread-plate enrichment culture exhibited solely *meta*-dechlorination, where PCP dechlorinated solely to 2,4,6-TCP. The separation of *ortho*- and *meta*-specific dechlorination reactions provides evidence that PCP dechlorination in the FBR enrichment culture was catalyzed by at least the following two separate groups of CP-dechlorinating bacteria: one *meta*-dechlorinating group and one primarily *ortho*-dechlorinating group.

Walt W. McNab Jr. David W. Rice, Cary Tuckfield. (Environmental Restoration Division, Lawrence Livermore National Laboratory, 7000 East Avenue, L-530, Livermore, California 94551, USA. Savannah River Technology Center, Savannah River Site, Bidg. 773-42A, Aiken, SC 29808, USA). **Evaluating Chlorinated Hydrocarbon Plume Behavior Using Historical Case Population Analyses.** *Bioremediation Journal*, 4(4) (2000), 311-335.

A nationwide survey of chlorinated volatile organic compound (CVOC) plumes was conducted across a spectrum of sites from diverse hydrogeologic environments and contaminant release scenarios. The goal was to evaluate significant trends in the data that relate plume behavior to site variables (e.g., source strength, mean groundwater velocity, reductive dehalogenation regime) through correlation and population analyses. Data from 65 sites (government facilities, dry cleaners, landfills) were analyzed, yielding 247 individual CVOC plumes by compound. Data analyses revealed several trends, notably correlations between plume length and maximum observed concentration (presumably reflecting the source term) and mean groundwater velocities. Reductive dehalogenation, indicated by daughter products and groundwater geochemistry, appears

to exert a relatively subtle effect on plume length, apparent only after the contributions of source strength and groundwater velocity are factored out. CVOC properties (K_{oc} , Henry's Law constant) exert significant effects on variability in maximum observed concentrations between sites but hold little influence on plume length. Probabilistic plume modeling, entailing Monte Carlo simulation of an analytical solution for average plume behavior with parameter distributions derived from site data, was used to produce a synthetic plume set for comparison with field data. Modeling results exhibited good agreement with field data in terms of parameter sensitivity.

Wilfred F. M. Roling, Michael G. Milner, D. Martin Jones, Kenneth Lee, Fabien Daniel, Richard J.P. Swannell, and Ian M. Head. Fossil Fuels and Environmental Geochemistry and Centre for Molecular Ecology, University of Newcastle, Newcastle upon Tyne NE1 7RU, National Environment Technology Centre, AEA Technology, Abingdon OX14 3ED, National Environment Technology Centre, AEA Technology, Didcot, Oxfordshire OX11 0QJ, United Kingdom, Bedford Institute of Oceanography, Dartmouth, Nova Scotia B2Y 4A2, Canada). **Robust Hydrocarbon Degradation and Dynamics of Bacterial Communities during Nutrient-Enhanced Oil Spill Bioremediation.** Applied and Environmental Microbiology, 68(11) (2002), 5537-5548.

Degradation of oil on beaches is, in general, limited by the supply of inorganic nutrients. In order to obtain a more systematic understanding of the effects of nutrient addition on oil spill bioremediation, beach sediment microcosms contaminated with oil were treated with different levels of inorganic nutrients. Oil biodegradation was assessed respirometrically and on the basis of changes in oil composition. Bacterial communities were compared by numerical analysis of denaturing gradient gel electrophoresis (DGGE) profiles of PCR-amplified 16S rRNA genes and cloning and sequencing of PCR-amplified 16S rRNA genes. Nutrient amendment over a wide range of concentrations significantly improved oil degradation, confirming that N and P limited degradation over the concentration range tested. However, the extent and rate of oil degradation were similar for all microcosms, indicating that, in this experiment, it was the addition of inorganic nutrients rather than the precise amount that was most important operationally. Very different microbial communities were selected in all of the microcosms. Similarities between DGGE profiles of replicate samples from a single microcosm were high ($95\% \pm 5\%$), but similarities between DGGE profiles from replicate microcosms receiving the same level of inorganic nutrients ($68\% \pm 5\%$) were not significantly higher than those between microcosms subjected to different nutrient amendments ($63\% \pm 7\%$). Therefore, it is apparent that the different communities selected cannot be attributed to the level of inorganic nutrients present in different microcosms. Bioremediation treatments dramatically reduced the diversity of the bacterial community. The decrease in diversity could be accounted for by a strong selection for bacteria belonging to the alkane-degrading *Alcanivorax/Fundibacter* group. On the basis of Shannon-Weaver indices, rapid recovery of the bacterial community diversity to preoil levels of diversity occurred. However, although the overall diversity was similar, there were considerable qualitative differences in the community structure before and after the bioremediation treatments.

William A. Smith, William A. Apel, James N. Petersen and Brent M. Peyton. (Biotechnology Department, Idaho National Engineering and Environmental Laboratory, P. O. Box 1625, Idaho Falls, ID 83415-2203, USA. Center for Multiphase Environmental Research, Department of Chemical Engineering, Washington State University, P. O. Box 642719, Pullman, WA 99164-2719, USA). **Effect of Carbon and Energy Source on Bacterial Chromate Reduction.** Bioremediation Journal, 6(3) (2002), 205-215.

Studies were conducted to evaluate carbon and energy sources suitable to support hexavalent chromium (Cr(VI)) reduction by a bacterial consortium enriched from dichromate-contaminated aquifer sediments. The consortium was cultured under denitrifying conditions in a minimal, synthetic groundwater medium that was amended

with various individual potential carbon and energy sources. The effects of these individual carbon and energy sources on Cr(VI) reduction and growth were measured. The consortium was found to readily reduce Cr(VI) with sucrose, acetate, L-asparagine, hydrogen plus carbon dioxide, ethanol, glycerol, glycolate, propylene glycol, or D-xylose as a carbon and energy source. Minimal Cr(VI) reduction was observed when the consortium was cultured with citrate, 2-oxo-ketoglutarate, L-lactate, pyruvate, succinate, or thiosulfate plus carbon dioxide as a carbon and energy source when compared with abiotic controls. The consortium grew on all of the above carbon and energy sources, with the highest cell densities reached using D-xylose and sucrose, demonstrating that the consortium is metabolically diverse and can reduce Cr(VI) using a variety of different carbon and energy sources. The results suggest that the potential exists for the enrichment of Cr(VI)-reducing microbial populations *in situ* by the addition of a sucrose-containing feedstock such as molasses, which is an economical and readily available carbon and energy source.

William J. Hunter. **Bioremediation of Chlorate or Perchlorate Contaminated Water Using Permeable Barriers Containing Vegetable Oil.** *Curr Microbiol*, 45 (2002), 287-292.

A scale model of an *in situ* permeable barrier, formed by injecting vegetable oil onto laboratory soil columns, was used to remove chlorate and perchlorate from flowing groundwater. The hypothesis that trapped oil would serve as a substrate enabling native microorganisms to reduce chlorate or perchlorate to chloride as water flowed through the oil-rich zone had merit. Approximately 96% of the 0.2 mM chlorate and 99% of the 0.2 mM perchlorate present in the water was removed as water was pumped through columns containing vegetable oil barriers. The product formed was chloride. When nitrate at 1.4 mM was added to the water, both nitrate and chlorate were removed. High concentrations of chlorate or perchlorate can be treated; 24 mM chlorate and 6 mM perchlorate were completely reduced to chloride during microcosm incubations. Microorganisms capable of reducing perchlorate are plentiful in the environment.

Y. Iimura, S. Ikeda, T. Sonoki, T. Hayakawa, S. Kajita, K. Kimbara, K. Tatsumi, Y. Katayama. (Institute for Environmental Management Technology, National Institute of Advanced Industrial Science and Technology, 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan. Department of Environment Symbiotic Production System, Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan. Environmental Engineering Division, Railway Technical Research Institute, Tokyo 185-8540, Japan). **Expression of a gene for Mn-peroxidase from *Coriolus versicolor* in transgenic tobacco generates potential tools for phytoremediation.** *Applied Microbiology and Biotechnology*, 59(2-3) (2002), 246-251.

In efforts aimed at the detoxification of contaminated areas, plants have many advantages over bacteria and fungi. We are attempting to enhance the environmental decontamination functions of plants by transferring relevant genes from microorganisms. When the gene for Mn-peroxidase (MnP) from *Coriolus versicolor* was expressed in transgenic tobacco plants, one line (designated fMnP21) expressed MnP activity at levels 54-fold higher than in control lines. When undamaged roots of transgenic plants were applied to liquid medium supplemented with 250 µM pentachlorophenol (PCP), the decrease in the level of PCP in fMnP21 (86% reduction) was about 2-fold higher than that in control lines (38% reduction). Expression of the gene for MnP in the transgenic plants had no obvious negative effects on their vegetative and sexual growth. Our system should contribute to the development of novel methods for the removal of hazardous chemicals from contaminated environments using transgenic plants.

Biosensor

C. Y. Shao, C. J. Howe, A. J. R. Porter, L. A. Glover. (Department of Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD,¹ Department of Biochemistry, University of Cambridge, Cambridge CB2 1QN, United Kingdom²). **Novel Cyanobacterial Biosensor for Detection of Herbicides.** Applied and Environmental Microbiology, 68(10) (2002), 5026-5033.

The aim of this work was to generate a cyanobacterial biosensor that could be used to detect herbicides and other environmental pollutants. A representative freshwater cyanobacterium, *Synechocystis* sp. strain PCC6803, was chromosomally marked with the luciferase gene *luc* (from the firefly *Photinus pyralis*) to create a novel bioluminescent cyanobacterial strain. Successful expression of the *luc* gene during growth of *Synechocystis* sp. strain PCC6803 cultures was characterized by measuring optical density and bioluminescence. Bioluminescence was optimized with regard to uptake of the luciferase substrate, luciferin, and the physiology of the cyanobacterium. Bioassays demonstrated that a novel luminescent cyanobacterial biosensor has been developed which responded to a range of compounds including different herbicide types and other toxins. This biosensor is expected to provide new opportunities for the rapid screening of environmental samples or for the investigation of potential environmental damage.

K. B. Riether, M. A. Dollard, P. Billard. (U.R. Ecotoxicité, Biodiversité, Santé Environnementale, Université de Metz, Campus Bridoux - Rue du Général Delestraint, 57070 Metz Borny, France). **Assessment of heavy metal bioavailability using *Escherichia coli zntAp::lux* and *copAp::lux*-based biosensors.** Applied Microbiology and Biotechnology, 57(5-6) (2001), 712-716.

To determine the amount of metals detectable by bacteria, two plasmids were constructed in which the metal-inducible *zntA* and *copA* promoters from *Escherichia coli* were fused to a promoterless *Vibrio fischeri luxCDABE* operon. The luminescence response of *E. coli* bearing these constructs was studied as a function of the concentration of several heavy metals and was shown to be influenced by cell growth phase. The *zntAp::lux* fusion is induced mainly by salts of cadmium, lead, mercury and zinc, with significant induction by other metal ions, whereas the specificity of *copA* induction is restricted to copper and silver. In optimized assay conditions, metals could be detected at threshold concentrations ranging from nanomolar to micromolar, with maximal induction observed after only 60-100 min incubation. The ability of these biosensor strains to distinguish bioavailable quantities of metals in a sample makes them good candidates as useful tools to monitor metal contamination in environmental samples.

Michal Koblížek, Jan Malý, Jiří Masojídek, Josef Komenda, Tomáš Kučera, Maria T. Giardi, Autar K. Mattoo, Roberto Pilloton. (Photosynthesis Research Center, Institute of Microbiology, 379 81 Třeboň, Czech Republic. Photosynthesis Research Center, Institute of Landscape Ecology, Nový Zámek 136, 373 33 Nové Hradky, Czech Republic. Faculty of Education, University of J.E. Purkyně, 400 96 Ústí n. Labem, Czech Republic. Department of Biochemistry, Charles University, Hlavova 2030, 128 40 Prague, Czech Republic. IBEV-CNR, Via Salaria Km 29.3, 00016 Monterotondo Scalo, Italy Vegetable Laboratory, USDA/ARS/BARC, Building 010A, Beltsville, Maryland 20705. Biotechnology and Agriculture Div., ENEA, Casaccia, Via Anguillarese 301, 00060 Rome, Italy). **A biosensor for the detection of triazine and phenylurea herbicides designed using Photosystem II coupled to a screen-printed electrode.** Biotechnology and Bioengineering, 78(1) (2002), 110-116.

A biosensor for the detection of triazine- and phenylurea-type herbicides was constructed using isolated Photosystem II (PS II) complexes as a biosensing element. PSII isolated from the thermophilic cyanobacterium *Synechococcus elongatus* was immobilized on the surface of a screen-printed sensor composed of a graphite working electrode and Ag/AgCl reference electrode deposited on a polymeric substrate. The biosensor was mounted in a flow microcell with illumination. The principle of the detection was based on the fact that herbicides selectively block PSII electron transport activity in a concentration-dependent manner. Changes of the activity were registered amperometrically as the rate of photoreduction of an artificial electron acceptor. The setup resulted in a reusable herbicide biosensor with a good stability (half-life of 24 h) and limit of detection of approximately 10^{-9} M for diuron, atrazine and simazine.

P.V Preejith, C.S Lim, A. Kishen, M.S John, A. Asundi. (School of Mechanical and Production Engineering, c/o School of Mechanical and Production Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798. Biomedical Engineering Research Centre, c/o School of Mechanical and Production Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798). **Total protein measurement using a fiber-optic evanescent wave-based biosensor.** *Biotechnology Letters*, 25(2) (2003), 105-110.

A novel method and instrumental system to determine the total protein concentration in a liquid sample is described. It uses a fiber optic total protein sensor (FOPS) based on the principles of fiber optic evanescent wave spectroscopy. The FOPS applies a dye-immobilized porous glass coating on a multi-mode optical fiber. The evanescent waves at the fiber optic core-cladding interface are used to monitor the protein-induced changes in the sensor element. The response time and reusability of the FOPS are evaluated. This unique sensing method presents a sensitive and accurate platform for the quantification of protein.

Raz Jelinek, and Sofiya Kolusheva. (Department of Chemistry, Stadler Minerva Center for Mesoscopic Macromolecular Engineering, Ben Gurion University of the Negev, Beersheba 84105, Israel). **Polymerized lipid vesicles as colorimetric biosensors for biotechnological applications.** *Biotechnology Advances*, 19(2) (2001), 109-118.

Supramolecular chemical assemblies composed of polydiacetylene (PDA) exhibit rapid colorimetric transitions upon specific interactions with a variety of biological analytes in aqueous solutions. Among the analytes that give rise to the unique blue-red color changes are lipophilic enzymes, antibacterial peptides, ions, antibodies, and membrane penetration enhancers. The chemical assemblies include conjugated PDA, responsible for the chromatic transitions, and the molecular recognition elements, which are either chemically or physically associated with the PDA. Thus, by incorporation of specific recognition elements, the system can be designed in ways allowing for highly selective identification of analytes. In particular, receptors and epitopes can be incorporated within the sensor assembly, which then determine the specificity of the colorimetric transitions. The PDA-based molecular assemblies are robust and can be readily applied to diagnosis of physiological molecules and for rapid screening of chemical and biological libraries, for example, in 96 well-plate platforms.

Rikke Louise Meyer, Lars Hauer Larsen, Niels Peter Revsbech. (Unisense A/S, Science Park, Aarhus, Department of Microbial Ecology, University of Aarhus, 8000 Aarhus C, Denmark¹). **Microscale Biosensor for Measurement of Volatile Fatty Acids in Anoxic Environments.** *Applied and Environmental Microbiology*, 68(3) (2002), 1204-1210.

A microscale biosensor for acetate, propionate, isobutyrate, and lactate is described. The sensor is based on the bacterial respiration of low-molecular-weight, negatively charged species with a concomitant reduction of NO_3^- to N_2O . A culture of denitrifying bacteria deficient in N_2O reductase was immobilized in front of the tip of an electrochemical N_2O microsensor. The bacteria were separated from the outside environment by an ion-permeable membrane and supplied with nutrients (except for electron donors) from a medium reservoir behind the N_2O sensor. The signal of the sensor, which corresponded to the rate of N_2O production, was proportional to the supply of the electron donor to the bacterial mass. The selectivity for volatile fatty acids compared to other organic compounds was increased by selectively enhancing the transport of negatively charged compounds into the sensor by electrophoretic migration (electrophoretic sensitivity control). The sensor was susceptible to interference from O_2 , N_2O , NO_2^- , H_2S , and NO_3^- . Interference from NO_3^- was low and could be quantified and accounted for. The detection limit was equivalent to about 1 μM acetate, and the 90% response time was 30 to 90 s. The response of the sensor was not affected by changes in pH between 5.5 and 9 and was also unaffected by changes in salinity in the range of 2 to 32%. The functioning of the sensor over a temperature span of 7 to 30°C was investigated. The concentration range for a linear response was increased five times by increasing the temperature from 7 to 19.5°C. The life span of the biosensor varied between 1 and 3 weeks after manufacturing.

Ryoichi Asai, Chikashi Nakamura, Kazunori Ikebukuro, Isao Karube, Jun Miyake. (Tissue Engineering Research Center, National Institute of Industrial Science and Technology, 1-1-4 Higashi, Tsukuba, Ibaragi 305-8562, Japan Research Centre for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan. Research Centre for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan. Research Centre for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan. Tissue Engineering Research Center, National Institute of Industrial Science and Technology, 1-1-4 Higashi, Tsukuba, Ibaragi 305-8562, Japan). **Detection technique of asymmetric RT-PCR-based amplified single-stranded DNA and its application to biosensor for detection of mRNA for cyanobacteria, *Anabaena variabilis*.** Biotechnology Letters, 24(20) (2002), 1677-1682.

A real-time detection technique for mRNA was developed using a specific DNA probe. The design of the probe was based on a sequence polymorphism within the expressed mRNA of *Anabaena variabilis* PCC 7120 by using the BIAcore 2000 biosensor, which uses surface plasmon resonance (SPR). The reverse transcription polymerase chain reaction (RT-PCR)-amplified single-stranded DNA (ssDNA), specific for ribonuclease P RNA B (*rnpB*) from *A. variabilis*, was determined by an SPR biosensor. Through these detection methods, the ssDNA amplified from *rnpB* could be detected within 10 min after asymmetric RT-PCR.

Willem Haasnoot, Elma E. M. G. Loomans, Geert Cazemier, Richard Dietrich, Ron Verheijen, Aldert A. Bergwerff, Rainer W. Stephany. **Direct Versus Competitive Biosensor Immunoassays for the Detection of (Dihydro)Streptomycin Residues in Milk.** Food and Agricultural Immunology, 14(1) (2002), 15-27.

A monoclonal antibody (MAb) against dihydrostreptomycin (4G8) was developed and its performance compared with a previously developed MAb against streptomycin (4E2) in biosensor immunoassays (BIAs) using a surface plasmon resonance (SPR)-based biosensor (BIACORE 3000). Direct BIAs for the detection of dihydrostreptomycin (DHS; 583 Da) and streptomycin (STREP; 581 Da) were developed by immobilising the MAbs on the sensor chip (CM5). These direct BIAs were compared with competitive inhibition

BIAs, using a STREP- protein conjugate immobilized on the chip. The sensitivities of the direct and competitive BIAs for both drugs in buffer were comparable ($10\text{--}20\text{ ng ml}^{-1}$ at 50% binding or inhibition). With milk, interferences, probably due to the nonspecific binding of proteins to the sensor chips, were observed in both BIAs. These interferences could be largely reduced using ultra filtration (UF) as sample pre-treatment. Another option was the use of a reference flow channel to correct for nonspecific binding. Using this option with five times diluted milk, MAb 4G8 was found to be suited for the direct BIA of both drugs with a limit of detection (LOD) of 20 ng ml^{-1} and both MAbs could be applied in the competitive BIA format with similar LODs.

Yan Y. Goh,¹ Bow Ho,² and Jeak L. Ding¹. (Department of Biological Sciences,¹ Department of Microbiology, National University of Singapore, Singapore 1175432). **A Novel Fluorescent Protein-Based Biosensor for Gram-Negative Bacteria**. Applied and Environmental Microbiology, 68(12) (2002), 6343-6352.

Site-directed mutagenesis of enhanced green fluorescent protein (EGFP) based on rational computational design was performed to create a fluorescence-based biosensor for endotoxin and gram-negative bacteria. EGFP mutants (EGFP_i) bearing one (G10) or two (G12) strands of endotoxin binding motifs were constructed and expressed in an *Escherichia coli* host. The EGFP_i proteins were purified and tested for their efficacy as a novel fluorescent biosensor. After efficient removal of lipopolysaccharide from the *E. coli* lysates, the binding affinities of the EGFP_i G10 and G12 to lipid A were established. The K_D values of $7.16 \times 10^{-7}\text{ M}$ for G10 and $8.15 \times 10^{-8}\text{ M}$ for G12 were achieved. With high affinity being maintained over a wide range of pH and ionic strength, the binding of lipid A/lipopolysaccharide to the EGFP_i biosensors could be measured as a concentration-dependent fluorescence quenching of the EGFP mutants. The EGFP_i specifically tagged gram-negative bacteria like *E. coli* and *Pseudomonas aeruginosa*, as well as other gram-negative bacteria in contaminated water sampled from the environment. This dual function of the EGFP_i in detecting both free endotoxin and live gram-negative bacteria forms the basis of the development of a novel fluorescent biosensor.

Biotechnology – Agricultural Issue

D P Patil, MV Kulkarni, V L Maheshwari, R M Kothari. (School of Life Sciences, North Maharashtra University, P.B. 80, Jalgaon 425 001, India). **A Sustainable Agro-biotechnology for Bioremediation of Saline Soil**. Journal of Scientific & Industrial Research, 51 (2002), 517-528.

To restore fertility and productivity of saline soil through bioremediation, a farm-scale trial was undertaken by exploring the effect of three factors, viz. soil conditioner (SC, recycled agrowaste), halophiles culture, and a plant growth regulator (PGR), modified industrial byproduct. A three factor factorial design was used-with each factor at two levels – the lower level indicated no treatment, while the upper level indicated treatment, and there were eight experiments in all, which were replicated thrice. One out of the eight treatment combinations was without SC, PGR, or-halophiles inputs served as control. The plantation and growth of *Casuarina equisetifolia*, in the treated soils as per the design, was monitored through various relevant parameters that included soil characteristics, level of (micro) nutrients, and exchangeable cations, and growth-related parameters. The analysis of data thus generated through appropriate ANOVA indicated an overwhelming role of SC in the bioremediation of soil salinity and growth parameters of the *C equisetifolia* plantation, followed by that of halophiles and then the PGR as envisaged. The role of PGR was however important in first establishing the plantation in the saline soil, when halophiles get enough time to establish favourable pattern and concentration of microbes in soil by up to more than a million times of the

initial microflora level of 5.9×10^3 /g soil. Data collected up to six months clearly indicated bioremediation of the otherwise saline soil, and effectiveness of the given treatments was evident. It is proposed that such bio-measures may go along way in converting large tracts of saline soils into fertile ones with healthy soil-microflora in an eco-friendly manner which is cost-effective as well.

H. C. Sharma, K. K. Sharma, N. Seetharama and Rodomiro Ortiz. **Genetic transformation of crop plants: Risks and opportunities for the rural poor.** Current Science, 80(12) (2001), 1495.

The world population is increasing at an alarming rate and is expected to increase from 6.5 billion at present to 7.5 billion by 2025. Most of this population lives in the rural areas in the developing countries where poverty, food insecurity and nutritional deficiencies are the major problems. Low crop productivity, limited use of inputs such as fertilizers and pesticides and losses due to biotic and abiotic stress factors are a major constraint to increase production and productivity of crops. With the advent of genetic engineering, it has become possible to clone and insert genes into the crop plants to confer resistance to insect pests and improve the nutritional quality. Genetically transformed crops with *Bacillus thuringiensis* and herbicide resistance genes have been deployed for cultivation in USA, Canada, China and Australia. However, very little has been done to use this technology for improving crop production in the harsh environments of the tropics, where the need for increasing food production is most urgent. However, there is a need to follow the biosafety regulations and a better presentation of the results to the general public for a rational deployment of the genetically transformed crops for improving the livelihoods of the rural poor.

Jeffrey Burkhardt. (Institute of Food and Agricultural Sciences, University of Florida, Gainesville FL 32611, USA). **Agricultural Biotechnology and the Future Benefits Argument.** Journal of Agricultural and Environmental Ethics, 14(2) (2001), 135-145.

In the face of criticisms about the current generation of agricultural biotechnology products, some proponents of agricultural biotechnology offer a "future benefits argument" (FBA), which is a utilitarian ethical argument that attempts to justify continued R&D. This paper analyzes several logical implications of the FBA. Among these are that acceptance of the FBA implies (1) acceptance of a precautionary approach to risk, (2) the need for a more proportional and equitable distribution of the benefits of agricultural biotechnology, and most important, (3) the need to reorient and restructure biotechnology R&D institutions (and the agricultural biotechnology community's values and attitudes) so that future benefits are indeed achieved through agricultural biotechnology.

Keith Douglass Warner. (Department of Environmental Studies, University of California Santa Cruz, Santa Cruz, CA 95064, USA). **Are Life Patents Ethical? Conflict between Catholic Social Teaching and Agricultural Biotechnology's Patent Regime.** Journal of Agricultural and Environmental Ethics, 14(3) (2001), 301-319.

Patents for genetic material in the industrialized North have expanded significantly over the past twenty years, playing a crucial role in the current configuration of the agricultural biotechnology industries, and raising significant ethical issues. Patents have been claimed for genes, gene sequences, engineered crop species, and the technical processes to engineer them. Most critics have addressed the human and ecosystem health implications of genetically engineered crops, but these broad patents raise economic issues as well. The Catholic social teaching tradition offers guidelines for critiquing the economic implications of this new patent regime. The Catholic principle of

the universal destination of goods implies that genes, gene sequences, and engineered crop varieties are ineligible for patent protection, although the processes to engineer these should be eligible. Religious leaders are likely to make a more substantive contribution to debates about agricultural biotechnology by addressing these life patents than by speculating that genetic engineering is "playing God".

M.H. EI-Masry, A.I. Khalil, M.S. Hassouna, H.A.H. Ibrahim. (Department of Bioscience and Technology, Institute of Graduate Studies and Research, University of Alexandria, 163 El-Horreya Avenue, El-Shatby, Alexandria 21526, Egypt. Department of Environmental Studies, Institute of Graduate Studies and Research, University of Alexandria, 163 El-Horreya Avenue, El-Shatb, Alexandria 21526, Egypt). ***In situ and in vitro suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi.*** World Journal of Microbiology and Biotechnology, 18(6) (2002), 551-558.

In situ and *in vitro* experiments were carried out to determine the effect of various composts (leafy fruit compost (LFC), garden compost (GC), and crops compost (CC)) and their water extract on *Pythium debaryanum*, *Fusarium oxysporum f. sp. lycopersici*, *Sclerotium bataticola*. Compost water extract (CWE) of LPC, GC, and CC were found to contain *Bacillus* spp., *Micrococcus* spp., *Staphylococcus* spp. and *Corynebacterium* spp., and the fungi *Aspergillus* spp., *Rhizopus* spp., and *Drechslera* spp., and various *Actinomycetes*. *In situ* results indicated considerable decrease in fungal growth around the unautoclaved compost especially in the case of *S. bataticola* and *F. oxysporum f.sp. lycopersici*, compared to the autoclaved compost. *In vitro* tests showed that concentration of CWE at 5, 10 and 15% (v/v) suppressed the hyphal growth of *S. bataticola* by 83% using 5% CC and by 94.4% using 5% LFC or 10% GC, and *F. oxysporum f.sp. lycopersici* by 94.4% using either composts. CWE of GC decreased fungal dry weight of *F. oxysporum f.sp. lycopersici* by 97.7%, *P. debaryanum* by 92.8%, and *S. bataticola* by 84.4%; CC decreased *F. oxysporum f.sp. lycopersici* by 94%, *P. debaryanum* by 86.2%, and *S. bataticola* by 63.3%, while CWE of LFC was the least effective against the tested fungi. CWE produced clear inhibition zones against all the tested fungi. Microflora found in CWE have an important role in suppressing the growth of tested fungi. CWE contained neither antibiotics nor siderophores. The presence of protease, chitinase, lipase and 2-1,3 glucanase (lysogenic enzymes) in CWE indicates a possible role in fungal degradation.

Parvinder Chawla. (National Institute of Science Communication (CSIR), New Delhi). **'Agricultural Biotechnology'- Challenges in the new millennium.** Science and Culture, (2002), 170.

Rapid advances in molecular biology have paved the way for new and better varieties of crop plants. The infusion of science and technology in agricultural practices has tremendously enhanced the yield of several useful crops needed not just for sustaining the fast growing human populations but also important for economic progress of the world nations. Further increase in yield and production of various crops is envisaged as improved technologies for crop management are developed. Agricultural productivity is much dependent on integrated systems of management of water, soil moisture and soil fertility besides selective plant breeding which are all essential for improved crop yield and further, developing diseases resistance and stress tolerance in the crop. Exactly alike copies of valuable plants can be therefore produced by employing the tissue culture method. This technique is immensely useful in enhancing the yield of several agricultural products. Plant modeling is yet another revolutionary development in the world agricultural science made in recent years. Scientists at the Plant Agriculture Modelling Laboratory, France are already working on mathematical models of some plants. Notwithstanding the limitations and many challenges that the agricultural scientists

worldwide might have to face in the 21st century, it is for certain that biotechnological advances have a rich store of promises for the agricultural sector assuring a fast economic progress for all the world nations.

Sarit Shalhevet, Nava Haruvyb, Ishai Spharima. (Department of Economics, Agricultural Research Organization, P.O. Box 6, Bet Dagan 50250, Israel. Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, P.O. Box 6, Bet Dagan 50250, Israel). **Management strategies for agricultural biotechnology in small countries-A case study of Israel.** *Biotechnology Advances*, 19(7), (2001), 539-554.

Agricultural biotechnology is concentrated in four major countries. This paper suggests strategies for developing it in small countries, based on analysis of the world trends and the characteristics of small countries. Israel is presented as a specific case study. The main relevant trends are domination by big companies, consumer concerns on genetically modified foods, and focusing on consumer benefits and specific market niches. Small countries' disadvantages include companies that are too small to benefit fully from research, difficulty in raising funds, lack of infrastructures and experienced management personnel, and public sector research organizations that are unsuitable for commercializing research. The recommended strategies include: developing a large number of low-volume products and small market niches, forming partnerships with intermediaries (such as food companies), specializing in intermediate products (such as the seed or the gene patent), and conducting market research and cost-benefit analysis in advance. Additional strategies include developing benefits that are unique to genetically modified foods and focusing on benefits specifically for consumers who accept genetically modified foods, rather than on benefits for the average consumer. A national representative organization could buy and rent out expensive equipment, finance specific projects in return for the commercial rights, and perform collective marketing research and marketing. Israel has the advantages of a successful agricultural sector and complementary scientific research, and should focus on those fruits, vegetables, and flowers for which it already has the experience and infrastructure.

Biotechnology Policy Issue

Donald M. Bruce. (Society, Religion and Technology Project, Church of Scotland, John Knox House, 45 High Street, Edinburgh, Scotland EH1 1SR, UK). **A Social Contract for Biotechnology: Shared Visions for Risky Technologies?** *Journal of Agricultural and Environmental Ethics*, 15(3) (2002), 279-289.

Future technological developments concerning food, agriculture, and the environment face a gulf of social legitimation from a skeptical public and media, in the wake of the crises of BSE, GM food, and foot and mouth disease in the UK (House of Lords, 2000). Key ethical issues were ignored by the bioindustry, regulators, and the Government, leaving a legacy of distrust. The paper examines agricultural biotechnology in terms of a social contract, whose conditions would have to be fulfilled to gain acceptance of novel applications. Various current and future GM applications are evaluated against these conditions. Success would depend critically on how far a shared vision can be found with the public. To re-establish trust, significant changes are identified in the planning and pursuit of biotechnology.

Ilan Levy and Oded Shoseyov. (The Institute of Plant Science and Genetics in Agriculture and The Otto Warburg Centre for Agricultural Biotechnology, The Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, PO Box

12, Rehovot 76100, Israel). **Cellulose-binding domains-Biotechnological applications.** *Biotechnology Advances*, 20(3-4) (2002), 191-213.

Many researchers have acknowledged the fact that there exists an immense potential for the application of the cellulose-binding domains (CBDs) in the field of biotechnology. This becomes apparent when the phrase "cellulose-binding domain" is used as the key word for a computerized patent search; more than 150 hits are retrieved. Cellulose is an ideal matrix for large-scale affinity purification procedures. This chemically inert matrix has excellent physical properties as well as low affinity for nonspecific protein binding. It is available in a diverse range of forms and sizes, is pharmaceutically safe, and relatively inexpensive. Present studies into the application of CBDs in industry have established that they can be applied in the modification of physical and chemical properties of composite materials and the development of modified materials with improved properties. In agro-biotechnology, CBDs can be used to modify polysaccharide materials both in vivo and in vitro. The CBDs exert nonhydrolytic fiber disruption on cellulose-containing materials. The potential applications of "CBD technology" range from modulating the architecture of individual cells to the modification of an entire organism. Expressing these genes under specific promoters and using appropriate trafficking signals, can be used to alter the nutritional value and texture of agricultural crops and their final products.

Joseph H Hulse. (Siemens Hulse IDA, Inc, Ottawa, K1H 6P2 Canada). **Ethical issues in biotechnologies and international trade.** *Journal of Chemical Technology & Biotechnology*, 77(5) (2002), 607-615.

Natural and physical sciences are based on determinable facts. What is ethical, as distinct from illegal, is largely a matter of opinion. Scientific and industrial activities related to ancient and modern biotechnologies are among the most critically scrutinised for ethical probity by social activists and journalists. The practices and products of biotechnologies should be judged both deontologically - by motivation and intention, and teleologically - by determinable consequence. Bioethical criteria have been proposed by governments, medical practitioners and philosophers for many centuries. During the past decade, various scientifically competent organisations, national and international, have formulated comprehensive protocols by which to determine effectiveness and safety of novel foods, pharmaceuticals and other biologicals, including those derived from genetically modified organisms. Means and opportunities by which to satisfy the health and nutritional needs of impoverished nations and communities differ significantly from those who enjoy greater affluence. It is distinctly unethical for Europeans and North Americans, whose food and health securities are not at risk, to impose their ethical predilections on poorer nations. Equally reprehensible are the diverse tariff and non-tariff barriers to equitable international trade, and acts of biopiracy inflicted upon poorer nations. As a wise Asian sage has observed, the planet's resources and scientific ingenuity are sufficient to satisfy everyone's need, but not everyone's greed. Present and predictable world-wide demand for bioscientists and bioengineers exceeds best estimates of supply. Systematically planned, long-term investments by governments and bioindustries to generate adequate qualified men and women are urgently needed.

K. Soyez, S. Plickert. **Mechanical-Biological Pre-Treatment of Waste: State of the Art and Potentials of Biotechnology.** *Acta Biotechnol*, 22(3-4) (2002), 271-284.

Mechanical-biological treatment of waste (MBP) is the processing or conversion of waste from human settlements with biologically degradable components via a combination of mechanical, other physical processes and biological processes. It is a technological alternative to waste incineration. It is applicable for the treatment of waste prior to depositing, but also for the production of refuse derived fuels (RDF). A capacity of about

2 million tons has been established in Europe over the fast ten years, and a broad technological variety including aerobic and anaerobic bioprocesses is available. The process design is mainly empirical, but biotechnological information is widely used. The following figures characterise the process results: Up to 95% of the degradable TOC and 94% resp. 86% of non-cellulosic carbohydrates resp. cellulose are metabolized. The stability of the treated waste is defined by a respiratory coefficient (AT4) or a gas production coefficient (GB 21); typical process results on the technical scale are 5 mg/g dry matter for AT4 and 20 l/kg dry matter for GB 21. Emissions from the processes include organic compounds metabolized or generated by bioprocesses, such as methane and carbon dioxide, as well as volatile organics, which are stripped out from the waste. A treatment by biofilters results in a 20% to 50% reduction; after a further treatment by incineration a value <55 g TOC per ton of waste is achieved. The level of contaminants in both leachate and gas emissions from the landfill is reduced up to 95% compared with untreated waste. One kilogram of treated waste potentially releases a total load of 1-3 g COD, 0.5-1.5 g TOC and 0.1-0.2 g NH₄-N into the leachate.

Lyle M. Vernell, David A. Evans, Kirk J. Havens. (Office of Research and Advisory Services and Wetlands Program, Center for Coastal Resources Management, Virginia Institute of Marine Science, College of William and Mary, Rt. 1208, Create Road, Gloucester Point, VA 23062, USA. Department of Coastal and Ocean Policy, Virginia Institute of Marine Science, College of William and Mary, Rt. 1208, Greate Road, Gloucester Point, VA 23062, USA. Wetlands Program, Center for Coastal Resources Management, Virginia Institute of Marine Science, College of William and Mary, Rt. 1208, Greate Road, Gloucester Point, VA 23062, USA). **A geomorphological model of Intertidal cave marshes with application to wetlands management.** Ecological Engineering, 19(5) (2003), 339-347.

Detailed topographic and hydrologic surveys were conducted in five intertidal cove marshes in an outer coastal plain landscape to test the hypothesis that the equilibrium geologic state of intertidal habitats residing in similar landscape situations conforms to a consistent geometric form. The equation $V=1571.84A^{1.70}$ ($R^2=96.2\%$) describes the relationship between hectares of marsh (A) and cubic meter volume at mean high tide (V). An empirical relationship between tide height and volume was found to obey the power series $V_p=L^{2.38}$ ($R^2=99.6\%$), where V_p is volume as a percent of full pool and L is water height as a percent of mean high tide. A dimensionless index describing the relationship between area and volume is consistent for each marsh and approaches 0.10. A channel form parameter describing width to channel depth ratios is of consistent value for four of the five marshes. These provide evidence of deterministic rather than stochastic geologic development. The benefits of applying natural basin shape patterns in the design and engineering of created/restored intertidal marshes are highlighted and a generic basin is modeled (based on the geometrical section of a paraboloid retained by ample integration) as an example of the potential applicability of the study.

M. Dua, A. Singh, N. Sethunathan, A. K. Johri. (Department of Zoology, University of Delhi, Delhi 110007, India. Department of Biology, University of Waterloo, Waterloo, N2L 3G1, Ontario, Canada. CSIRO Land and Water, PMB 2, Glen Osmond, SA 5064, Australia. Channing Laboratory, Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, USA. Department of Biomedical Research, Division of Hematology/Oncology, St. Elizabeth Medical Center, School of Medicine, Tufts University, Boston, MA 02135, USA). **Biotechnology and bioremediation: successes and limitations.** Applied Microbiology and Biotechnology, 59(2-3) (2002), 143-152.

With advances in biotechnology, bioremediation has become one of the most rapidly developing fields of environmental restoration, utilizing microorganisms to reduce the concentration and toxicity of various chemical pollutants, such as petroleum

hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters, nitroaromatic compounds, industrial solvents, pesticides and metals. A number of bioremediation strategies have been developed to treat contaminated wastes and sites. Selecting the most appropriate strategy to treat a specific site can be guided by considering three basic principles: the amenability of the pollutant to biological transformation to less toxic products (biochemistry), the accessibility of the contaminant to microorganisms (bioavailability) and the opportunity for optimization of biological activity (bioactivity). Recent advances in the molecular genetics of biodegradation and studies on enzyme-tailoring and DNA-shuffling are discussed in this paper.

Manju Sharma, K. S. Charak and T. V. Ramanaiah. (Department of Biotechnology, Block-2, CGO Complex, New Delhi 1 10 003, India). **Agricultural biotechnology research in India:status and policies**. Current Science, 84(3) (2003), 297.

Agriculture is a way of life for more than sixty per cent of India's population. The cultivation of land not only sustains their livelihood but also provides a social milieu for their day-to-day living. No wonder the hopes, despairs, joys and sorrows of rural communities are woven around what the land provides. Around 35 years ago, agricultural production in India got a major boost with the introduction of dwarf varieties of wheat and rice. The introduction of these varieties led to a dramatic increase in the yields of the two crops. Some productivity enhancement came through the use of hybrids in corn, sorghum and millet although the area under cultivation of these crops has steadily decreased in the last decade. In the last 10 years the yields of rice and wheat have also plateaued out. The productive agricultural areas in the North, due to continuous rice-wheat cultivation are encountering serious problems of sub-soil water depletion, deficiency of micronutrients in the soil and increase in the use of pesticides, fungicides and herbicides to control pests, pathogens and persistent weeds. Agricultural production is becoming more and more dependent on agrochemicals, thereby increasing input costs and causing significant damage to the environment and human health. Both farmers and consumers are at the receiving end -farmers by exposure to agrochemicals and consumers due to residues of agrochemicals in the consumed food. While there is self-sufficiency in cereal grains at present, the yields and productivity of dryland crops, mostly grain legumes and oilseeds remain low and no major breakthroughs in productivity enhancement and yield stabilization have been achieved. Currently, India is importing both grain legumes and edible oils to meet internal demands. India's population is expected to reach approximately 1.5 billion by 2050. It is estimated that around 300 million (roughly 30%) of India's population is suffering from malnutrition. Due to socio-economic reasons, women and children are more at the receiving end in terms of micronutrient deficiency and low calorie intake. Nutritional security for everyone would require more extensive availability of grain legumes, edible oils, fruits and vegetables, milk and poultry products. The challenges of malnutrition, enhanced productivity and crop diversification can be met by better resource management and by breeding more productive, more nutritious and at the same time less resource input demanding crops. Crop biotechnology, which broadly includes areas of development of transgenic crops, structural and functional genomics and marker-assisted breeding could provide us with the vital breakthroughs to achieve improvements in both quality and quantity in a sustainable manner. With the advent of techniques of genetic engineering in the early seventies, the natural barrier to gene exchange has been removed. Sequences from varied sources like bacteria, viruses and eukaryotic systems can be transferred to plants to develop transgenic crop varieties. Achievements, to date, in plant biotechnology have surpassed all previous expectations and with the development of high throughput instruments, the future is even more promising. In this article we outline the contributions made by the Department of Biotechnology in promoting research and development in the area of crop biotechnology and in establishing proper regulatory regimes for transgenic crops.

Nicole C. Karafyllis. (Goethe University Frankfurt am Main, Institute of Polytechnics (PO Box 248), Division of Philosophy of Technology, Department of Social Studies, D-60054 Frankfurt am Main, Germany). **Renewable Resources and the Idea of Nature – What Has Biotechnology Got to Do With It?** *Journal of Agricultural and Environmental Ethics*, 16(1) (2003), 3-28.

The notion that the idea of nature is not quite the unbiased rule to design sustainable futures is obvious. But, nevertheless, questions about nature, how it functions and what it might aim at, is leading the controversial debates about both sustainability and biotechnology. These two research areas hardly have the same theory background. Whereas in the first concept, the idea of eternal cyclical processes is basic, the latter focuses on optimization. However, both concepts can work together, but only under a narrow range of public acceptance in Europe. The plausibility of arguments for using biotechnology within sustainable technologies varies according to the assumed part nature itself plays for reaching optimized states. The culture related vision of nature's functions has impact on agricultural biotechnology, dealing not only with food crops but also with non-food plants like renewable resources that are used for energy or fiber production. These plants are grown to reach sustainable development. However, there is a fundamental difference between regarding biofuels as "renewable" and "regenerative," due to the tension between the concepts of "the natural" and "the sustainable". Arguments of optimization, efficiency, and efficacy are critically discussed in order to take the present need for sustainable technologies for serious.

Rita R. Colwell. (US National Science Foundation, 4201 Wilson Boulevard, Suite 1205, Arlington, VA 22230, USA). **Fulfilling the promise of biotechnology.** *Biotechnology Advances*, 20(3-4) (2002), 215-228.

Genetic engineering has produced pharmaceuticals, disease-resistant plants, cloned animals and research and industrial products. While the comparably mature field of medical biotechnology now reveals its true potential, marine biotechnology is still in the realm of the future. As we explore the earth for new sources of natural chemicals, we now search the waters. Myriad organisms, most unknown to us, live there. Many produce compounds that can be commercialized, or the organisms themselves may be commercialized, through genetic engineering methods. For decades, scientists studied the ocean depths searching for unique molecules and organisms. But not until the early 1980s was there a synthesis uniting marine natural products, ecology, aquaculture and bioremediation research under the heading of marine biotechnology. As harvesting enough products from marine sources to produce sufficient amounts, even for study, is nearly impossible, we need to use genomics techniques to identify biologically active compounds. As we damage our oceanic ecosystems through pollution, overfishing and destructive fishing methods, opportunities to learn more about marine organisms and their commercial potential may be limited. Although governments and intergovernmental agencies are committed to funding and expanding oceanic research, more funding is needed to discover and study the ocean's vast, unplumbed resources.

Th. Bley, S. Muller. **How Should Microbial Life be Quantified to Optimise Bioprocesses.** *Acta Biotechnol*, 22(3-4) (2002), 401-409.

Quantification of microbial life is necessary for optimizing bioprocesses. The scientific background of biologists, chemists and engineers, who are engaged in this field, is very specific and therefore the approaches to quantification are extremely different as well. The roots for quantification and *the* constraints of microbial performance are discussed, and 'it is shown that only a holistic approach, the approach of systems biology, can lead to satisfactory results. The essential contribution of Wolfgang Babel to this holistic

approach is emphasized.

Biotransformation

A. Amanullah, C. J. Hewitt, A. W. Nienow, C. Lee, M. Chartrain, B. C. Buckland, S. W. Drew, J. M. Woodley. (Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London W1CE 7JE, United Kingdom. Centre for Bioprocess Engineering, School of Chemical Engineering, The University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom. Department of Bioprocess Research and Development, Merck Research Laboratories, Merck & Co. Inc., Rahway, New Jersey). **Application of multi-parameter flow cytometry using fluorescent probes to study substrate toxicity in the indene bioconversion.** *Biotechnology and Bioengineering*, 80(3) (2002), 239-249.

The bioconversion of indene to *cis*-(1S,2R) indandiol, a potential key intermediate in the synthesis of Merck's HIV protease inhibitor, CRIXIVAN™, can be achieved using a *Rhodococcus* strain. This study using *Rhodococcus* I24 reports on the application of multiparameter flow cytometry for the measurement of cell physiological properties based on cytoplasmic membrane (CM) integrity and membrane depolarization as indicators of toxic effects of the substrate, indene. Quantification of intact polarized CM, intact depolarized CM and permeabilized CM of a large population of bacterial cells has been conducted using specific intracellular and membrane-binding fluorescent stains. Measurements of oxygen uptake rate (OUR) and optical density (OD) as indicators of metabolic activity and biomass growth, respectively, were also made. Indene concentrations of up to 0.25 g/L (0.037 g indene/g dry cell weight) did not significantly (<5% compared to control) affect cell light-scattering properties, intact CM, membrane polarization, respiratory activity, or biomass growth. Between this value and 1.5 g/L (0.221 g indene/g dry cell weight), the changes in intact CM, respiratory activity and biomass growth were relatively insignificant (<5% compared to control), although dissipation of the membrane potential of a significant proportion of the cell population occurred at 0.50 g/L (0.074 g indene/g dry cell weight). At 2.5 g/L (0.368 g indene/g dry cell weight) there was a significant increase in the dead cell population, accompanied by changes in the extracellular cationic concentrations and substantial decrease in respiratory activity. The primary effect of indene toxicity was the disruption of the proton motive force across the cytoplasmic membrane which drives the formation of ATP. The disruption of the proton motive force may have been due to the measured changes in proton permeability across the membrane. In addition, indene may have directly inhibited the membrane-bound enzymes related to respiratory activity. The overall consequence of this was reduced respiratory activity and biomass growth. The cell physiological properties measured via flow cytometry are important for understanding the effects of toxicity at the cellular level which neither measurements of biomass growth or indandiol formation rates can provide since both are cell averaged measurements. The technique described here can also be used as a generic tool for measuring cell membrane properties in response to toxicity of other indene-resistant strains that may be possible to use as recombinant hosts to perform the biotransformation of indene. This study has demonstrated that flow cytometry is a powerful tool for the measurement of cell physiological properties to assess solvent toxicity on whole cell biocatalysts.

A. Amanullah, C. J. Hewitt, A. W. Nienow, C. Lee, M. Chartrain, B. C. Buckland, S. W. Drew, J. M. Woodley. (Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London W1CE 7JE, United Kingdom. Centre for Bioprocess Engineering, School of Chemical Engineering, The University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom. Department of Bioprocess Research and Development, Merck Research Laboratories, Merck & Co. Inc., Rahway, New Jersey 07065). **Measurement of strain-dependent toxicity in the indene bioconversion using multiparameter flow cytometry.** *Biotechnology and Bioengineering*, 81(4) (2003), 405-420.

The bioconversion of indene to *cis*-(1*S*,2*R*)-indandiol, a potential key intermediate in the synthesis of Merck's HIV protease inhibitor, CRIVAN™, can be achieved using *Rhodococcus*, *Pseudomonas putida*, and *Escherichia coli* strains. This study reports on the application of multiparameter flow cytometry for the measurement of cytoplasmic membrane integrity and membrane depolarization as indicators of toxic effects of the substrate, product, and by-products using each of these strains. Measurements of oxygen uptake rate (OUR) and optical density (OD) as indicators of metabolic activity and biomass growth, respectively, were also made. Measurements of the cytoplasmic membrane potential, cell viability, and respiratory activity provided a sensitive set of parameters to assess toxicity in the indene bioconversion and provided the basis for process improvements and strain selection. The toxic concentrations of the substrate, product, and by-products for each strain have been determined. The results show that it is possible to accumulate *cis*-(1*S*,2*R*)-indandiol and *cis*-1-amino-2-indanol up to 20 g/L without significant negative effects on cell physiology using any of the strains tested. The Gram-negative *P. putida* (421-5 and GM 730) and *E. coli* strains were more resistant to indene and the isolated chemicals of the biotransformation than the Gram-positive *Rhodococcus* I24 strain, possibly due to the presence of the outer membrane and efflux pump mechanisms. *P. putida* GM 730 and the *E. coli* TDO 123 strains responded similarly to toxic effects, and the *E. coli* TDO 123 strain was more resistant than the *P. putida* 421-5 strain. In addition to the recommendations for strain selection, the identified targets for bioprocess improvement include a combination of genetic as well as process engineering approaches.

Archana Giria, Vikas Dhingra, C. C. Giri¹, Ajay Singh^c, Owen P. Ward^c and M. Lakshmi Narasu. (Centre for Biotechnology, Institute of PG Studies and Research, Jawaharlal Nehru Technological University, Mahaveer Marg, Hyderabad 500 028, India. Centre for Plant Molecular Biology, Department of Genetics, Osmania University, Hyderabad 500 007, India. Department of Biology, University of Waterloo, Waterloo, ON, Canada N2L 3G1). **Biotransformations using plant cells, organ cultures and enzyme systems: current trends and future prospects.** *Biotechnology Advances*, 19(3) (2001), 175-199.

Plants are valuable sources of a variety of chemicals including drugs, flavours, pigments and agrochemicals. Some of the biochemical reactions occurring in plant cells are complex and cannot be achieved by synthetic routes. In vitro plant cell and organ cultures and plant enzymes act as suitable biocatalysts to perform these complex reactions. A wide variety of chemical compounds including aromatics, steroids, alkaloids, coumarins and terpenoids can undergo biotransformations using plant cells, organ cultures and enzymes. The biocatalyst-mediated reactions are regiospecific and stereospecific. Reaction types include oxidations, reductions, hydroxylations, methylations, acetylations, isomerizations, glycosylations and esterifications. Genetic manipulation approaches to biotransformation offer great potential to express heterologous genes and to clone and overexpress genes for key enzymes. Biotransformation efficiencies can further be improved using molecular techniques involving site-directed mutagenesis and gene manipulation for substrate specificity.

B. Junker, A. Seeley, M. Lester, M. Kovatch, J. Schmitt, S. Boryscwicz, J. Lynch, J. Zhang, R. Greasham. (Fermentation Pilot Plant Operations, Bioprocess R&D, P.O. Box 2000, Merck and Co., Inc., Rahway, New Jersey 07065, USA). **Use of frozen bagged seed inoculum for secondary metabolite and bioconversion processes at the pilot scale.** *Biotechnology and Bioengineering*, 79(6) (2002), 628-640.

Frozen bagged seed inoculum was prepared, thawed and tested for seven cultures. Thawing techniques were developed and other key influences on thawing rate were quantified; seed bag thawing without a water bath rarely required more than 4 to 5 h and was as short as 0.5 to 1 h for lower fill volume bags. Testing included growth of bagged seed as a function of bag fill volume (0.5, 1.0, 2.0, and 3.5 L), comparison of culture age at time of bagging, growth of bagged versus laboratory-prepared seed, productivity of production cultures derived from bagged versus laboratory-prepared seed, growth of bagged seed as a function of volume percent glycerol added at time of bagging, and growth of bagged seed as a function of frozen storage time and temperature. For each culture tested, conditions were developed such that seed tanks inoculated with bagged seed showed only minimal delay in attaining the target oxygen uptake rate (OUR) relative to seed tanks inoculated with laboratory-prepared inoculum. Although the bag fill volume did influence culture growth in some cases, bag fill volumes required were reasonable (typically 2.0 to 3.5 L) compared with laboratory seed inoculum volumes of 2.0 L. In the most remarkable example, frozen bagged seed was prepared from a second-stage seed-tank cultivation of *Glarca lozoyensis*, then thawed and inoculated into first-stage seed medium. It grew to the desired OUR in a similar timeframe as laboratory-prepared inoculum inoculated into first-stage seed medium. Thus, the frozen bagged seed replaced an existing laboratory inoculum preparation period of 7 days without an appreciable delay in either of the two subsequent seed-tank growth stages. Furthermore, productivities were found to be comparable for bagged-seed-derived and laboratory-seed-derived production cultivations for four different fermentation processes.

Corey W Radtke, R. Michael Lehman and Francisco F. Roberto. (Biotechnology Department, Idaho National Engineering and Environmental Laboratory, P.O. Box 1625, Mailstop 2203, Idaho Falls, Idaho 83415, USA). **Increased Biotransformation Efficiency of Chunk-TNT-Contaminated Soil Using Acetone Pretreatments.** *Bioremediation Journal*, 4(1) (2000), 57-67.

Particulate, or chunk 2,4,6-trinitrotoluene (TNT), in soil was found to be recalcitrant to composting down to particle sizes of approximately 2 mm. Evidence for the colonization of TNT surfaces was obtained, but no pitting or otherwise preferential solid TNT solubilization was observed. Acetone pretreatments were used to make the chunk-TNT-contaminated soil more amenable to bioremediation. A pretreatment of acetone slurring to dissolve and redisperse solid TNT in soil before applying remedial treatments was developed. The well-described treatment of composting was subsequently applied to native and acetone-pretreated contaminated soils. Acetone-pretreated soil responded to composting significantly better than untreated soil. After evaporating off the acetone used as pretreatment, composting microcosms held at 55°C showed sporadic removal from 3000 ppm to 300 ppm TNT in 24 days for untreated soil, while pretreated soil demonstrated conclusive removal from 3000 ppm to 18.1 ppm TNT in 6 days. Separate results indicated that residual acetone from pretreatment without subsequent evaporation was found to delay, but not otherwise inhibit, the compost's ability to degrade TNT. Community level physiological profile testing of 13-day-old composts, with pretreatment and residual acetone, suggests that three significantly different microbiological compost communities were equally adept at degrading the repartitioned TNT. The superior removal rates and efficiencies in the acetone-pretreated systems are likely to be due to the increased availability of TNT to the necessary microflora.

E. Hammer, L. Schoefer, A. Schäfer, K. Hundt, Schauer. (Institut für Mikrobiologie, Ernst-Moritz-Arndt-Universität Greifswald, F.-L.-Jahn-Strasse 15, 17487 Greifswald, Germany. Deutsches Institut für Ernährungsforschung, Artur-Scheunert-Allee 114-116, 14558 Potsdam-Rehbrücke, Germany. Plasma Select, Robert-Koch-Strasse 1, 17166 Teterow, Germany). **Formation of glucoside conjugates during biotransformation of dibenzofuran by *Penicillium canescens* SBUG-M 1139.** Applied Microbiology and Biotechnology, 57(3) (2001), 390-394.

Penicillium canescens oxidises dibenzofuran (DBF) to produce monohydroxylated derivatives and other more hydrophilic metabolites. These substances are water-soluble but unstable in organic solvents such as ethyl acetate, acetone or dichloromethane. Both extraction with ethyl acetate and enzymatic treatment of the aqueous culture filtrate with β -glucuronidase led to decay of the hydrophilic metabolites and indicated these products to be glycoside conjugates. The glycosyl residue was identified as glucose both by liquid chromatography and by the use of glucose oxidase. The conjugate pattern formed was the same in type and amount, independent of the carbon source used for cultivation of the fungus. Clearly, DBF transformation in *P. canescens* occurred in two phases: first the conversion to 2-, 3-, and 4-hydroxydibenzofuran (phase I), followed by the formation of the corresponding glucosyl conjugates (phase II). In contrast, 2,3-dihydroxydibenzofuran added to the cultures was transformed by ring cleavage producing a muconic acid-like dead-end product.

Hyo-Bong Hong, Yoon-Seok Chang, In-Hyun Nam, Peter Fortnagel, and Stefan Schmidt. (School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang 790-784, Korea, Institut für Allgemeine Botanik der Universität Hamburg, Abteilung für Mikrobiologie, D-22609 Hamburg, Germany). **Biotransformation of 2,7-Dichloro- and 1,2,3,4-Tetrachlorodibenzo-*p*-Dioxin by *Sphingomonas wittichii* RW1.** Applied and Environmental Microbiology, 68(5) (2002), 2584-2588.

Aerobic biotransformation of the diaryl ethers 2,7-dichlorodibenzo-*p*-dioxin and 1,2,3,4-tetrachlorodibenzo-*p*-dioxin by the dibenzo-*p*-dioxin-utilizing strain *Sphingomonas wittichii* RW1, producing corresponding metabolites, was demonstrated for the first time. Our strain transformed 2,7-dichlorodibenzo-*p*-dioxin, yielding 4-chlorocatechol, and 1,2,3,4-tetrachlorodibenzo-*p*-dioxin, producing 3,4,5,6-tetrachlorocatechol and 2-methoxy-3,4,5,6-tetrachlorophenol; all of these compounds were unequivocally identified by mass spectrometry both before and after *N,O*-bis(trimethylsilyl)-trifluoroacetamide derivatization by comparison with authentic standards. Additional experiments showed that strain RW1 formed a second metabolite, 2-methoxy-3,4,5,6-tetrachlorophenol, from the original degradation product, 3,4,5,6-tetrachlorocatechol, by methylation of one of the two hydroxy substituents.

I. Estrada Alvarado, A. Lomascolo, D. Navarro, M. Delattre, M. Asther, L. Lesage-Meessen. (Unité INRA de Biotechnologie des Champignons Filamenteux, IFR de Biotechnologie Agro-Industrielle de Marseille, Universités de Provence et de la Méditerranée, ESIL, 163 Avenue de Lunimy, CP 925, 13288 Marseille Cedex 09, France). **Evidence of a new biotransformation pathway of *p*-coumaric acid into *p*-hydroxybenzaldehyde in *Pycnoporus cinnabarinus*.** Applied Microbiology and Biotechnology, 57(5-6) (2001), 725-730.

Pycnoporus cinnabarinus MUCL39533 was shown to be able to convert *p*-coumaric acid into *p*-hydroxybenzaldehyde, a component of high organoleptic note present in natural vanilla aroma. Use of phospholipid-enriched medium led to high-density cultures of *P.*

cinnabarinus, since dry mycelial biomass was increased three-fold as compared to glucose medium. In the presence of phospholipids, 155 mg l⁻¹ *p*-hydroxybenzaldehyde was produced as the major compound on culture day 13 with a molar yield of 26%. The degradation pathways of *p*-coumaric acid were investigated. Based on the different metabolites identified, an oxidative side-chain degradation pathway of *p*-coumaric acid conversion to *p*-hydroxybenzoic acid was suggested. This acid was further reduced to *p*-hydroxybenzaldehyde and *p*-hydroxybenzyl alcohol, or hydroxylated and reduced to protocatechyl derivatives. Additionally, a reductive pathway of *p*-coumaric acid with 3-(4-hydroxyphenyl)-propanol as the terminal product occurred.

Jian-Shen Zhao, Owen P. Ward, Piotr Lubicki, James D. Cross, Peter Huck. (Department of Biology, University of Waterloo, Ontario, N2L 3G1, Canada. Department of Electrical and Computer Engineering, University of Waterloo, Ontario, N2L 3G1, Canada. Department of Civil Engineering, University of Waterloo, Ontario, N2L 3G1, Canada). **Process for degradation of nitrobenzene: Combining electron beam irradiation with biotransformation.** *Biotechnology and Bioengineering*, 73(4) (2001), 306-312.

Electron beam irradiations of aqueous solutions containing 15-30 mg/L of nitrobenzene at 60 kGy dose removed 78% of the contaminant. Three mononitrophenols were detected as by-products of electron beam treatment of nitrobenzene. A mixed culture enriched on a mixture of 2-, 3-, and 4-nitrophenol degraded both the residual nitrobenzene and the nitrophenol products. Percentage removal of nitrobenzene increased with increasing electron beam dose. This observation led to the conceptual design of a two-stage electron beam microbial process for degradation of nitrobenzene. Three groups of pure isolates were characterized from the mixed culture based on their abilities to grow on corresponding nitrophenol substrates: Group A, 2NP⁻3NP⁻4NP⁺; Group B, 2NP⁺3NP⁺4NP⁻; and Group C, 2NP⁻3NP⁺4NP⁻. Bacteria that grew on 3-NP transformed nitrobenzene into ammonia in the electron beam-treated nitrobenzene samples.

Jörg Overhage, Alexander Steinbüchel, Horst Priefert. (Institut für Mikrobiologie der Westfälischen Wilhelms-Universität Münster, D-48149 Münster, Germany). **Biotransformation of Eugenol to Ferulic Acid by a Recombinant Strain of *Ralstonia eutropha* H16.** *Applied and Environmental Microbiology*, 68(9) (2002), 4315-4321.

The gene loci *ehyAB*, *calA*, and *calB*, encoding eugenol hydroxylase, coniferyl alcohol dehydrogenase, and coniferyl aldehyde dehydrogenase, respectively, which are involved in the first steps of eugenol catabolism in *Pseudomonas* sp. strain HR199, were amplified by PCR and combined to construct a catabolic gene cassette. This gene cassette was cloned in the newly designed broad-host-range vector pBBR1-JO2 (pBBR1-JO2*ehyABcalAcalB*) and transferred to *Ralstonia eutropha* H16. A recombinant strain of *R. eutropha* H16 harboring this plasmid expressed functionally active eugenol hydroxylase, coniferyl alcohol dehydrogenase, and coniferyl aldehyde dehydrogenase. Cells of *R. eutropha* H16(pBBR1-JO2*ehyABcalAcalB*) from the late-exponential growth phase were used as biocatalysts for the biotransformation of eugenol to ferulic acid. A maximum conversion rate of 2.9 mmol of eugenol per h per liter of culture was achieved with a yield of 93.8 mol% of ferulic acid from eugenol within 20 h, without further optimization.

K. M. Lai, M. D. Scrimshaw, and J. N. Lester. (Environmental Processes and Water Technology Group, Department of Environmental Science and Technology, Imperial College of Science, Technology and Medicine, London SW7 2BP, United Kingdom). **Biotransformation and Bioconcentration of Steroid Estrogens by *Chlorella***

vulgaris. Applied and Environmental Microbiology, 68(2) (2002), 859-864.

The biotransformation and bioconcentration of natural and synthetic steroid estrogens by *Chlorella vulgaris* were investigated by using batch-shaking experiments with incubation for 48 h in the light or dark. Estradiol and estrone were interconvertible in both light and dark conditions; however, this biotransformation showed a preference for estrone. In the light, 50% estradiol was further metabolized to an unknown product. Apart from biotransformation, estrone, as well as hydroxyestrone, estriol, and ethinylestradiol, was relatively stable in the algal culture, whereas estradiol valerate was hydrolyzed to estradiol and then to estrone within 3 h of incubation. All of the tested estrogens exhibited a degree of partitioning to *C. vulgaris*; however, the concentrations of estriol, hydroxyestrone, ethinylestradiol, and estradiol valerate were always below the quantification limits. For estradiol and estrone, the partitioning of these estrogens in the algal extracts to the filtrates was <6% of the total amount present. The average concentration factor for estrone was ca. 27; however, the concentration factor for estradiol was not reported since no equilibrium was reached between the aqueous solution and that within the cells due to continuing biotransformation.

K. Thomas Klassonl, Eric M. Just. (Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6226, USA). **Computer Tool For Evaluation of Anaerobic Microbial PCB Transformations**. Bioremediation Journal, 6(1) (2002), 77-85.

Several researchers have demonstrated the transformation of polychlorinated biphenyls (PCBs) by both aerobic and anaerobic bacteria. This transformation, or conversion, is characteristic and often dependent on PCB congener structure and, in addition, dictates the products or extent of degradation. Because transformation is linked to microbial activities, bioremediation has been hailed as a possible solution for PCB-contaminated soils and sediments, and several demonstration activities have verified laboratory results. This article presents results from mathematical modeling of anaerobic microbial PCB transformation. Because transformation can be influenced by both starting composition of the PCBs and microbial activity, this article systematically evaluates several of the most common transformation patterns. The predicted data are also compared with experimental results. For example, the correlation between laboratory-observed and predicted products was, in some cases, as good as 0.96 (perfect correlation = 1.0). In addition to predicting extent of transformation, the water solubility and the possible human effects of the PCBs are discussed through the use of documented dioxin-like toxicity and accumulation in humans before and after transformation.

Keshetty Srisilam and Ciddi Veeresham. (Faculty of Pharmaceutical Sciences, Kakatiya University, Warangal 506 009, India). **Biotransformation of drugs by microbial cultures for predicting mammalian drug metabolism**. Biotechnology Advances, 21(1) (2003), 3-39.

This review discusses the microbial transformation studies of drugs, correlating them with the corresponding metabolism (biotransformation) in animal systems. Approaches are provided for development of microbial models for mammalian metabolism. Emphasis is placed on the potential of microorganisms to mimic mammalian metabolism and provide ways for structural elucidation and toxicological and pharmacological studies of metabolites. Microorganisms can provide difficult-to-synthesize drugs and assist in identifying metabolic pathways of drugs.

L. Serrano-Carreón, K. Balderas-Ruíz, E. Galindo¹ and M. Rito-Palomares. (Instituto de Biotecnología, Depto. de Bioingeniería, Universidad Nacional Autónoma de México, Av. Universidad 2001, Col. Chamilpa, Cuernavaca, 62271, Morelos, México. Centro de Biotecnología, Instituto Tecnológico y de Estudios Superiores de Monterrey, Av. Eugenio Garza Sada 2501-Sur, Monterrey, 64849, Nuevo León, México). **Production and biotransformation of 6-pentyl-pyrone by *Trichoderma harzianum* in two-phase culture systems.** Applied Microbiology and Biotechnology, 58(2) (2002), 170-174.

The final concentration of 6-pentyl-pyrone (6PP) produced in cultures of *Trichoderma* spp. is limited by the fact that inhibition of biomass growth occurs at 6PP concentrations as low as 100 mg/l. The aim of this work was to evaluate liquid-liquid extractive fermentation systems as an alternative to overcome the toxicity problems and to increase the production of 6PP by this fungus. Two alkanes (n-decane and n-hexadecane) and two dicarboxylic esters (dibutyl phthalate and dioctyl phthalate) were evaluated in shake flask cultures. The highest 6PP production (173 ppm) was achieved when n-hexadecane was used, being 3.5-fold the maximum 6PP concentration of a culture without the solvent. Cultivation of *Trichoderma harzianum* in a 10-l bioreactor with n-hexadecane yielded 6PP production ninefold higher than that from control cultures. However, 6PP production in the bioreactor (83 ppm) was lower than in shake flasks. Differences in the power drawn to the fluid at each scale could account for such behavior. Even in the presence of the solvent, 6PP content decreased after reaching its maximal concentration.

M. Bertau. (Institut für Biochemie, Technische Universität Dresden, D-01062 Dresden, Germany). **How Cell Physiology Affects Enantioselectivity of the Biotransformation of Ethyl 4-chloro-acetoacetate with *Saccharomyces cerevisiae*.** Biocatalysis and Biotransformation, 20(5) (2002), 363 – 367.

Saccharomyces cerevisiae (baker's yeast) reduces ethyl 4-chloro-acetoacetate enantioselectively to (R)- or (S)-ethyl 4-chloro-3-hydroxybutyrate depending on the reaction conditions and the physiological state of the yeast cells. The (S)-enantiomer is obtained under batch conditions with resting cells (55%, enantiomeric excess [ee]), and 4-chloro-acetate fed-batch actively metabolising yeast affords the (R)-isomer (54%, ee). The enantioselective reduction of the substrate is accompanied by competing enzyme actions. Of the metabolites formed from the substrate, chloroacetone and the target compound (R)-ethyl 4-chloro-3-hydroxybutyrate emerged as most important effectors of enantioselectivity of the microbial reduction. As a minor side-reaction, an aerobic reductive dehalogenation of the substrate was observed. The unusual high enantiopurity of the dehalo-product (S)-ethyl 3-hydroxybutyrate confirms the stereodirecting effect of chloroacetone impressively. Hence, with *S. cerevisiae* either enantiomer can be obtained by variation of reaction conditions. The yeast further turned out to be a promising biocatalyst for dehalogenations.

M. Bramucci, M. Singh, V. Nagarajan. (Central Research and Development, DuPont Company, P.O. Box 80328, Wilmington, DE 19880-0328, USA). **Biotransformation of *p*-xylene and 2,6-dimethylnaphthalene by xylene monooxygenase cloned from a *Sphingomonas* isolate.** Applied Microbiology and Biotechnology, 59(6) (2002), 679-684.

Sphingomonas strain ASU1 was isolated from an industrial wastewater bioreactor and grew on 2,6-dimethylnaphthalene (2,6-DMN) as the sole carbon/energy source. The genes for a xylene monooxygenase were cloned from strain ASU1. Expression of the ASU1 xylene monooxygenase was compared to expression of the pWVO xylene monooxygenase in *Escherichia coli*. Both monooxygenases transformed *p*-xylene and 2,6-DMN by initially hydroxylating one methyl group. In addition, the ASU1

monooxygenase also hydroxylated the second methyl group on *p*-xylene and 2,6-DMN whereas the pWVO monooxygenase hydroxylated the second methyl group only on *p*-xylene. Endogenous *E. coli* enzymes contributed to further oxidation of the resulting aromatic alcohols to form aromatic carboxylates.

M.D. Salokhe, S.P. Govindwar. (Department of Biochemistry, Shivaji University, Kolhapur 416 004, India). **Inducibility of biotransformation enzymes in *Serratia marcescens***. World Journal of Microbiology and Biotechnology, 19(2) (2003), 199-200.

A significant increase in the levels of electron transport components and biotransformation enzymes was observed when cells of *Serratia marcescens* were incubated for 3 h or cells grown for 8 h (exponential growth phase) in synthetic medium containing either 1.0% ethanol or 5.0% veratrole when compared to peptone-glucose medium. Phenobarbital was found to be a poor inducer of monooxygenase.

Mitsuo Miyazawa, Hideki Kawazoe, Mitsuro Hyakumachi. (Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Kowakae, Higashiosaka-shi, Osaka 577-8502, Japan. Laboratory of Plant Disease, Faculty of Agriculture, Gifu University, 1-1, Yanagido, Gifu, 501-1112, Japan). **Biotransformation of *l*-menthol by soil-borne plant pathogenic fungi (*Rhizoctonia solani*)**. Journal of Chemical Technology & Biotechnology, 77(1) (2002), 21-24.

The biotransformation of *l*-menthol was investigated by using nine isolates of *Rhizoctonia solani* (AG-1-IA Rs24, Joichi-2, RRG97-1; AG-1-IB TR22, R147, 110.4; AG-1-IC F-1, F-4 and P-1) as a biocatalyst. In the cases of *Rhizoctonia solani* F-1, F-4 and P-1, almost all of the substrate was consumed in 3 days and the major metabolite increased rapidly for the first of 3 days incubation. The structure of the major metabolite was elucidated on the basis of its spectral data. The major metabolite was determined to be (-)-(1*S*,3*R*,4*S*,6*S*)-6-hydroxymenthol which indicated that *l*-menthol was hydroxylated at the C-6 position. From the main component analysis, the nine isolates of *Rhizoctonia solani* were divided into two groups based on their ability to transform *l*-menthol to (-)-(1*S*,3*R*,4*S*,6*S*)-6-hydroxymenthol. This is the first report on the biotransformation of *l*-menthol by *Rhizoctonia solani*.

Mitsuo Miyazawa, Yoshiki Miyasato. (Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Kowakae, Higashiosaka, Osaka 577-8502, Japan). **Biotransformation of (+)- and (-)-bornyl acetate using the plant parasitic fungus *Glomerella cingulata* as a biocatalyst**. Journal of Chemical Technology & Biotechnology, 76(2) (2001), 220-224.

The microbial transformations of (+)- and (-)-bornyl acetate were investigated using the plant parasitic fungus, *Glomerella cingulata*. As a result, (+)- and (-)-bornyl acetate were converted to (+)- and (-)-5-*exo*-hydroxybornyl acetate, (+)- and (-)-5-oxobornyl acetate and (+)- and (-)-borneol respectively. The structures of the metabolic products were determined by spectroscopic data.

R. Saini, S.S. Kanwar, O.P. Sharma, M.K. Gupta. (Department of Microbiology, College of Basic Sciences, CSK Himachal Pradesh Agricultural University, Palampur 176 062, India. Biochemistry Laboratory, Indian Veterinary Research Institute, Regional Station, Kangra Valley, Himachal Pradesh, Palampur 176 061, India). **Biomethanation of Lantana weed and biotransformation of its toxins**. World Journal of Microbiology and Biotechnology, 19(2) (2003) 209-213.

The utility of *Lantana camara* as a substrate for biogas production and the fate of its toxins after biomethanation process was studied. Both fresh and predigested Lantana leaves along with cattle dung were subjected to anaerobic batch digestion for a period of 50 days. Fresh Lantana did not produce any biogas. However, predigested Lantana did produce biogas but only up to a concentration of 50% (w/w, on dry weight basis). Both, the quantity and quality of biogas was better when cattle dung was supplemented with predigested Lantana. Biotransformation of Lantana toxins (lantadenes) during the biomethanation process was noticed.

Sarah J. Todd, Ronald B. Cain, Stefan Schmidt. (Author for correspondence Department of Biological and Nutritional Science, The University of Newcastle, Agriculture Building, Kings Walk, Newcastle upon Tyne, NE1 7RU, UK Abteilung für Mikrobiologie, Institut für Allgemeine Botanik der Universität Hamburg, Ohnhörstraße 18, D-22609 Hamburg, Germany. Abteilung für Mikrobiologie, Institut für Allgemeine Botanik der Universität Hamburg, Ohnhörstraße 18, D-22609 Hamburg, Germany). **Biotransformation of naphthalene and diaryl ethers by green microalgae.** Biodegradation, 13(4) (2002), 229-238.

The role of green microalgae in the biotransformation of naphthalene (a polycyclic aromatic hydrocarbon) and diaryl ethers was investigated using axenic cultures of *Chlorella vulgaris* and two environmental isolates, *Scenedesmus* S11 and *Ankistrodesmus* S12. Biotransformation experiments with dense cell cultures showed that these green algae transformed toxic xenobiotics to more polar metabolites. *Chlorella vulgaris* is metabolized naphthalene to 1-naphthol (0.36-0.65%). *Ankistrodesmus* S12 biotransformed dibenzofurans to six metabolites (total over 7%), three of which (possibly four) were identified as monohydroxylated dibenzofurans, the remaining two may be dihydroxylated derivatives. *Scenedesmus* S11 biotransformed dibenzo-*p*-dioxin to three metabolites, one of which was tentatively identified as 2-hydroxydibenzo-*p*-dioxin (approximately 3.8%), the remainder may be dihydroxylated derivatives. This is the first time that the biotransformation of diaryl ethers by green microalgae has been investigated.

Steven Pratt, Zhiguo Yuan, Daniel Gapes, Marco Dorigo, Raymond J. Zeng, Jurg Keller. (Advanced Wastewater Management Centre, The University of Queensland, QLD 4072, Australia). **Development of a novel titration and off-gas analysis (TOGA) sensor for study of biological processes in wastewater treatment systems.** Biotechnology and Bioengineering, 81(4) (2003), 482-495.

The development of the new TOGA (titration and off-gas analysis) sensor for the detailed study of biological processes in wastewater treatment systems is outlined. The main innovation of the sensor is the amalgamation of titrimetric and off-gas measurement techniques. The resulting measured signals are: hydrogen ion production rate (HPR), oxygen transfer rate (OTR), nitrogen transfer rate (NTR), and carbon dioxide transfer rate (CTR). While OTR and NTR are applicable to aerobic and anoxic conditions, respectively, HPR and CTR are useful signals under all of the conditions found in biological wastewater treatment systems, namely, aerobic, anoxic and anaerobic. The sensor is therefore a powerful tool for studying the key biological processes under all these conditions. A major benefit from the integration of the titrimetric and off-gas analysis methods is that the acid/base buffering systems, in particular the bicarbonate system, are properly accounted for. Experimental data resulting from the TOGA sensor in aerobic, anoxic, and anaerobic conditions demonstrates the strength of the new sensor. In the aerobic environment, carbon oxidation (using acetate as an example carbon source) and nitrification are studied. Both the carbon and ammonia removal rates measured by the sensor compare very well with those obtained from off-line chemical analysis. Further, the aerobic acetate removal process is examined at a fundamental

level using the metabolic pathway and stoichiometry established in the literature, whereby the rate of formation of storage products is identified. Under anoxic conditions, the denitrification process is monitored and, again, the measured rate of nitrogen gas transfer (NTR) matches well with the removal of the oxidised nitrogen compounds (measured chemically). In the anaerobic environment, the enhanced biological phosphorus process was investigated. In this case, the measured sensor signals (HPR and CTR) resulting from acetate uptake were used to determine the ratio of the rates of carbon dioxide production by competing groups of microorganisms, which consequently is a measure of the activity of these organisms. The sensor involves the use of expensive equipment such as a mass spectrometer and requires special gases to operate, thus incurring significant capital and operational costs. This makes the sensor more an advanced laboratory tool than an on-line sensor.

Thierry Maugard, Sylvain Lamare, Marie Dominique Legoy. (Laboratoire de Génie Protéique et Cellulaire, EA3169, Université de La Rochelle, Avenue Michel Crépeau, 17042 La Rochelle Cedex 1, France). **Gas phase biotransformation reaction catalyzed by baker's yeast.** *Biotechnology and Bioengineering*, 73(2) (2001), 164-168.

The gas phase continuous production of acetaldehyde from ethanol and hexanol from hexanal using dried baker's yeast was studied as an alternative approach to conventional processes. The effects of water activity, activity of substrates, and amount of yeast on the performance of the continuous bioreactor were investigated. The extent of yeast hydration and ethanol activity are the most important factors affecting yeast activity and stability.

Vilas B Shukla, Virendra R Madyar, Bhushan M Khadilkar, Pushpa R Kulkarni. (Food & Fermentation Technology Division, University Dept of Chemical Technology, University of Mumbai (UDCT), Nathalal Parekh Marg, Matunga, Mumbai - 400 019, India. Organic Chemistry Division, University Dept of Chemical Technology, University of Mumbai (UDCT), Nathalal Parekh Marg, Matunga, Mumbai - 400 019, India). **Biotransformation of benzaldehyde to L-phenylacetylcarbinol (L-PAC) by *Torulaspora delbrueckii* and conversion to ephedrine by microwave radiation.** *Journal of Chemical Technology & Biotechnology*, 77(2) (2002), 137-140.

In a 5 dm³ stirred tank reactor, bioconversion of 30 g benzaldehyde by cells of *Torulaspora delbrueckii* yielded 22.9 g of pure L-phenylacetylcarbinol (L-PAC). Facile functional group transformation of 4.5 g of L-PAC to 2-(methylimino)-1-phenyl-1-propanol by exposure to microwave irradiation for 9 min resulted in 2.48 g of product. Conversion of 4.8 g of 2-(methylimino)-1-phenyl-1-propanol to 3.11 g of ephedrine was achieved by exposure to microwaves in a reaction time of 10 min. The identity of all the products was confirmed by ¹H NMR and FT-IR analysis.

W. A. M. Wolken, M. J. van der Werf. (Division of Industrial Microbiology, Department of Food Technology and Nutritional Sciences, Wageningen University and Research Centre, P.O. Box 8129, 6700 EV Wageningen, The Netherlands. Department of Applied Microbiology and Gene Technology, TNO Nutrition and Food Research, Zeist, The Netherlands). **Geraniol biotransformation-pathway in spores of *Penicillium digitatum*.** *Applied Microbiology and Biotechnology*, 57(5-6) (2001), 731-737.

Spores of *Penicillium digitatum* ATCC 201167 transform geraniol, nerol, citral, and geranic acid into methylheptenone. Spore extracts of *P. digitatum* convert geraniol and nerol NAD⁺-dependently into citral. Spore extract also converts citral NAD⁺-dependently into geranic acid. Furthermore, a novel enzymatic activity, citral lyase, which cofactor-

independently converts citral into methylheptenone and acetaldehyde, was detected. These results show that spores of *P. digitatum* convert geraniol via a novel biotransformation pathway. This is the first time a biotransformation pathway in fungal spores has been substantiated by biochemical studies. Geraniol and nerol are converted into citral by citral dehydrogenase activity. The citral formed is subsequently deacetylated by citral lyase activity, forming methylheptenone. Moreover, citral is converted reversibly into geranic acid by citral dehydrogenase activity.

Y. Teramoto, S. Yoshida, S. Ueda. (National Museum of Ethnology, Senri Expo Park, Suita, Osaka 565-8511, Japan. Department of Applied Microbial Technology, Sojo University, Ikeda 4-22-1, Kumamoto 860-0082, Japan). **Characteristics of a rice beer (zutho) and a yeast isolated from the fermented product in Nagaland, India.** World Journal of Microbiology and Biotechnology, 18(9) (2002), 813-816.

Rice beer, known locally as zutho was collected in the Kohima district in Nagaland, India, and subjected to analytical and microbiological characterization. Zutho was a whitish porridge-like slurry containing 5.0% (v/v) ethanol. Volatile esters and higher alcohols, such as ethyl acetate and 3-methylbutanol, were detected in this indigenous alcoholic beverage by gas chromatography. The pH and acidity of zutho were 3.6 and 5.1, respectively. Zutho had a fruity aroma and sour taste and its unique aroma had characteristics similar to those of Japanese sake and sprouted rice sake. A fermentation yeast isolated from zutho was identified as being a strain of *Saccharomyces cerevisiae* and was found to be suitable as the brewing yeast for ethanol fermentation.

Zhi Feng Wang, Yu Liang Huang, James F Rathman, Shang-Tian Yang. (Department of Chemical Engineering, The Ohio State University, 140 West 19th Avenue, Columbus, Ohio 43210, USA). **Lecithin-enhanced biotransformation of cholesterol to androsta-1,4-diene-3,17-dione and androsta-4-ene-3,17-dione.** Journal of Chemical Technology & Biotechnology, 77(12) (2002), 1349-1357.

A biotransformation process using *Mycobacterium* sp was studied for androsta-1, 4-diene-3,17-dione (ADD) and androsta-4-ene-3,17-dione (AD) production from cholesterol. Cholesterol has a poor solubility in water (1.8 mg dm^{-3} at $25 \text{ }^\circ\text{C}$), which makes it difficult to use as the substrate for biotransformation. Lecithin is a mixture of phospholipids of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which behave like surfactants and can form planar bi-layer structures in an aqueous medium. Therefore, a small amount of lecithin ($<1 \text{ g dm}^{-3}$) can be used to form stable colloids with cholesterol at a relatively high concentration (20 g dm^{-3}) in water. In this work, an energy density of 1000 J cm^{-3} from sonication was provided to overcome the self-association of cholesterol and to generate a stable lecithin-cholesterol suspension that could be used for enhanced biotransformation. The lecithin-cholesterol suspension was stable and could withstand typical autoclaving conditions ($121 \text{ }^\circ\text{C}$, 15 psig, 20 min). In contrast to conventional surfactants, such as Tween 80, that are commonly used to help solubilize cholesterol, lecithin did not change the surface tension of the aqueous solution nor cause any significant foaming problem. Lecithin was also biocompatible and showed no adverse effect on cell growth. Compared with the medium with Tween 80 as the cholesterol-solubilizing agent, lecithin greatly improved the biotransformation process in regard to its final product yield (59% w/w), productivity ($0.127\text{-}0.346 \text{ g dm}^{-3} \text{ day}^{-1}$), ADD/AD ratio (6.7-8), as well as the long-term process stability. Cells can be reused in repeated batch fermentations for up to seven consecutive batches, but then lose their bioactivity due to aging problems, possibly caused by product inhibition and nutrient depletion.

Pollen Biotechnology

A. Modak, G.K. Saha. (Department of Medical Entomology, Calcutta School of Tropical Medicine, Kolkata 700073, West Bengal, India). **Effect of certain socio-ecological factors on the population density of house dust mites in mattress-dust of asthmatic patients of Calcutta, India.** *Aerobiologia*, 18 (3-4) (2002), 239-244.

Allergy to house dust mites, particularly to the genus *Dermatophagoides* is a fairly common problem in Calcutta and its adjoining areas since last two decades. Both the common species of the genus *Dermatophagoides* i.e. *D. farinae* and *D. pteronyssinus* are found to be abundant in the dust samples collected from beds of patients suffering from nasobronchial allergic disorders. The presence of these mites in quite a good number in the patients' beds are clinically correlated with the aetiopathology of various allergic manifestations like bronchial asthma. *Dermatophagoides* mites may occupy different niches in the homes of asthmatics and are more common in beds than elsewhere in the house, however, the distribution and abundance of these mites are influenced by some socio-ecological factors. The aim and objective of the present study is to assess the impact of some common socio-ecological management practices like the age of house, age of mattress, type of mattress, frequency of cleaning of the mattress and even the economic status of the patients on the growth, multiplication and finally accumulation of these mites in the patients' surroundings. Proper identification of offending allergens and subsequent reduction of load of these mite allergens from the patient's environment may be helpful for the prophylactic management of these diseases in Calcutta metropolis.

Andrea Ranzi, Paolo Lauriola, Vittorio Marletto, Franco Zinoni. (*Environmental Epidemiology Unit, Scientific Management – Regional Agency for Prevention and Environment of Emilia Romagna (ARPA-ER). Regional Meteorological Service – Regional Agency for Prevention and Environment of Emilia Romagna (ARPA-ER)*). **Forecasting airborne pollen concentrations: Development of local models.** *Aerobiologia*, 19 (1) (2003), 39-45.

People's sensitivity to allergies may represent one of the most important health factors of the next century to which attention must be paid in order to reduce the incidence of social costs and improve the quality of life. Taking into consideration the earnest requests of the medical-scientific community Emilia-Romagna ARPA (Regional Agency for the Prevention of the Environment) moved the attention from the monitoring to a short and medium term prediction of the concentration of allergenic pollens in the air in order to achieve a more effective therapeutic action. Our main objectives are to improve seasonal forecasts and to interpret anomalous years. A neural network model for grass pollen forecasting has been implemented. Input variables were meteorological situations, i.e., daily temperature (max., min. and average) and rainfall, in addition to combinations of individual variables and their thresholds. The output was daily pollen concentration. The model was able to understand and predict anomalous years. We demonstrate that the relationships between pollen concentrations and meteorological situations are independent from site. This means that such models can understand the differences in different areas.

Caroline Duchaine, Anne Mériaux, Paul Comtois. (Département de Biochimie-Microbiologie, Université Laval, Québec, Canada; Centre de Recherche, Hôpital Laval, Institut Universitaire de Cardiologie et de Pneumologie de l'Université, Québec, Canada. Centre de Recherche, Hôpital Laval, Institut Universitaire de Cardiologie et de Pneumologie de l'Université, Québec, Canada. Laboratoire d'Aérobiologie, Département de Géographie, Université de Montréal, Montréal, Québec, Canada). **Usefulness of**

using three different culture media for mold recovery in exposure assessment studies. *Aerobiologia*, 18 (3-4) (2002), 245-251.

The choice of culture media when performing exposure assessment for mold quantification is difficult since no standard methods exist and, in many cases, there is no way to predict, before hand, the mold population present and thus, select the most appropriate media. The aim of this project was to compare the efficiency and usefulness of 3 widely used culture media during the recovery of molds in sawmills. Andersen microbial samplers (AMS) and all-glass impingers-30 (AGI-30) were used to sample 51 work sites within 17 sawmills. Rose Bengal Agar (RBA), Czapek Solution Agar (CZA) and Sabouraud Dextrose Agar (SDA) were used directly in AMS and to plate AGI-liquid. The results show that there was no significant difference between the three culture media concerning the counts and the species recovery. However, SDA often lead to non usable counts. The variations obtained with the different culture media when AGI-30 were plated in triplicate was also not significantly different. However, only 19% of the species recovered at one site were present on all culture media. We believe the use of various media increases the chances of obtaining valid counts and an adequate species recovery.

Jacques Lavoie, Christopher J. Dunkerley. (Institut de recherche en sante et en securite du travail du Quebec (IRSST). McGill University, Joint Departments of Epidemiology, Biostatistic, and Occupational Health, Faculty of Medicine, Montreal, Canada). **Assessing waste collectors exposure to bioaerosols.** *Aerobiologia*, 18 (3-4) (2002), 277-285.

Published studies on household waste collectors' exposure to airborne biological agents (bioaerosols) do not indicate high exposures to these agents. However, these studies did not consider several factors. The objective of this study was to characterize the exposure of waste collectors to bioaerosols and to propose solutions to control exposures to these agents. Personal exposures of waste collectors to bioaerosols (total bacteria, endotoxins and molds) were measured for seven types of collection during the summer, which represents the worst conditions. The effect of truck cleaning was also evaluated. Mean concentrations of bacteria were all in the order of 10^3 - 10^4 CFU/m³ of air. The intervention threshold was exceeded for endotoxins during the collection of compost once every two weeks in the country. Mean concentrations varied from 8.5 to 100 endotoxin units per cubic meter of air (EU/m³). Measured mean concentrations of molds were between 8,300 and 98,170 CFU/m³ of air. Also, the cleaning of an empty garbage truck does not improve the quality of the air. On the other hand, a dirty truck is not a major source of bioaerosols. The sources of these bioaerosols are leachate, particularly if the waste in the truck is of organic origin, as well as the garbage pails that contain this waste. Unnecessary exposures to these sources should therefore be avoided. For bioaerosols, stringent personal hygiene measures remain one of the best means of prevention.

Meindert D. de Jong, Graeme W. Bourdot, Geoff A. Hurrell, David J. Saville, Hans J. Erbrink, Jan C. Zadoks. (Biological Farming Systems, Wageningen University and Research Centre, Marijkeweg 22, 6709 PG Wageningen, The Netherlands. New Zealand Pastoral Agriculture Research Institute Ltd., P.O. Box 60, Lincoln, New Zealand. KEMA Power Generation and Sustainable, P.O. Box 9035, 6800 ET Arnhem, The Netherlands. Herengracht 96C, 1015 BS Amsterdam, The Netherlands). **Risk analysis for biological weed control-simulating dispersal of *Sclerotinia sclerotiorum* (Lib.) de Bary ascospores from a pasture after biological control of *Cirsium arvense* (L.) Scop.** *Aerobiologia*, 18(3-4) (2002), 211-222.

Biological control of *Cirsium arvense* (L.) Scop. in pasture by the plurivorous plant pathogenic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary may result in the formation, escape and aerial dispersal of ascospores, creating an additional disease risk in down-

wind market garden crops. To determine the width of a safety zone for a pasture subjected to this form of weed control, we simulated the spatial pattern in the ratio of added (due to biocontrol) to naturally occurring airborne ascospores (due to market garden crops) around a 1 ha virtual biocontrol pasture under either sheep or dairy cattle grazing over a 91 -day emission period in 1996 in Canterbury, New Zealand. This was achieved using a unique combination of two computer models; SPORESIM-1 D (for spore escape from a vegetation source) and PC-STACKS (a modern Gaussian plume model for dispersal beyond a source). Plumes of dispersing ascospores were modelled for each hour of the emission period for both the virtual market garden and biocontrol sites, and the aerial density of the ascospores was averaged over the period. Assuming that a 1:1 ratio of added to naturally present spores is acceptable, no safety zone was necessary for either of the modeled pastures. A ten-fold ratio (1:10 added to natural) necessitated safety zones of 300 and 150 m for the sheep and dairy pasture respectively. Uncertainties associated with extrapolation of this conclusion to individual pasture management scenarios, and to other years and climatically different regions are discussed.

S. K. Bera, Anjali Trivedi, Chhaya Sharma. (Birbal Sahni Institute of Palaeobotany, 53, University Road, Lucknow 226007, India). **Trapped pollen and spores from spider webs of Lucknow environs.** Current Science, 83(12) (2002), 1580.

Study of pollen and spores retrieved from the spider webs provides interesting new frontiers to evaluate the aerospora of a region. Such studies carried out for Lucknow environs have yielded a variety of palynomorphs such as pollen grains and fungal spores besides insect body fragments, etc. These studies are of immense significance to understand the aerospora for meaningful comparison with the data on the differential pollen dispersal and deposition in a particular region, generated through other conventional methods, viz. pollen catchers, moss cushions, etc.

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
14. Applied Bacteriology
15. Applied And Environmental Microbiology
16. Applied Microbiology & Biotechnology
17. Aquaculture
18. Australian Journal of Plant Physiology
19. Biocatalysis And Transformation
20. Biocontrol
21. Biocontrol Potential and its exploitation in sustainable Agriculture
22. Biodegradation
23. Biodeterioration & Biodegradation
24. Biodiversity And Conservation
25. Biological Agriculture and Horticulture
26. Biomass and Bioenergy
27. Biomedical and Environmental Sciences
28. Biomedical Engineering
29. Bioremediation Journal
30. Bioscience, Biotechnology And Biochemistry
31. Biosensors-and -Bioelectronics
32. Bioseparation
33. Biotechnolgy Letters
34. Biotechnolgy Techniques
35. Biotechnology Advances
36. Biotechnology And Applied Biochemistry
37. Biotechnology And Bioengineering
38. Biotechnology Letters
39. Botanical Review
40. Canadian Journal Of Microbiology
41. Cell & Tissue Banking
42. Clinical Microbiology Reviews
43. Critical Reviews In Biotechnology
44. Crop research Hisar
45. Current Microbiology
46. Current Opinion In Biotechnology
47. Current Science
48. Cytotechnology
49. Ecology and Environmental Corner
50. Ecological Engineering
51. Ecotoxicology
52. Environmental Conservation
53. Fems Microbiology & Ecology
54. Food & Agricultural Immunology

55. Global Environmental Change
56. Immunological Research
57. Indian Agriculturist
58. Indian Farming
59. Indian Journal Of Agricultural Science
60. Indian Journal Of Biotechnology
61. Indian Journal Of Ecology
62. Indian Journal of Experimental Biology
63. Indian Journal of Environmental Toxicology
64. Indian Journal of Environmental Health
65. Indian Journal of Plant Physiology
66. International Biodeterioration & Biodegradation
67. International Journal Of Biotechnology
68. International Journal Of Phytoremediation
69. Journal of Agriculture and Environmental Ethics
70. Journal Biological Control
71. Journal Of Bacteriology
72. Journal of Chemical Technology & Biotechnology
73. Journal of Environmental Management
74. Journal of Food Science and Technology-Mysore
75. Journal Indian Association Environment Management
76. Journal Indian Pollution Control
77. Journal Of Indian Soil Science
78. Journal Of Industrial Microbiology & Biotechnology
79. Journal of Scientific And Industrial Research
80. Microbial Review
81. Microbiological Research
82. Molecular Biotechnology
83. Mycological Research
84. Mycorrhizal Biology
85. Nature
86. Nature Biotechnology
87. New Biotechnology
88. Perspectives-in-Biotechnology
89. Pesticide research Journal
90. Pestology
91. Plants And Soil
92. Pollution
93. Pollution Research
94. Reviews In Environmental Science And Biotechnology
95. Research Journal Chemistry & Environment
96. Reviews in Environmental Science and Biotechnology
97. Sciences
98. Science & Culture
99. Shaspa
100. The Indian Forester
101. Trends In Biotechnology
102. Water, Air and Soil Pollution
103. World Journal Of Biotechnology
104. World Journal Of Microbiology And Biotechnology

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