EMCB-ENVIS Node
on
ENVIRONMENTAL BIOTECHNOLOGY

Abstract, Vol. V

Sponsored by

MINISTRY OF ENVIRONMENT AND FOREST
GOVERNMENT OF INDIA
NEW DELHI

Department of Environmental Science
University of Kalyani
Nadia, West Bengal

December 2004

ENVIS's staff

EMCB-ENVIS NODE

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C O N T E N T S

BACKGROUND

Environmental Information System (ENVIS) is established in the year 1984 as a network of Information 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 75 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 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 Fifth publication of this ENVIS Centre. This contains the abstract of research papers collected in the area of Environmental Biotechnology from various journals published during January 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 2000-2005.

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, anddegrade 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 varity 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.

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 warm. Biological insecticides against both these insects are being prepared by transfer of a gene from *Bacillus thuringiensis*

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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 varity of environments. These specific substances may include sugars, proteins, or humas and varity of toxins in the industrial effluents. In designing a biosensor an enzyme or an antibody or even microbial cells are associated with microchip devices which are used for quantitative estimate of a substance.

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	Biotechnique(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
Geogri	Geographical	Occ	Occassional
Geol	Geological	Occupl	Occupational
Geosci	Geoscience	Oceanogr	Oceanogoraphy
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	Immunlogical	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

Del Campo JA, Rodriguez H, Moreno J, Vargas MA, Rivas J, Guerrero MG. (Instituto de Bioquimica Vegetal y Fotosintesis, Centro de Investigaciones Cientificas Isla de la Cartuja, Consejo Superior de Investigaciones Cientificas-Universidad de Sevilla, Avda. Americo Vespucio s/n, 41092 Sevilla, Spain) Accumulation of astaxanthin and lutein in Chlorella zofingiensis (Chlorophyta). Appl Microbiol Biotechnol. 2004 Jun;64(6): 848-54.

When grown photoautotrophically, Chlorella zofingiensis strain CCAP 211/14 accumulates a significant amount of valuable carotenoids, namely astaxanthin and lutein, of increasing demand for use as feed additives in fish and poultry farming, as colorants in food, and in health care products. Under standard batch-culture conditions, this microalgal strain exhibits high values of both growth rate (about 0.04 h(-1)) and standing cell population (over 10(11) cells l(-1), or 7 g dry weight l(-1)). Lutein, in a free (unesterified) form, was the prevalent carotenoid during early stages of cultivation (over 0.3 pg cell(-1), equal to 4 mg g(-1) dry weight, or 20 mg l(-1) culture), whereas esterified astaxanthin accumulated progressively, to reach a maximum (over 0.1 pg cell(-1), equal to 1.5 mg g(-1) dry weight, or 15 mg l(-1) culture) in the late stationary phase. A differential response of lutein and astaxanthin accumulation was also recorded with regard to the action of some environmental and nutritional factors. C. zofingiensis CCAP 211/14 represents a unique model system for analyzing the differential regulation of the levels of primary (lutein) and secondary (astaxanthin) carotenoids. Relevant also from the biotechnological viewpoint, this photosynthetic organism, with outstanding attributes for fast photosynthetic growth and carotenoid accumulation, might prove most valuable for its application to the mass production of either or both lutein and astaxanthin.

Jan Kostal,[1] Rosanna Yang,[1] Cindy H. Wu,[1,2] Ashok Mulchandani,[1] and Wilfred Chen[1*] (Department of Chemical and Environmental Engineering,[1] Environmental Toxicology Program, University of California, Riverside, California 92521[2]). Enhanced Arsenic Accumulation in Engineered Bacterial Cells Expressing ArsR. Applied and Environmental Microbiology, August 2004, p. 4582-4587, Vol.70, No. 8.

The metalloregulatory protein ArsR, which offers high affinity and selectivity toward arsenite, was overexpressed in *Escherichia coli* in an attempt to increase the bioaccumulation of arsenic. Overproduction of ArsR resulted in elevated levels of arsenite bioaccumulation but also a severe reduction in cell growth. Incorporation of an elastin-like polypeptide as the fusion partner to ArsR (ELP153AR) improved cell growth by twofold without compromising the ability to accumulate arsenite. Resting cells overexpressing ELP153AR accumulated 5- and 60-fold-higher levels of arsenate and arsenite than control cells without ArsR overexpression. Conversely, no significant improvement in Cd^{2+} or Zn^{2+} accumulation was observed, validating the specificity of ArsR. The high affinity of ArsR allowed 100% removal of 50 ppb of arsenite from contaminated water with these engineered cells, providing a technology useful to comply with the newly approved U.S. Environmental Protection Agency limit of 10 ppb. These results open up the possibility of using cells overexpressing ArsR as an inexpensive, high-affinity ligand for arsenic removal from contaminated drinking and ground water.

Ozdemir G, Baysal SH. (Biology Department, Faculty of Science, Ege University, 35100 Bornova/Izmir, Turkey) Chromium and aluminum biosorption on Chryseomonas luteola TEM05. Appl Microbiol Biotechnol. 2004 May;64(4):599-603.

Cr (VI) and Al (III) are environmental pollutants that are frequently encountered together in industrial wastewaters, e.g., from mining iron-steel, metal cleaning, plating, metal processing, automobile parts, and the manufacturing and dye industries. In this work, several variables that affect the capacity for chromium and aluminum biosorption by Chryseomonas luteola TEM05 were studied, particularly the effects of pH, metal concentration and contact time. Optimum adsorption pH values of Cr(VI) and Al (III) were determined as 4.0 and 5.0, respectively. The biosorption equilibrium was described by Freundlich and Langmuir adsorption isotherms. The value of Qo appears to be significantly higher for the Al (III) C. luteola TEM05 system. Langmuir parameters of C. luteola TEM05 also indicated a maximum adsorption capacity of 55.2 mg g(-1) for Al(III) and 3.0 mg g(-1) for Cr(VI).

BIOREMEDIATION

Agneta Backman,[1,2] Ninwe Maraha,[1,2] and Janet K. Jansson[1,3*]. (Section for Natural Sciences, Södertörn University College, Huddinge,[1] Department of Laboratory Medicine, Karolinska Institute, Huddinge University Hospital, Stockholm,[2] Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden[3]) Impact of Temperature on the Physiological Status of a Potential Bioremediation Inoculant, *Arthrobacter chlorophenolicus* A6. Applied and Environmental Microbiology, May 2004, p. 2952-2958, Vol. 70, No. 5.

Arthrobacter chlorophenolicus A6 (A6) can degrade large amounts of 4-chlorophenol in soil at 5 and 28°C. In this study, we investigated the effects of temperature on the physiological status of this bacterium in pure culture and in soil. A derivative of A6 tagged with the *gfp* gene (encoding green fluorescent protein [GFP]) was used to specifically quantify A6 cells in soil. In addition, cyano-ditolyl-tetrazoliumchloride was used to stain GFP-fluorescent cells with an active electron transfer system ("viable cells") whereas propidium iodide (PI) was used to stain cells with damaged membranes ("dead cells"). Another derivative of the strain (tagged with the firefly luciferase gene [*luc*]) was used to monitor the metabolic activity of the cell population, since the bioluminescence phenotype is dependent on cellular energy reserves. When the cells were incubated in soil at 28°C, the majority were stained with PI, indicating that they had lost their cell integrity. In addition, there was a corresponding decline in metabolic activity and in the ability to be grown in cultures on agar plates after incubation in soil at 28°C, indicating that the cells were dying under those conditions. When the cells were incubated in soil at 5°C, by contrast, the majority of the cells remained intact and a large fraction of the population remained metabolically active. A similar trend towards better cell survival at lower temperatures was found in pure-culture experiments. These results make *A. chlorophenolicus* A6 a good candidate for the treatment of chlorophenol-contaminated soil in cold climates.

Annette C. Dietz and Jerald L. Schnoor. (Department of Civil and Environmental Engineering, University of Iowa, Iowa City, Iowa, USA.) Advances in Phytoremediation. Environ Health Perspect,Vol.109 (suppl 1) :163–168 (2001).

Phytoremediation is the use of plants to remedy contaminated soils, sediments, and/or groundwater. Sorption and uptake are governed by physicochemical properties of the compounds, and moderately hydrophobic chemicals (logarithm octanol–water coefficients = 1.0–3.5) are most likely to be bioavailable to rooted, vascular plants. Some hydrophilic compounds,

such as methyl*tert*-butylether and 1,4-dioxane, may also be taken up by plants via hydrogen bonding with transpiration water. Organic chemicals that pass through membranes and are translocated to stem and leaf tissues may be converted (e.g., oxidized by cytochrome P450s), conjugated by glutathione or amino acids, and compartmentalized in plant tissues as bound residue. The relationship between metabolism of organic xenobiotics and toxicity to plant tissues is not well understood. A series of chlorinated ethenes is more toxic to hybrid poplar trees (*Populus deltoides x nigra*, DN-34) than are the corresponding chlorinated ethanes. Toxicity correlates best with the number of chlorine atoms in each homologous series. Transgenic plants have been engineered to rapidly detoxify and transform such xenobiotic chemicals. These could be used in phytoremediation applications if issues of cost and public acceptability are overcome.

Barton, JW; Klasson, KT; Koran, LJ; Davison, BH. Microbial removal of alkanes from dilute gaseous waste streams- kinetics and mass-transfer considerations. Biotechnology Progress, 13(6) 814-821 1997.

Treatment of dilute gaseous hydrocarbon waste streams remains a current need for many industries, particularly as increasingly stringent environmental regulations and oversight force emission reduction. Biofiltration systems hold promise for providing low-cost alternatives to more traditional, energy-intensive treatment methods such as incineration and adsorption. Elucidation of engineering principles governing the behavior of such systems, including mass transfer limitations, will broaden their applicability. Our processes exploit a microbial consortium to treat a mixture of 0.5% n-pentane and 0.5% isobutane in air. Since hydrocarbon gases are sparingly soluble in water, good mixing and high surface area between the gas and liquid phases are essential for biodegradation to be effective. One liquid-continuous columnar bioreactor was operated for more than 30 months with continued degradation of n-pentane and isobutane as sole carbon and energy sources. The maximum degradation rate observed in this gas-recycle system was 2 g of volatile organic compounds (VOC)/m(3).h). A trickle-bed bioreactor was operated continuously for over 24 months to provide a higher surface area (using a structured packing) with increased rates. Degradation rates consistently achieved were approximately 50 g of VOC/(m(3).h) via single pass in this gas-continuous columnar system. Effective mass transfer coefficients comparable to Literature values were also measured for this reactor; these values were substantially higher than those found in the gas-recycle reactor. Control of biomass levels was implemented by limiting the level of available nitrogen in the recirculating aqueous media, enabling long-term stability of reactor performance.

Bondada BR, Tu S, Ma LQ. (Soil and Water Sciences Department, University of Florida, 2169 McCarty Hall, P.O. Box 110290, Gainesville, FL 32611, USA). Absorption of foliar-applied arsenic by the arsenic hyperaccumulating fern (Pteris vittata L.). Sci Total Environ. 2004 Oct 1; 332(1-3):61-70.

The fact that heavy metals can enter various domains of the plant system through foliar pathways spurred us to explore if the fronds of the Chinese brake fern (Pteris vittata L.), a hyperaccumulator of arsenic, a carcinogenic metalloid, was proficient in absorbing arsenic in the form of sprays. The specific objective of this study was to investigate the impact of frond age, form of arsenic, and time of application on the absorption of foliar-applied arsenic by the brake fern; also examined were the effects of foliar sprays on surface ultrastructure and arsenic speciation in the frond following absorption. Foliar sprays of different arsenic concentrations (0, 50, 100, 200, and 400 ppm) were applied to young and fertile fronds. A positive linear relationship existed between arsenic concentration and absorption; the arsenic concentration of

fronds increased from 50 to 200 ppm. Time-course analysis with excised pinnae indicated an initial linear increase followed by a plateau at 48 h. The young fronds with immature sori absorbed more arsenic (3100 ppm) than the fertile mature fronds (890 ppm). In the frond, the arsenic absorption was greatest in the lamina of the pinnae followed by the sori and the rachis. Applying arsenic during night (20:00-22:00 h) or afternoon (12:00-14:00 h) resulted in greater absorption of arsenic than the application in the morning (08:00-10:00 h). The arsenic absorption was greater through abaxial surfaces than through adaxial surfaces. The brake fern absorbed more arsenic when it was applied in the form of arsenite. Regardless of the form of arsenic and the surface it was applied to, arsenic occurred as arsenite, the reduced and the most toxic form of arsenic, after having been absorbed by the fronds. Scanning electron microscopy revealed no surface morphological alterations following all arsenic sprays. The study unequivocally illustrated that the Chinese brake fern absorbed foliar-applied arsenic with great efficiency. Consequently, the arsenic concentrations in the fronds transcended the levels of hyperaccumulation; such a characteristic could be exploited in the phytoremediation of groundwater contaminated with arsenic.

Cavalca L, Dell'Amico E, Andreoni V. (Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Universita degli Studi di Milano, via Celoria 2, 20133 Milan, Italy) Intrinsic bioremediability of an aromatic hydrocarbon-polluted groundwater: diversity of bacterial population and toluene monoxygenase genes. Appl Microbiol Biotechnol. 2004 May;64(4):576-87. Epub 2003 Nov 18.

The functional and phylogenetic biodiversity of bacterial communities in a benzene, toluene, ethylbenzene and xylene (BTEX)-polluted groundwater was analysed. To evaluate the feasibility of using an air sparging treatment to enhance bacterial degradative capabilities, the presence of degrading microorganisms was monitored. The amplification of gene fragments corresponding to toluene monooxygenase (tmo), catechol 1,2-dioxygenase, catechol 2,3-dioxygenase and toluene dioxygenase genes in DNA extracted directly from the groundwater samples was associated with the presence of indigenous degrading bacteria. Five months of air injection reduced species diversity in the cultivable community (as calculated by the Shannon-Weaver index), while little change was noted in the degree of biodiversity in the total bacterial community, as characterised by denaturing gradient gel electrophoresis (DGGE) analysis. BTEX-degrading strains belonged to the genera Pseudomonas, Microbacterium, Azoarcus, Mycobacterium and Bradyrhizobium. The degrading capacities of three strains in batch liquid cultures were also studied. In some of these microorganisms different pathways for toluene degradation seemed to operate simultaneously. Pseudomonas strains of the P24 operational taxonomic unit, able to grow only on catechol and not on BTEX, were the most abundant, and were present in the groundwater community at all stages of treatment, as evidenced both by cultivation approaches and by DGGE profiles. The presence of different tmo-like genes in phylogenetically distant strains of Pseudomonas, Mycobacterium and Bradyrhizobium suggested recent horizontal gene transfer in the groundwater.

Ghabbour EA, Davies G, Lam YY, Vozzella ME. (Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115-5000, USA). Metal binding by humic acids isolated from water hyacinth plants (Eichhornia crassipes [Mart.] Solm-Laubach: Pontedericeae) in the Nile Delta, Egypt. Environ Pollut. 2004 Oct;131(3):445-51.

Humic acids (HAs) are animal and plant decay products that confer water retention, metal and organic solute binding functions and texture/workability in soils. HAs assist plant nutrition with minimal run-off pollution. Recent isolation of HAs from several live plants prompted us to investigate the HA content of the water hyacinth (Eichhornia crassipes [Mart.] Solm-Laubach: Pontedericeae), a delicately flowered plant from Amazonian South America that has invaded temperate lakes, rivers and waterways with devastating economic effects. Hyacinth thrives in nutrient-rich and polluted waters. It has a high affinity for metals and is used for phytoremediation. In this work, HAs isolated from the leaves, stems and roots of live water hyacinth plants from the Nile Delta, Egypt were identified by chemical and spectral analysis and by comparison with authentic soil and plant derived HAs. Similar carbohydrate and amino acid distributions and tight metal binding capacities of the HAs and their respective plant components suggest that the presence of HAs in plants is related to their metal binding properties.

J. Sabaté, M. Viñas and A. M. Solanas. (Department of Microbiology, Faculty of Biology, University of Barcelona, Diagonal 645, E-08028, Barcelona, Spain) Laboratory-scale bioremediation experiments on hydrocarbon-contaminated soils. International Biodeterioration & Biodegradation Volume 54, Issue 1, July 2004, Pages 19-25.

Successful application of bioremediation technology to contaminated soil requires knowledge of the characteristics of the site and the parameters that affect the microbial biodegradation of pollutants. Here, we propose a simple protocol for biotreatability assays in two phases. In the first phase of the assays we examined the type and metabolic activity of the indigenous microorganisms at the site, and the presence of possible inhibitors. The biodegradability of contaminants in soil slurries under optimal conditions was also tested. In the second phase several parameters, such as the influence of nutrients and the addition of surfactant and specialized inocula, were evaluated in microcosms with 2.5 kg soil. The application of this protocol to two hydrocarbon-contaminated soils is described. In the first phase of the protocol, the results obtained with the first soil indicated high metabolic activity of indigenous microbial populations and a total petroleum hydrocarbon (TPH) decrease of 46%. Assays of the second soil indicated low indigenous microbial metabolic activity and limited biodegradation of TPH. In the second phase of the protocol, which lasted 360 days, assay of microcosms showed that the first soil responded to several treatments with a large decrease in TPH, while none of the treatments applied to the second soil showed a reduction in TPH. The information obtained from the results in the first phase of the protocol indicates whether a biological treatment of contaminated soil is appropriate. In the second phase of the protocol, we attempted to identify the most appropriate treatment through the evaluation of various conditions and additives.

Marchiol L, Assolari S, Sacco P, Zerbi G. (Department of Agriculture and Environmental Science, University of Udine, Via delle Science 208, 33100, Italy). Phytoextraction of heavy metals by canola (Brassica napus) and radish (Raphanus sativus) grown on multicontaminated soil. Environ Pollut. 2004 Nov;132(1): 21-7.

Phytoextraction can provide an effective in situ technique for removing heavy metals from polluted soils. The experiment reported in this paper was undertaken to study the basic potential of phytoextraction of Brassica napus (canola) and Raphanus sativus (radish) grown on a multi-

metal contaminated soil in the framework of a pot-experiment. Chlorophyll contents and gas exchanges were measured during the experiment; the heavy metal phytoextraction efficiency of canola and radish were also determined and the phytoextraction coefficient for each metal calculated. Data indicated that both species are moderately tolerant to heavy metals and that radish is more so than canola. These species showed relatively low phytoremediation potential of multicontaminated soils. They could possibly be used with success in marginally polluted soils where their growth would not be impaired and the extraction of heavy metals could be maintained at satisfying levels.

M. Humar[a], M. Bokan[a], S. A. Amartey[b], M. entjurc[c], P. Kalan[d] and F. Pohleven[a]. a (Department of Wood Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; b Forest Products Research Center, Buckinghamshire Chilterns, University College, High Wycombe, UK; c Institute Jozef Stefan, Ljubljana, Slovenia d Slovenian Forestry Institute, Ljubljana, Slovenia). Fungal bioremediation of copper, chromium and boron treated wood as studied by electron paramagnetic resonance. International Biodeterioration & Biodegradation Volume 53, Issue 1 , January 2004, Pages 25-32.

In future years, problems concerning the disposal of waste copper/chromium-treated wood will increase significantly. One of the environmentally friendly options of dealing with such treated wood is through bioremediation with copper-tolerant wood decay fungi in order to recycle both the wood fibers and the heavy metals. To study changes during the bioremediation process, Norway spruce (*Picea abies*) samples were vacuum impregnated with 5% CCB solution. Some samples were also impregnated with copper or chromium solution of the same concentration as in the CCB preservative. Following conditioning of the samples, they were then exposed to two copper-tolerant brown rot fungi, (*Antrodia vaillantii*, *Leucogyrophana pinastri*) and two copper-sensitive brown rot fungi, (*Gloeophyllum trabeum*, *Poria monticola*) for a period of 4–8 weeks. After exposure, the samples were cleaned of the mycelia and leached with water or 1.25% ammonia solution for 4 days. The concentrations of Cr and Cu in the leachates were determined. After the leaching process, the samples were studied using electron paramagnetic resonance (EPR). The results obtained showed the important role oxalic acid produced by the decay fungi plays during leaching of the metals from the treated wood. Furthermore, it was also found that though excretion of oxalic acid is necessary for the leaching of metals, it does not fully explain fungal ability to decay copper preserved wood.

Ohtsubo Y, Kudo T, Tsuda M, Nagata Y. (Department of Environmental Life Sciences, Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, 980-8577, Sendai, Japan). Strategies for bioremediation of polychlorinated biphenyls. Appl Microbiol Biotechnol. 2004 Aug;65(3):250-8.

Polychlorinated biphenyls (PCBs) are serious environmental pollutants that threaten both the natural ecosystem and human health. For remediation of environments contaminated with PCBs, several approaches that exploit the potential of microbes to degrade PCBs have been developed. These approaches include improvement of PCB solubilization and entry into the cell, pathway and enzyme engineering, and control of enzyme expression. In this mini-review, we briefly summarize these strategies and provide potentially useful knowledge for the further improvement of the bacterial breakdown of PCBs.

Okuno, K; Hirai, M. Microbial removal of nitrogen monoxide (NO) under aerobic conditions. Biotechnology Letters, 22(1): 77-79. 2000

Nitrogen oxide gas (NOx), consisting of nitrogen monoxide (NO) and nitrogen dioxide (NO2), at a low concentration corresponding to that on roads as a result of exhaust from automobiles, was supplied for 25 days through a laboratory-scale biofilter packed with soil as a packing material. The removal efficiency of NO2 by soil was almost 100%, and the removal efficiency of NO was 60% on average and 86% at maximum. By using gamma-irradiated soil as a packing material, NO2 was completely removed mainly by adsorption onto or absorption into the packing material. However, the removal efficiency of NO in the sterilized soil was only 20%, suggesting that NO in soil was removed microbiologically under aerobic conditions.

Olivier Potin , Catherine Rafin and Etienne Veignie. (Université du Littoral-Côte d'Opale, 50 rue Ferdinand Buisson, BP 699, 62228, Calais Cédex, France). Bioremediation of an aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil by filamentous fungi isolated from the soil. International Biodeterioration & Biodegradation Volume 54, Issue 1, July 2004, Pages 45-52.

Twenty-one filamentous fungi were isolated from the soil of an old polycyclic aromatic hydrocarbon (PAH)-contaminated gaswork site and tested in their native soil for PAH degradation. This degradation study was performed for each isolate with two inoculation treatments, by spore or mycelial inoculum. An improvement in the extent of total PAH degradation occurred with mycelial inoculum. The greatest degradation was obtained with *Coniothyrium* sp. (26.5%) and *Fusarium* sp. (27.5%) inoculum, especially for PAHs of high-molecular-weight that contained more than three fused aromatic rings. Correlations between mycobiota capacity (in treatments inoculated with mycelium) and PAH characteristics (structure, water solubility, bioavaibility) were obtained from correspondence factorial analysis. This study suggests that such filamentous fungi could be used in clean-up of long-term contamination of soils by PAHs.

Wilfred F. M. Röling,[1,†] Ivana R. Couto de Brito,[1] Richard P. J. Swannell,[2] and Ian M. Head[1*] (School of Civil Engineering and Geosciences and Centre for Molecular Ecology, University of Newcastle, Newcastle upon Tyne NE1 7RU,[1] AEA Technology, Didcot, Oxfordshire OX11 OQJ, United Kingdom[2]). Response of Archaeal Communities in Beach Sediments to Spilled Oil and Bioremediation. Applied and Environmental Microbiology, May 2004, p. 2614-2620, Vol. 70, No. 5.

While the contribution of *Bacteria* to bioremediation of oil-contaminated shorelines is well established, the response of *Archaea* to spilled oil and bioremediation treatments is unknown. The relationship between archaeal community structure and oil spill bioremediation was examined in laboratory microcosms and in a bioremediation field trial. 16S rRNA gene-based PCR and denaturing gradient gel analysis revealed that the archaeal community in oil-free laboratory microcosms was stable for 26 days. In contrast, in oil-polluted microcosms a dramatic decrease in the ability to detect *Archaea* was observed, and it was not possible to amplify fragments of archaeal 16S rRNA genes from samples taken from microcosms treated with oil. This was the case irrespective of whether a bioremediation treatment (addition of inorganic nutrients) was applied. Since rapid oil biodegradation occurred in nutrient-treated microcosms, we concluded that *Archaea* are unlikely to play a role in oil degradation in beach ecosystems. A clear-cut relationship between the presence of oil and the absence of *Archaea* was not apparent in the field experiment. This may have been related to continuous inoculation of beach sediments in the field with *Archaea* from seawater or invertebrates and shows that the reestablishment of *Archaea* following bioremediation cannot be used as a determinant of ecosystem recovery

following bioremediation. Comparative 16S rRNA sequence analysis showed that the majority of the *Archaea* detected (94%) belonged to a novel, distinct cluster of group II uncultured *Euryarchaeota*, which exhibited less than 87% identity to previously described sequences. A minor contribution of group I uncultured *Crenarchaeota* was observed.

BIOTRANSFORMATION

Barton JW, Kuritz T, O'Connor LE, Ma CY, Maskarinec MP, Davison BH. (Life Sciences Division, Oak Ridge National Laboratory, 37831, Oak Ridge, TN, USA). Reductive transformation of methyl parathion by the cyanobacterium Anabaena sp. strain PCC7120. Appl Microbiol Biotechnol. 2004 Aug;65(3):330-5.

Organophosphorus compounds are toxic chemicals that are applied worldwide as household pesticides and for crop protection, and they are stockpiled for chemical warfare. As a result, they are routinely detected in air and water. Methods and routes of biodegradation of these compounds are being sought. We report that under aerobic, photosynthetic conditions, the cyanobacterium Anabaena sp. transformed methyl parathion first to o, o-dimethyl o- p-nitrosophenyl thiophosphate and then to o, o-dimethyl o- p-aminophenyl thiophosphate by reducing the nitro group. The process of methyl parathion transformation occurred in the light, but not in the dark. Methyl parathion was toxic to cyanobacteria in the dark but did not affect their viability in the light. Methyl parathion transformation was not affected by mutations in the genes involved in nitrate reduction in cyanobacteria.

Brooks SJ, Doyle EM, Hewage C, Malthouse JP, Duetz W, O' Connor KE. (Department of Industrial Microbiology, University College Dublin, Belfield Dublin 4, Republic of Ireland). Biotransformation of halophenols using crude cell extracts of Pseudomonas putida F6. Appl Microbiol Biotechnol. 2004 May;64(4):486-92.

Crude cell extracts of Pseudomonas putida F6 transformed 4-substituted fluoro-, chloro-, bromo- and iodo-phenol without the exogenous addition of cofactors. The rate of substrate consumption decreased with increasing substituent size (F>Cl>Br>I). Biotransformations resulted in greater than 95% utilisation of the halogenated substrate. Product accumulation was observed in incubations with 4-chloro, 4-bromo- and 4-iodo-phenol. These products were identified as the corresponding 4-substituted catechols. Transformation of 4-fluorophenol did not result in the accumulation of the corresponding catechol; however, manipulation of the reaction conditions by incorporation of ascorbic acid culminated in the formation of 4-fluorocatechol. Cell extracts of P. putida F6 also showed activity towards a 3-substituted phenol, namely 3-fluorophenol, resulting in the formation of a single product, 4-fluorocatechol.

Chakraborty R, Coates JD. (Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA 94720,USA). Anaerobic degradation of monoaromatic hydrocarbons. Appl Microbiol Biotechnol. 2004 May;64(4):437-46.

Over the last two decades significant advances have been made in our understanding of the anaerobic biodegradability of monoaromatic hydrocarbons. It is now known that compounds such as benzene, toluene, ethylbenzene, and all three xylene isomers can be biodegraded in the absence of oxygen by a broad diversity of organisms. These compounds have been shown to serve as carbon and energy sources for bacteria growing phototrophically, or respiratorily with nitrate, manganese, ferric iron, sulfate, or carbon dioxide as the sole electron acceptor. In addition, it has also been recently shown that complete degradation of monoaromatic

hydrocarbons can also be coupled to the respiration of oxyanions of chlorine such as perchlorate or chlorate, or to the reduction of the quinone moieties of humic substances. Many pure cultures of hydrocarbon-degrading anaerobes now exist and some novel biochemical and genetic pathways have been identified. In general, a fumarate addition reaction is used as the initial activation step of the catabolic process of the corresponding monoaromatic hydrocarbon compounds. However, other reactions may alternatively be involved depending on the electron acceptor utilized or the compound being degraded. In the case of toluene, fumarate addition to the methyl group mediated by benzylsuccinate synthase appears to be the universal mechanism of activation and is now known to be utilized by anoxygenic phototrophs, nitrate-reducing, Fe(III)-reducing, sulfate-reducing, and methanogenic cultures. Many of these biochemical pathways produce unique extracellular intermediates that can be utilized as biomarkers for the monitoring of hydrocarbon degradation in anaerobic natural environments.

Lu Sun,[1] Hai-Hua Huang,[2] Lei Liu,[2] and Da-Fang Zhong[1*] (Laboratory of Drug Metabolism and Pharmacokinetics,[1] Department of Microbiology, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China[2]). Transformation of Verapamil by *Cunninghamella blakesleeana*. Applied and Environmental Microbiology, May 2004, p. 2722-2727, Vol. 70, No. 5.

A filamentous fungus, *Cunninghamella blakesleeana* AS 3.153, was used as a microbial model of mammalian metabolism to transform verapamil, a calcium channel antagonist. The metabolites of verapamil were separated and assayed by the liquid chromatography-ion trap mass spectrometry method. After 96 h of incubation, nearly 93% of the original drug was metabolized to 23 metabolites. Five major metabolites were isolated by semipreparative high-performance liquid chromatography and were identified by proton nuclear magnetic resonance and electrospray mass spectrometry. Other metabolites were characterized according to their chromatographic behavior and mass spectral data. The major metabolic pathways of verapamil transformation by the fungus were N dealkylation, O demethylation, and sulfate conjugation. The phase I metabolites of verapamil (introduction of a functional group) by *C. blakesleeana* paralleled those in mammals; therefore, *C. blakesleeana* could be a useful tool for generating the mammalian phase I metabolites of verapamil.

Mark Dopson,[1,†] Craig Baker-Austin,[1] Andrew Hind,[1] John P. Bowman,[2] and Philip L. Bond[1,3*]. (School of Biological Sciences,[1] Centre for Ecology, Evolution and Conservation, University of East Anglia, Norwich NR4 7TJ, United Kingdom,[3] School of Agricultural Science, University of Tasmania, Hobart 7001, Tasmania, Australia[2]). Characterization of *Ferroplasma* Isolates and *Ferroplasma acidarmanus* sp. nov., Extreme Acidophiles from Acid Mine Drainage and Industrial Bioleaching Environments. Applied and Environmental Microbiology, April 2004, p. 2079-2088, Vol. 70, No. 4.

Three recently isolated extremely acidophilic archaeal strains have been shown to be phylogenetically similar to *Ferroplasma acidiphilum* Y^T by 16S rRNA gene sequencing. All four *Ferroplasma* isolates were capable of growing chemoorganotrophically on yeast extract or a range of sugars and chemomixotrophically on ferrous iron and yeast extract or sugars, and isolate "*Ferroplasma acidarmanus*" $Fer1^T$ required much higher levels of organic carbon. All four isolates were facultative anaerobes, coupling chemoorganotrophic growth on yeast extract to the reduction of ferric iron. The temperature optima for the four isolates were between 35 and 42°C and the pH optima were 1.0 to 1.7, and "*F. acidarmanus*" $Fer1^T$ was capable of growing at pH 0. The optimum yeast extract concentration for "*F. acidarmanus*" $Fer1^T$ was higher than that for the other three isolates. Phenotypic results suggested that isolate "*F. acidarmanus*" $Fer1^T$ is of a

different species than the other three strains, and 16S rRNA sequence data, DNA-DNA similarity values, and two-dimensional polyacrylamide gel electrophoresis protein profiles clearly showed that strains DR1, MT17, and Y^T group as a single species. "*F. acidarmanus*" $Fer1^T$ groups separately, and we propose the new species "*F. acidarmanus*" $Fer1^T$ sp. nov.

Richard E. Williams,[1,†] Deborah A. Rathbone,[1,‡] Nigel S. Scrutton,[2] and Neil C. Bruce[1*] (Institute of Biotechnology, University of Cambridge, Cambridge CB2 1QT,[1] Department of Biochemistry, University of Leicester, Leicester LE1 7RH, United Kingdom[2]). Biotransformation of Explosives by the Old Yellow Enzyme Family of Flavoproteins. Applied and Environmental Microbiology, June 2004, p. 3566-3574, Vol. 70, No. 6.

Several independent studies of bacterial degradation of nitrate ester explosives have demonstrated the involvement of flavin-dependent oxidoreductases related to the old yellow enzyme (OYE) of yeast. Some of these enzymes also transform the nitroaromatic explosive 2,4,6-trinitrotoluene (TNT). In this work, catalytic capabilities of five members of the OYE family were compared, with a view to correlating structure and function. The activity profiles of the five enzymes differed substantially; no one compound proved to be a good substrate for all five enzymes. TNT is reduced, albeit slowly, by all five enzymes. The nature of the transformation products differed, with three of the five enzymes yielding products indicative of reduction of the aromatic ring. Our findings suggest two distinct pathways of TNT transformation, with the initial reduction of TNT being the key point of difference between the enzymes. Characterization of an active site mutant of one of the enzymes suggests a structural basis for this difference.

BIOMARKER

Aarab N, Champeau O, Mora P, Daubeze M, Garrigues P, Narbonne JF. (Laboratoire de Physico-Toxicochimie des systemes Naturels, UMR 5472 CNRS, Universite Bordeaux I, F-33405 Talence, France). Scoring approach based on fish biomarkers applied to French river monitoring. Biomarkers. 2004 May-Jun;9(3):258-70.

The aim was to apply a multimarker scoring approach as complementary to freshwater monitoring programmes carried out by the Water Agency Adour-Garonne. Fish (chub, barbel and trout) were collected in 11 sites in rivers in south-west France. Five biomarkers of response were measured either in muscle or brain for acetylcholinesterase (AChE) and in liver for glutathione S-transferase, catalase and 7-ethoxyresorufine O-deethylase. As a result of multivariate analysis, sites were clearly discriminated mainly by 7-ethoxyresorufine O-deethylase and acetylcholinesterase activities. According to the scoring approach, a multimarker pollution index was calculated for each sampling site as the sum of the response index of the five measured biomarkers (pollution index). Sorting was established by ranging the sites from lightly to highly contaminated locations.

Anderson JW, Hartwell SI, Hameedi MJ. (Columbia Analytical Services, Kelso, Washington 98626, USA). Regional comparisons of coastal sediment contamination detected by a biomarker (P450 HRGS; EPA Method 4425). Environ Sci Technol. 2005 Jan 1;39(1):17-23.

Pollution investigations by the Center for Coastal Monitoring and Assessment of the National Oceanic and Atmospheric Administration (NOAA) have been conducted since 1984 and have incorporated extensive biological and chemical analyses. Since 1993, one of the biological tests utilized in these studies has been the biomarker P450HRGS, which is more recently described as

EPA Method 4425. Extracts of sediments are applied to a human cell line with a reporter gene (firefly luciferase) at the CYP1A1 site. Light produced by the extracts is a function of the concentrations and potencies of those compounds with an affinity for Ah-receptor (certain polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and dioxins/ furans). These compounds are carcinogenic and can produce chronic toxicity, and those containing chlorine are persistent and bioaccumulated. Nineteen coastal regions and 1309 samples from the three U.S. coasts have been evaluated as part of the NOAA investigations. The stratified random sampling approach used by NOAA provides estimates of the areas (km2) of each region containing levels of the compounds above thresholds. From analysis of the database, sediments with concentrations at or below 11 microg benzo[a]pyrene equivalents (B[a]PEq)/g would not be expected to produce effects on the benthos. At 32 microg B[a]PEq/g and above there is the potential for impacts on the biota, and above 60 microg/g, the degradation of the benthic community has been observed. Several of the regional surveys found no samples at or above 60 microg B[a]PEq/g, but 60% of the samples from New York Harbor (280 km2) were above this level. Analyses of data from Puget Sound and Chesapeake Bay demonstrate an increase in samples above 32 microg B[a]PEq/g in more populated and industrial regions. Method 4425 serves as both a biomarker, simulating the response of an organism (with CYP1A) exposed to inducing compounds, and as a bioanalytical technique measuring the levels of these chemicals in the samples. A targeted investigation of the distribution of the three important classes of compounds identified by Method 4425 would be more cost-effective by first screening samples by this method before expending substantial funds in the detailed chemical analysis of all samples.

Berthoin K, Broeckaert F, Robin M, Haufroid V, De Burbure C, Bernard A. (Unit of Industrial Toxicology and Occupational Medicine, Faculty of Medicine, Catholic University of Louvain, B-12 Brussels, Belgium). Serum pneumoproteins and biomarkers of exposure to urban air pollution: a cross-sectional comparison of policemen and foresters. Biomarkers. 2004 Jul-Oct;9(4-5):341-52.

Very few biomarkers are available for the non-invasive detection of effects of urban air pollution on the respiratory tract. The objective was to evaluate whether Clara cell protein (CC16) and surfactant-associated protein-A (SP-A), two pulmonary secretory proteins, were useful in the detection of effects of urban air pollutants on the pulmonary epithelium. These proteins were determined in the serum of 53 policemen working in Brussels, Belgium, and a control group of 59 foresters working in the countryside. Except for ozone (O(3)), annual concentrations of the main air pollutants (PM(10), NO(2), CO, SO(2) and benzene) were significantly higher in Brussels than in the country. The proportion of smokers was lower in urban policemen compared with foresters, but they smoked on average a similar number of cigarettes per day as confirmed by their urinary excretion of cotinine. Muconic acid, a marker of benzene exposure, was significantly higher in urban policemen than in foresters, in both smokers and non-smokers. Multiple regression analysis showed that the type of work, smoking habits and time spent outdoors and in a car were significant determinants of benzene uptake. Tobacco smoking impaired lung function to a similar extent in urban policemen and foresters. The serum levels of SP-A were significantly increased in smokers but were not different between policemen and foresters. Serum CC16 was significantly reduced by tobacco smoking and slightly decreased in policemen compared with foresters. Interestingly, the reduction of serum CC16 was more pronounced in the subgroup of traffic compared with survey policemen, the latter being also less exposed to benzene. The results suggest that serum pneumoproteins and especially serum CC16 could be useful in the detection of chronic effects of urban air pollutants on the respiratory epithelium of populations particularly at risk.

Broeg K, Westernhagen HV, Zander S, Korting W, Koehler A. (Alfred-Wegener-Institute for Polor and Marine Research, Ecotoxicology, Am Handelshafen 12, 27570 Bremerhaven, Germany). The "bioeffect assessment index" (BAI) A concept for the quantification of effects of marine pollution by an integrated biomarker approach. Mar Pollut Bull. 2005 May;50(5):495-503.

The "bioeffect assessment index" (BAI) is based on the integration of several pathological endpoints measured in the liver of European flounder (Platichthys flesus (L.) during a long term study of biological effects of pollution in the German Bight. The BAI represents a modification of the "health assessment index" since it includes solely validated biomarkers reflecting toxically induced alterations at different levels of biological organisation in order to quantify the effects of environmental pollution. The concept of the BAI is based on the observation of progressive deleterious effects from early responses to late effects. Specific "key events" were detected, representing progressive stages of functional deterioration. The biomarkers selected from a whole battery of cellular markers for the BAI calculation reflect deleterious effects of various classes of contaminants such as heavy metals, organochlorines, pesticides, PAHs, and therefore reflect general toxicity in an integrative manner. Selected biomarkers were: lysosomal perturbations (reduced membrane stability), storage disorders (lipid accumulation) as early markers for toxic effects of liver cells, and the size of macrophage aggregates and their acid phosphatase activity. The latter two markers are indicative for the modulation of non-specific immune response which represents longer time scale responses after chronic exposure.

Carballo M, Jimenez JA, de la Torre A, Roset J, Munoz MJ. (Animal Health Research Centre-INIA, Division of Environmental Toxicology, 28130 Valdeolmos, Madrid, Spain). A survey of potential stressor-induced physiological changes in carp (Cyprinus carpio) and barbel (Barbus bocagei) along the Tajo River. Environ Toxicol. 2005 Apr;20(2):119-25.

The objective of this study was to evaluate fish response to acute stress induced by confinement after capture. Because of the previously reported presence of chemical compounds in the Tajo River basin where the study samplinig took place, an exposure biomarker to organic chemicals (retinol) was used. Cortisol and glucose were used as stress biomarkers. Plasma levels of cholesterol were used as lipidic metabolism indicators, and retinol level was used as a specific exposure biomarker. A reference site was established along 300 km of the Tajo River, and nine sampling sites were selected on the basis of whether various human activities and hydrographic characteristics were present. A total of 55 carp (Cyprinus carpio) and 52 barbel (Barbus bocagei) were examined. Cortisol and glucose levels were considered acceptable indicators of the response of the fish to induced stress. In the barbel, plasma retinol levels decreased at two of the sampling sites indicating possible exposure to organic compounds. The overall evaluation of these parameters enabled us to identify three sampling sites at which more studies should be carried out. The possible relationship between the health state of wild fish and the presence of organic compounds or sources of pollution was considered.

Cavas T, Ergene-Gozukara S. Faculty of Sciences and Letters, (Department of Biology, Mersin University, Mersin, Turkey). Micronucleus test in fish cells: A bioassay for in situ monitoring of genotoxic pollution in the marine environment. Environ Mol Mutagen. 2005 May 2.

To evaluate the use of native fish species for assessing genotoxic pollution in the marine environment, micronucleus (MN) analysis was performed in peripheral blood erythrocytes and gill cells of the grey mullet (Mugil cephalus) from three sampling stations off the southeastern Mediterranean coast of Turkey. The frequencies of blebbed, notched, and lobed nuclei and binucleated cells also were evaluated in peripheral erythrocytes. The sampling sites were chosen

on the basis of pollution levels; Karaduvar harbor, contaminated by different types of industrial effluents, and Mersin harbor, mainly contaminated by aromatic hydrocarbons, were selected as polluted areas. Erdemli harbor, a relatively unpolluted site, was used as the control area. Sampling was carried out at four different seasons. The frequencies of both micronuclei and other nuclear abnormalities (NAs) in mullets captured from polluted areas were significantly higher than those in mullets from the reference area. In general, gill cells had considerably higher MN frequencies than did erythrocytes, and genotoxic responses were higher in summer than in winter. The results of this study indicate that the MN test in fish is a suitable biomarker for in situ monitoring of genotoxic pollution in the marine environment. As demonstrated in this study, NAs other than micronuclei are also useful indices of chemical exposure and toxic responses. Therefore, measuring both micronuclei and NAs may increase the sensitivity of the test system. Environ. Mol. Mutagen., 2005. (c) 2005 Wiley-Liss, Inc.

de la Torre FR, Ferrari L, Salibian A. (Applied Ecophysiology Program, Basic Sciences Department, National University of Lujan, Casilla de Correo 221, B6700ZBA-Lujan, Argentina). Freshwater pollution biomarker: response of brain acetylcholinesterase activity in two fish species. Comp Biochem Physiol C Toxicol Pharmacol. 2002 Mar;131(3):271-80.

The effect of prolonged exposure at two sites along the Reconquista River (Argentina), a highly polluted peri-urban water body, on brain acetylcholinesterase (AChE, EC 3.1.1.7, acetylcholine acetylhydrolase) of two teleosts was examined. Caged Cyprinus carpio and field-captured Cnesterodon decemmaculatus were used as sentinel organisms. Eserine concentration inhibiting 50% of AChE activity (IC50) and inhibition kinetic parameters were also evaluated. Interspecies IC50 differences were found to agree with observed kinetic parameters (KA, ki and kc), indicating that carps were more sensitive to eserine. Data obtained disclosed spatial differences and demonstrated the high sensitivity of AChE activity as an exposure biomarker. Marked species-related differences were detected, showing that enzyme determination of C. decemmaculatus is more effective in highly polluted sites. Considering the river water physicochemical profile, observed changes in AChE activities can be partly attributed to long-lasting raised concentrations of dissolved heavy metals.

de la Torre FR, Ferrari L, Salibian A. (Applied Ecophysiology Program, Basic Sciences Department, National University of Lujan, Casilla de Correo 221, B6700ZBA Lujan, Argentina). Biomarkers of a native fish species (Cnesterodon decemmaculatus) application to the water toxicity assessment of a peri-urban polluted river of Argentina. Chemosphere. 2005 Apr;59(4) : 577-83.

Environmental monitoring by means of biomarker parameters assessed in different species is a useful tool. It has the advantage of providing a quantitative response as well as valuable information of ecological relevance on the chronic adverse effects caused by water pollution. The aim of this study was to assess the response of biochemical and physiological parameters of Cnesterodon decemmaculatus, a native teleost, simultaneously caught in two sites of Reconquista river, a highly polluted peri-urban river. This study compared the measured parameters with that of specimens of the same species captured in an unpolluted body water, and correlated the detected changes with the physicochemical profile of the water at each site. A comparison was made of selected parameters of gill, brain and liver and of somatic indices of fish collected from polluted and reference sites. The main parameters whose changes allowed to discriminate between sampling sites were gill (Na(+),K(+))-ATPase, brain acetylcholinesterase (AChE) and liver aminotransaminases activities; tissues' protein content and liver somatic index (LSI) were also sensitive biomarkers in brain and liver, respectively. The results showed that the

response of the measured biomarkers allowed for the differentiation of sampling sites according to their water quality and confirmed that Cnesterodon decemmaculatus may be a useful test organism for the biomonitoring of freshwater environments. In addition, the simultaneous measurement of the physicochemical parameters of the water samples showed a good correspondence between the biomarkers responses and the environmental chemical stress conditions.

Ford T, Jay J, Patel A, Kile M, Prommasith P, Galloway T, Sanger R, Smith K, Depledge M. (Department of Microbiology, Montana State University, Bozeman, Montana 59717, USA). Use of ecotoxicological tools to evaluate the health of New Bedford Harbor sediments: a microbial biomarker approach. Environ Health Perspect. 2005 Feb;113(2):186-91.

We have been investigating microbial communities in sediments from New Bedford Harbor (NBH), Massachusetts, USA, for a number of years. NBH is a U.S. Environmental Protection Agency-designated Superfund site heavily contaminated with polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and heavy metals. Microorganisms are thought to contribute to the fate and distribution of contaminants in NBH through a variety of mechanisms, including direct transformations and formation of soluble and insoluble species. Our more recent research has focused on changes in microbial community structure and function in response to exposure to toxic contaminants, with the ultimate goal of using microbes as ecotoxicological tools. Microbial diversity, as measured by restriction fragment-length polymorphism analysis, changes along pollution gradients, with an apparent increase in diversity at the most contaminated sites, concomitant with an increase in genetic relatedness. Current work on microbial communities examines the presence of arsenic-resistance genes in NBH isolates. In collaboration with the Plymouth Environmental Research Center, Plymouth University, United Kingdom, we have also used more conventional ecotoxicological approaches to examine the health of the NBH biota.

Geffard A, Amiard-Triquet C, Amiard JC. (Service d'Ecotoxicologie, CNRS GDR 1117, ISOMer, SMAB, 2, rue de la Houssiniere, BP 92208, 44322 Nantes Cedex 3, France). Do seasonal changes affect metallothionein induction by metals in mussels, Mytilus edulis? Ecotoxicol Environ Saf. 2005 Jun;61(2):209-220.

Mussels have been proposed as biomonitors of metal pollution based on the determination of metallothionein (MT) concentrations as a biomarker, but a comprehensive study taking into account both intersite and long-term temporal variations in MT and metal concentrations in different organs is lacking. Thus, the present study was designed to examine the concentrations of cytosolic and insoluble Cd, Cu, Zn, and MT in gills and digestive gland of mussels (Mytilus edulis) of homogeneous size and age obtained from aquaculture and kept on a reference site or translocated to a metal-rich site throughout their reproductive season (March-October 1997). Relatively significant binding of metals to the insoluble fraction was observed in both tissues. In the digestive gland, monthly MT concentrations were strongly correlated to cytosolic metal levels. Moreover, despite significant temporal variations, the grand mean MT concentration based on all individual determinations in the digestive gland (reference, n=54; transplants, n=50) was significantly higher in mussels from the metal-rich site. On the other hand, gill MT concentrations did not reflect metal contamination as reliably.

Kulhanek A, Trapp S, Sismilich M, Janku J, Zimova M. (Center of Environmental Hygiene, National Institute of Public Health, Srobarova 48, Prague 100 42, Czech Republic). Crop-specific human exposure assessment for polycyclic aromatic hydrocarbons in Czech soils. Sci Total Environ. 2005 Mar 1;339(1-3):71-80.

Polycyclic aromatic hydrocarbons (PAH) are pollutants frequently found in soils, particularly in urban areas. From polluted soils, the PAH can be taken up into crops and the consumption of these crops can result in a human health risk. We estimated the bioconcentration factors (BCF) in the edible plant tissues for PAH using crop-specific models for leafy vegetables, root vegetables, potatoes and tree fruits. The estimates were compared with results from the empirical regression of Travis and Arms (T&A) for above-ground vegetation. The comparison shows that the use of crop-specific models resulted in lower BCF values of pollutant concentrations in fruits, potatoes and leafy vegetables, particularly for the heavier PAH (M>220 g mol(-1)). However, the crop-specific models yielded higher BCF values for root vegetables (carrot) and leafy vegetables if the attached soil particles (1%) were considered. Consequently, the average daily intake of benzo(a)pyrene (BaP) by an adult Czech through fruits and vegetables was estimated with the crop-specific models to be 190 ng BaP per person, and 460 ng BaP per person with the T&A regression, for a soil concentration of 1 mg BaP kg(-1) soil (wet wt.). A virtually safe oral dose of BaP, as a marker of the carcinogenic PAH, was suggested by a European expert commission to be below 4.2 to 35 ng per person and day. Using these figures, an acceptable soil concentration of BaP was estimated for the purpose of crop production to be below 0.02 or 0.18 mg kg(-1) (wet wt.) with the crop-specific models, and below 0.01 or 0.08 mg kg(-1) (wet wt.) with T&A. The results demonstrate clearly the advantage of the crop-specific exposure assessment: it can be adapted to different food baskets and allows more effective risk assessment and management of soil pollution.

Pampanin DM, Marangon I, Volpato E, Campesan G, Nasci C. (Institute of Marine Science, ISMAR-CNR, Venice, Italy). Stress biomarkers and alkali-labile phosphate level in mussels (Mytilus galloprovincialis) collected in the urban area of Venice (Venice Lagoon, Italy). Environ Pollut. 2005 Jul;136(1):103-7.

In this study, a spatial and temporal survey at three sites located in the "canals" of the Venice historic centre (Italy) and at a reference site was undertaken to evaluate stress effects on mussels sampled in the Venice urban area, where raw sewage is discharged without treatment directly into the water. A battery of biomarkers (metallothionein, micronuclei, condition index and survival in air) was used to evaluate the stress condition of the animals. At the same time the alkali-labile phosphate assay (ALP) was performed in mussel' hemolymph with the aim to find an estrogenic effect biomarker in this mussel species. Biomarker results showed an impairment of the general health condition in the mussels coming from the urban area, in agreement with the chemical analysis. Significantly higher level of the ALP was found in male mussels sampled in April in the urban area, in comparison with the ones from the reference site ($P<0.001$). Finally, the PCA proved an easy and useful tool to summarize the obtained results, also able to classify the data to indicate a pollution gradient in the Venice urban area.

Sun Q, Wang XR, Ding SM, Yuan XF. (State Key Laboratory of Pollution Control and Resources Reuse, School of the Environment, Nanjing University, Nanjing 210093, People's Republic of China). Effects of interactions between cadmium and zinc on phytochelatin and glutathione production in wheat (*Triticum aestivum* L.). Environ Toxicol. 2005 Apr; 20(2):195-201.

It has been proposed that phytochelatins (PCs) act as a biomarker for the evaluation of metal toxicity. Little attention has been paid to the effects on metal combinations and glutathione (GSH), the most abundant cellular thiol. In the present study the effects of interactions between cadmium (Cd) and zinc (Zn) on PC and GSH production were examined in wheat tissue over 14 days' exposure. The results showed that the presence of Zn alleviated Cd toxicity, accompanied by a reduction of Cd uptake. Cd and Zn exposure increased PC-SH levels in concentration-, tissue- and time-dependent manners. Of the two metals, Cd was more effective than Zn in PC-SH production. Interactions of Cd and Zn with respect to PC-SH production may be synergistic or inhibitory, strongly depending on duration of exposure and concentration of the metal combinations. Cd also stimulated GSH production in concentration-, tissue- and time-dependent manners, whereas Zn had no significant effects on GSH levels. Compared to the presence of Cd alone, the presence of Zn reduced GSH levels in a tissue-dependent manner over the growth period. The results of the study suggest that metal interactions should be highly considered in the application of PCs and GSH as potential biomarkers for the evaluation of metal toxicity, as most metal-polluted natural environments are contaminated with more than one metal.

Sun Q, Wang XR, Ding SM, Yuan XF. (State Key Laboratory of Pollution Control and Resources Reuse, School of the Environment, Nanjing University, 22 Hankou Road, Nanjing 210093, PR China). Effects of exogenous organic chelators on phytochelatins production and its relationship with cadmium toxicity in wheat (*Triticum aestivum* L.) under cadmium stress. Chemosphere. 2005 Jun; 60(1):22-31.

Phytochelatins (PCs) have been proposed as a potential biomarker for metal toxicity. In this study, cadmium (Cd) toxicity, PCs production and their relationship in wheat under Cd stress were examined using various exogenous organic chelator-buffered nutrient solutions. Single Cd stress produced strong toxic effects, as indicated by decreases of growth parameters, high level of lipid peroxidation in leaf and overproduction of PCs in root. Exogenous organic chelators with proper dose more or less reduced Cd toxicity by increasing growth parameters and decreasing lipid peroxidation in leaves. Of organic chelators (EDTA, DTPA, citric acid, malic acid and oxalic acid), EDTA was the most effective in decreasing Cd toxicity in plants, followed by DTPA and citric acid. Simultaneously, the concentrations of Cd-induced PCs in roots decreased, and the greatest decrease was caused by application of EDTA and DTPA. Linearly positive relationships were observed between Cd toxicity and root PCs concentrations under the influences of organic chelators, particularly EDTA, DTPA and citric acid. Furthermore, present results provide stronger evidence that PCs synthesis in plant cells was related to free Cd ion concentrations, not total Cd, and demonstrate that the levels of PCs production in plants correlated well with toxic effects caused by the bioavailable Cd levels.

Swierzcek S, Abuknesha RA, Chivers I, Baranovska I, Cunningham P, Price RG. (Silesian Technical University, M. strzody str.9, 44-100 Gliwice, Poland). Enzyme-immunoassay for the determination of metallothionein in human urine: application to environmental monitoring. Biomarkers. 2004 Jul-Oct;9(4-5):331-40.

The objectives of this study were to develop an enzyme immunoassay for metallothioneins in human urine using a polyclonal antiserum and to demonstrate a possible relationship between the

level of this biomarker and heavy metal exposure. The antiserum was raised in sheep against horse metallothionein conjugated to carboxylated bovine serum albumin. The antibody was used to construct a two-step competitive ELISA procedure. Human urine was treated with activated charcoal powder to remove traces of metallothioneins and known amounts of pure metallothioneins were added to provide standards for a standard curve. Metallothionein levels were measured in two groups of children living in areas of mild and high environmental pollution due mainly to heavy metals. A comparison was made between the biomarker levels and the levels of cadmium and lead in urine samples in the two groups. A group of children from a non-polluted area acted as controls. The results show that the detected levels of metallothioneins appear to correspond to levels of the two heavy metals studied and that there was an apparent relationship to the environmental exposure. Thus according to results of this study the increase in the metallothionein excretion seems to provide an indication of previous of exposure to metals. The ELISA procedure is sensitive and robust and can be used to screen large numbers of samples and is more rapid than the physical procedures currently used for analysis of these proteins. The assay can therefore be used as an additional tool for screening at-risk populations where either environmental or occupational exposure to divalent heavy metals is suspected.

Wu RS, Siu WH, Shin PK. (Centre for Coastal Pollution and Conservation and Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong). Induction, adaptation and recovery of biological responses: Implications for environmental monitoring. Mar Pollut Bull. 2005 May 10.

A wide range of biological responses have been used to identify exposure to contaminants, monitor spatial and temporal changes in contamination levels, provide early warning of environmental deterioration and indicate occurrences of adverse ecological consequences. To be useful in environmental monitoring, a biological response must reflect the environmental stress over time in a quantitative way. We here argue that the time required for initial induction, maximum induction, adaptation and recovery of these stress responses must first be fully understood and considered before they can be used in environmental monitoring, or else erroneous conclusions (both false-negative and false-positive) may be drawn when interpreting results. In this study, data on initial induction, maximum induction, adaptation and recovery of stress responses at various biological hierarchies (i.e., molecular, biochemical, physiological, behavioral, cytological, population and community responses) upon exposure to environmentally relevant levels of contaminants (i.e., metals, oil, polycyclic aromatic hydrocarbons (PAHs), organochlorines, organophosphates, endocrine disruptors) were extracted from 922 papers in the biomarker literature and analyzed. Statistical analyses showed that: (a) many stress responses may decline with time after induction (i.e., adaptation), even if the level of stress remains constant; (b) times for maximum induction and recovery of biochemical responses are positively related; (c) there is no evidence to support the general belief that time for induction of responses at a lower biological hierarchy (i.e., molecular responses and biochemical responses) is shorter than that at higher hierarchy (i.e., physiological, cytological and behavioral responses), although longer recovery time is found for population and community responses; (d) there are significant differences in times required for induction and adaptation of biological responses caused by different types of contaminants; (e) times required for initial and maximum induction of physiological responses in fish are significantly longer than those in crustaceans; and (f) there is a paucity of data on adaptation and recovery of responses, especially those at population and community levels. The above analyses highlight: (1) the limitations and possible erroneous conclusions in the present use of biomarkers in biomonitoring programs, (2) the importance of understanding the details of temporal changes of biological responses before employing them in

environmental management, and (3) the suitability of using specific animal groups as bioindicator species.

Yamaoka K, Mitsunobu F, Kojima S, Shibakura M, Kataoka T, Hanamoto K, Tanizaki Y. (Department of Radiological Technology, Okayama University Medical School, Okayama, Japan). The elevation of p53 protein level and SOD activity in the resident blood of the Misasa radon hot spring district. J Radiat Res (Tokyo). 2005 Mar;46(1):21-4.

To clarify the mechanism by which radon hot springs prevent cancer or not, in this study, blood was collected from residents in the Misasa hot spring district and in a control district. The level of a representative cancer-suppressive gene, p53, and the activity of a representative antioxidant enzyme, superoxide dismutase (SOD), were analyzed as indices. The level of serum p53 protein in the males in the Misasa hot spring district was found to be 2-fold higher than that in the control district, which is a significant difference. In the females in the Misasa hot spring district, SOD activity was approximately 15% higher than that in the control district, which is also statistically significant, and exceeded the reference range of SOD activity despite advanced age. These results suggested that routine exposure of the residents in the Misasa hot spring district to radon at a concentration about 3 times higher than the national mean induces trace active oxygen in vivo, potentiating products of cancer-suppressive gene and antioxidant function. As the p53 protein level was high in the residents in the Misasa hot spring district, apoptosis of cancer cells may readily occur.

BIOFERTILIZER

Egamberdiyeva D, Qarshieva D, Davranov K. (Institute of Microbiology, Uzbek Academy of Sciences, A. Kadiriy str. 7B, Tashkent 700128, Uzbekistan). The Use of Bradyrhizobium to Enhance Growth and Yield of Soybean in Calcareous Soil in Uzbekistan. J Plant Growth Regul. 2004 Jun 1.

In this work the effect of inoculation with Bradyrhizobium japonicum S2492 on soybean (Glycine max (L) Merr) growth, nodulation and yield in nitrogen-deficient soil of Uzbekistan was studied. The field experiments were carried out in Tashkent Province of Uzbekistan in a randomized complete block design with four replicates of each treatment. The results revealed positive effects on growth, nodule number and yields of soybean after inoculation with B. japonicum S2492. The yield of soybean varieties was 48% higher for inoculated than for uninoculated plants. The effect of the inoculation was specific for variety but not for growth type. The protein and oil contents of seeds also increased after inoculation. It was concluded that B. japonicum S2492 can be considered as a biofertilizer for increasing the productivity of soybean in nitrogen-deficient soils in Uzbekistan.

Galleguillos C, Aguirre C, Miguel Barea J, Azcon R. (Instituto de Biologia, Universidad Catolica de Valparaiso, Avenida Brasil 2950, Casilla 4950, Valparaiso, Chile). Growth promoting effect of two Sinorhizobium meliloti strains (a wild type and its genetically modified derivative) on a non-legume plant species in specific interaction with two arbuscular mycorrhizal fungi. Plant Sci. 2000 Oct 16;159(1):57-63.

In the present study, we have investigated whether the ubiquitous rhizosphere soil organism Sinorhizobium meliloti has a plant growth promoting (PGP) effect on non-leguminous plant species. Such PGP activity was investigated for both a wild type strain and its genetically modified (GM) derivative, which had an enhanced biofertilizer capability. The PGP effect of

these rhizobial strains was tested in interaction with two arbuscular-mycorrhizal (AM) fungi: G. mosseae or G. intraradices on lettuce (Lactuca sativa L.) plants. Both rhizobial strains were efficient in increasing lettuce biomass and also induced modifications on root morphology, particularly in mycorrhizal plants; thus these strains behave as plant growth promoting rhizobacteria. In non-mycorrhizal plants, both strains exhibited a similar growth promoting effect on lettuce. However, both rhizobial strains differed in mycorrhizal plants with regard to (i) biomass production, (ii) the length of axis and lateral roots, and (iii) the number of lateral roots formed; effects which were, in turn, affected by the AM fungus involved. Microbial treatments were more effective on root growth and morphology at earlier developmental stages (20 days of plant growth) but, in a later stage (after 40 days), the microbial effects were more relevant at increasing plant biomass. The interaction between the GM rhizobial strain and G. mosseae produced the highest growth promoting effect (476% over control), in spite of the fact that G. intraradices showed a quicker and higher colonization ability than G. mosseae. Microbial interactions inducing PGP effects did not benefit AM colonization nor the succinate dehydrogenase activity in the AM fungal mycelium. Irrespective of the underlying mechanisms, which are being now investigated, the interactions between rhizobial strains, as free-living saprophs, and AM fungi are noteworthy, and depend on the microbial genotype involved.

Giri S, Pati BR. Microbiology Laboratory, (Department of Botany and Forestry, Vidyasagar University Midnapore-721102, West Bengal, India). A comparative study on phyllosphere nitrogen fixation by newly isolated Corynebacterium sp. & Flavobacterium sp. and their potentialities as biofertilizer. Acta Microbiol Immunol Hung. 2004;51(1-2):47-56.

A number of nitrogen fixing bacteria has been isolated from forest phyllosphere on the basis of nitrogenase activity. Among them two best isolates are selected and identified as Corynebacterium sp. AN1 & Flavobacterium sp. TK2 able to reduce 88 and 132 n mol of acetylene (10(8)cells(-1)h(-1)) respectively. They were grown in large amount and sprayed on the phyllosphere of maize plants as a substitute for nitrogenous fertilizer. Marked improvements in growth and total nitrogen content of the plant have been observed by the application of these nitrogen-fixing bacteria. An average 30-37% increase in yield was obtained, which is nearer to chemical fertilizer treatment. Comparatively better effect was obtained by application of Flavobacterium sp.

Ghosh C. (Centre for Environmental Management and Degraded Ecosystem, School of Environmental Studies, University of Delhi, New Delhi 110 007, India). Integrated vermi-pisciculture--an alternative option for recycling of solid municipal waste in rural India. Bioresour Technol. 2004 May;93(1):71-5.

Vermicomposts as a biofertilizer can be a great option for pond manuring as they never cause any long term harm to the soil like chemical fertilizer. In this study vegetable and horticulture waste was used as an important media for vermiculture. Three separate cemented tanks (6 m(3) each) were used in the system as control tank, vermicompost fertilized tank and inorganic fertilizer manured tank. Monoculture of fish was carried out with cat fish, Clarias batrachus. The produced earthworms were used as fish feed. Regular monitoring of water parameter was conducted in three different ponds. Specifically, the algal biomass variation was quite helpful in analyzing the behavior of the ponds. NPK value of soil samples were analyzed intermittently to know the eutrophication level. Despite the hot summer temperature in northern part of India, which is not ideal for fish growth, we have recorded an encouraging growth performance in organic manured pond along with inorganic fertilizer treated and control pond. Among

eutrophicated pond, the fish biomass from vermicompost fed pond showed an increasing trend compared to inorganic fertilizer treated pond. Water retention capacity of vermicompost pond soil was better in comparison to other ponds. Result shows that the low cost model by integrating two production system vermiculture and pisciculture could be a commercially and environmentally viable option.

Jayaraj J, Muthukrishnan S, Liang GH. (Department of Biochemistry, Kansas State University, Manhattan 66506, USA). Transfer of a plant chitinase gene into a nitrogen-fixing Azospirillum and study of its expression. Can J Microbiol. 2004 Jul;50(7):509-13.

Azospirillum is used extensively in rice and other cereal crops as a biofertilizer. There is a substantial opportunity to improve the efficiency of this bacterium through the transfer of genes of agricultural importance from other organisms. Chitinases are antifungal proteins, and expression of chitinase genes in Azospirillum would help to develop strains with potential antifungal activities. So far there are no reports about transfer of plant genes into Azospirillum and their expression. The present study was aimed at expressing an antifungal gene (a rice chitinase) of plant origin in Azospirillum brasilense. A rice chitinase cDNA (RC 7) that codes for a 35 kDa protein was subcloned into a broad host range plasmid pDSK519 under the control of LacZ promoter. The plasmid was mobilized into the nitrogen-fixing bacterium, Azospirillum brasilense strain SP51eFL1, through biparental mating. The conjugation frequency was in the range of 35-40 x 10(-6). The transconjugants grew in nitrogen-free media and fixed gaseous nitrogen in vitro. However, their growth and nitrogen-fixing ability were slightly less than those of the wild-type. Expression of the protein was demonstrated through western blotting of the total cell protein, which detected a 35 kDa band that was immuno-reactive to a barley chitinase antibody. The cell lysates also hydrolyzed various chitin substrates, which resulted in release of free sugars demonstrating the chitinase activity of transconjugants. The expressed protein also had antifungal activity as demonstrated by inhibition of growth of the plant pathogenic fungus, Rhizoctonia solani.

Kumar RS, Ayyadurai N, Pandiaraja P, Reddy AV, Venkateswarlu Y, Prakash O, Sakthivel N. (Department of Biotechnology, Pondicherry University, Kalapet, Pondicherry, India). Characterization of antifungal metabolite produced by a new strain Pseudomonas aeruginosa PUPa3 that exhibits broad-spectrum antifungal activity and biofertilizing traits. J Appl Microbiol. 2005;98(1):145-54.

To study the antifungal activity and plant beneficial traits of a broad-spectrum antagonistic fluorescent pseudomonad strain, PUPa3. METHODS AND RESULTS: Strain PUPa3 was isolated from the rhizosphere soil of rice and identified as Pseudomonas aeruginosa on the basis of biochemical tests and by comparison of 16S rDNA sequences. This bacterium exhibits a broad-spectrum antifungal activity towards phytopathogenic fungi. The antifungal metabolite by PUPa3 was extracted, purified and characterized using nuclear magnetic resonance (NMR) and mass spectroscopy (MS). Production of indole-3-acetic acid (IAA), siderophores, phosphatase and protease in PUPa3 was determined. Strain PUPa3 did not produce hydrogen cyanide, cellulase and pectinase. CONCLUSION: The antifungal metabolite produced by PUPa3 has been identified as phenazine-1-carboxamide (PCN) on the basis of NMR and MS data. Strain PUPa3 showed a broad-spectrum antifungal activity towards a range of phytopathogenic fungi. This bacterium also showed several plant growth-promoting traits but did not show the traits attributed to deleterious rhizobacteria. SIGNIFICANCE AND IMPACT OF THE STUDY: Present study reports the production of PCN as well as IAA for the first time by a saprophytic P.

aeruginosa strain PUPa3. Because of the production of siderophore, growth hormone, protease and phosphatase and its innate fungicidal potential, this strain can be used as biofertilizer and antagonist against a range of phytopathogenic fungi that infect rice, groundnut, tobacco, chili, mango, sugarcane, tea, cotton and banana.

Lem NW, Glick BR. (Biology Department, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1). Biotechnological uses of cyanobacteria. Biotechnol Adv. 1985;3(2):195-208.

Cyanobacteria (blue-green algae) are O(2)-evolving photosynthesizing prokaryotes that have an extensive history of use as a human food source and as a fertilizer in rice fields. They have also been recognized as an excellent source of vitamins and proteins and as such are found in health food stores in North America and elsewhere. Cyanobacteria have a great deal of potential as a source of fine chemicals, as a biofertilizer and as a source of renewable fuel. This potential is being realized as data from research in the areas of the physiology and chemistry of these organisms are gathered and as the knowledge of cyanobacterial genetics and genetic engineering increases. We review, here, the present (and possible future) uses of cyanobacteria and assess the state of the art with respect to the genetic manipulation of cyanobacteria.

Omel'ianets TG, Guloian TE, Filatova IN. A toxicological evaluation of an Agrobacterium radiobacter-based biological fertilizer. Mikrobiol Zh. 1992 May-Jun;54(3):40-3.

Pathogenic properties (for warm-blooded organisms) of the industrial strain Agrobacterium radiobacter (strain 204) and toxicity of biofertilizer on its base--rhizoagrin--have been studied. It is established that the studied microorganisms are avirulent, nontoxic, nontoxicogenic and may be recommended for making biopreparations. The preparation rhizoagrin is not toxic for warm-blooded animals and may be used as an alternative of chemical mineral fertilizers.

Rai UN, Pandey K, Sinha S, Singh A, Saxena R, Gupta DK. (Ecotoxicology and Bioremediation Laboratory, National Botanical Research Institute, Lucknow 226 001, India). Revegetating fly ash landfills with Prosopis juliflora L.: impact of different amendments and Rhizobium inoculation. Environ Int. 2004 May;30(3) : 293-300.

A revegetation trial was conducted to evaluate the feasibility of growing a legume species, Prosopis juliflora L., on fly ash ameliorated with combination of various organic amendments, blue-green algal biofertilizer and Rhizobium inoculation. Significant enhancements in plant biomass, photosynthetic pigments, protein content and in vivo nitrate reductase activity were found in the plants grown on ameliorated fly ash in comparison to the plants growing in unamended fly ash or garden soil. Higher growth was obtained in fly ash amended with blue-green algae (BGA) than farmyard manure or press mud (PM), a waste from sugar-processing industry, due to the greater contribution of plant nutrients, supply of fixed nitrogen and increased availability of phosphorus. Nodulation was suppressed in different amendments of fly ash with soil in a concentration-duration-dependent manner, but not with other amendments. Plants accumulated higher amounts of Fe, Mn, Cu, Zn and Cr in various fly ash amendments than in garden soil. Further, inoculation of the plant with a fly ash tolerant Rhizobium strain conferred tolerance for the plant to grow under fly ash stress conditions with more translocation of metals to the above ground parts. The results showed the potential of P. juliflora to grow in plantations on fly ash landfills and to reduce the metal contents of fly ash by bioaccumulation in its tissues.

BIOCOMPOSTING

Bohnel H, Lube K. (Institutes for Applied Biotechnology in the Tropics and for Tropical Animal Health, Georg-August-University, Gottingen, Germany). Clostridium botulinum and bio-compost. A contribution to the analysis of potential health hazards caused by bio-waste recycling. J Vet Med B Infect Dis Vet Public Health. 2000 Dec;47(10):785-95.

Bio-waste recycling and the production and use of bio-compost are politically encouraged in Europe. Quality control takes no consideration of pathogenic anaerobic spore formers, e.g. Clostridium botulinum. A protocol for health hazard analysis concerning this pathogen has been developed. Samples of marketed bio-compost were tested and results showed that about 50% of the tested samples contained C. botulinum. For the first time it has been shown that the use of bio-compost represents a health hazard to humans and animals, especially in the future when spores will have accumulated in the environment. The use of household bio-waste collected in 'bio-bins' is apparently one factor involved in the production of contaminated compost end-products. Environmental factors in the propagation of C. botulinum are discussed. The improvement of bio-waste recycling technology and management should be encouraged in order to minimize the health hazard caused by contaminated bio-compost.

BIOPESTICIDE

Yi-Hu Dong, Xi-Fen Zhang, Jin-Ling Xu, and Lian-Hui Zhang*. (Institute of Molecular and Cell Biology, National University of Singapore, Singapore 117609). Insecticidal *Bacillus thuringiensis* Silences *Erwinia carotovora* Virulence by a New Form of Microbial Antagonism, Signal Interference. Applied and Environmental Microbiology, February 2004, p. 954-960, Vol. 70, No. 2.

It is commonly known that bacteria may produce antibiotics to interfere with the normal biological functions of their competitors in order to gain competitive advantages. Here we report that *Bacillus thuringiensis* suppressed the quorum-sensing-dependent virulence of plant pathogen *Erwinia carotovora* through a new form of microbial antagonism, signal interference. *E. carotovora* produces and responds to acyl-homoserine lactone (AHL) quorum-sensing signals to regulate antibiotic production and expression of virulence genes, whereas *B. thuringiensis* strains possess AHL-lactonase, which is a potent AHL-degrading enzyme. *B. thuringiensis* did not seem to interfere with the normal growth of *E. carotovora*; rather, it abolished the accumulation of AHL signal when they were cocultured. In planta, *B. thuringiensis* significantly decreased the incidence of *E. carotovora* infection and symptom development of potato soft rot caused by the pathogen. The biocontrol efficiency is correlated with the ability of bacterial strains to produce AHL-lactonase. While all the seven AHL-lactonase-producing *B. thuringiensis* strains provided significant protection against *E. carotovora* infection, *Bacillus fusiformis* and *Escherichia coli* strains that do not process AHL-degradation enzyme showed little effect in biocontrol. Mutation of *aiiA*, the gene encoding AHL-lactonase in *B. thuringiensis*, resulted in a substantial decrease in biocontrol efficacy. These results suggest that signal interference mechanisms existing in natural ecosystems could be explored as a new version of antagonism for prevention of bacterial infections.

BIODEGRADATION

Agnes Pierwola,[1] Tomasz Krupinski,[1] Peter Zalupski,[2] Michael Chiarelli,[2] and Domenic Castignetti[1*]. (Departments of Biology,[1] Chemistry, Loyola University of Chicago, Chicago, Illinois 60626[2]). Degradation Pathway and Generation of Monohydroxamic Acids from the Trihydroxamate Siderophore Deferrioxamine B. Applied and Environmental Microbiology, February 2004, p. 831-836, Vol. 70, No. 2.

Siderophores are avid ferric ion-chelating molecules that sequester the metal for microbes. Microbes elicit siderophores in numerous and different environments, but the means by which these molecules reenter the carbon and nitrogen cycles is poorly understood. The metabolism of the trihydroxamic acid siderophore deferrioxamine B by a *Mesorhizobium loti* isolated from soil was investigated. Specifically, the pathway by which the compound is cleaved into its constituent monohydroxamates was examined. High-performance liquid chromatography and mass-spectroscopy analyses demonstrated that *M. loti* enzyme preparations degraded deferrioxamine B, yielding a mass-to-charge (*m/z*) 361 dihydroxamic acid intermediate and an *m/z* 219 monohydroxamate. The dihydroxamic acid was further degraded to yield a second molecule of the *m/z* 219 monohydroxamate as well as an *m/z* 161 monohydroxamate. These studies indicate that the dissimilation of deferrioxamine B by *M. loti* proceeds by a specific, achiral degradation and likely represents the reversal by which hydroxamate siderophores are thought to be synthesized.

Ahmet Colak and Saadettin Güner. (Department of Chemistry, Karadeniz Technical University, Trabzon 61080, Turkey). Polyhydroxyalkanoate degrading hydrolase-like activities by *Pseudomonas* sp. isolated from soil. International Biodeterioration & Biodegradation Volume 53, Issue 2, March 2004, Pages 103-109.

Degradation of polyhydroxyalkanoate (PHA) by three *Pseudomonas* spp., isolated from fuel-oil contaminated soil and identified as *Ps. fluorescens*, *Ps. aeruginosa* and *Ps. putida* on the basis of 16S rRNA sequence analysis, was investigated. Degradation of PHAs was determined as molecular weight loss measured by gel permeation chromatography and morphological changes in poly-3-hydroxybutyrate films observed by scanning electron microscopy. Both, decrease in molecular weight (7–17%) and weight loss (up to 29%) of solution-cast films were evidence of the isolates secreting an active depolymerase responsible for degradation of PHA. The kinetic parameters, *V*max and *K*m, for depolymerase activity of *Ps. aeruginosa* in the presence of *p*-nitrophenylbutyrate as substrate were determined to be 2.8 mol min-1 and 2.8 mM, respectively, in 50 mM phosphate buffer, pH 7.9, at 30°C. Stimulation of activity by Na+, K+, Ca2+ and Mg2+ at 1 mM concentration indicated a requirement for metal ions as a cofactor for activity. Inhibition of the extracellular depolymerases of the three strains in the presence of *p*-methylphenyl sulfonylfluoride, cyanide, azide, deoxycholate or EDTA confirms the presence of an enzyme in the isolates similar to poly(3-hydroxybutyrate) degrading hydrolases reported earlier.

Alois Orlita. (Laboratory of Industrial Microbiology, Janá kova 111O, Otrokovice 765 02, Czech Republic). Microbial biodeterioration of leather and its control: a review. International Biodeterioration & Biodegradation Volume 53, Issue 3 , April 2004, Pages 157-163.

Hides and leather can be damaged by bacteria, which are mainly responsible for the decomposition of untanned proteins (in raw hides and during soaking), and fungi, which thrive on tanned leathers containing carbohydrates, fats and proteins. A number of fungicides,

including 2-(thiocyanomethylthio)benzothiazole, in the wide range of biocidal products now available for preventing defects of biological origin in hides and leathers have been compared in efficacy tests and their penetration, absorption and distribution in tanned leather has been investigated. For protection against moulds it is possible to use a single active ingredient, but it is recommended that a combination of fungicides is used to improve performance by synergistic effects and by broadening the activity spectrum against moulds.

Anna A. Gorbushina[a], Jeroen Heyrman[b], Thomas Dornieden[a], M. Gonzalez-Delvalle[c], Wolfgang E. Krumbein[a], Leonila Laiz[c], Karin Petersen[a], Cesareo Saiz-Jimenez[c] and Jean Swings[b], (a AG Geomikrobiologie, ICBM, Oldenburg University, Postfach 2503, Oldenburg 26111, Germany; b Laboratory for Microbiology, Ghent University, K.L. Ledeganckstraat 35, Gent 9000, Belgium; c CSIC, Instituto de Recursos Naturales y Agrobiologia (IRNAS), CSIC, Apartado 1052, Sevilla 41080, Spain; d BCCM/LMG Culture Collection, K.L. Ledeganckstraat 35, Gent 9000, Belgium). Bacterial and fungal diversity and biodeterioration problems in mural painting environments of St. Martins church (Greene–Kreiensen, Germany). International Biodeterioration & Biodegradation ,Volume 53, Issue 1, January 2004, Pages 13-24.

Microbial biofilms were massively developing on the surfaces and within the painting layers of mural paintings of a parish church in Lower Saxony, which were exposed and restored in the end of 1970s. The causes of the heavy infections remained unclear. Within the frame of an European research project (ENV4-CT98-0705) these microbial infections were documented and analyzed. By scanning electron microscopy (SEM) and dissecting microscope analysis of mural painting fragments it was shown that the main biofilm formers were microscopic fungi with strong pigment development. Thirty-two fungal and 139 heterotrophic bacterial isolates were obtained by cultivation methods. Most of the fungi (32 isolates) were characterized by morphological methods and nutritional physiology (BIOLOG system) and identified as *Acremonium*, *Aspergillus*, *Cladosporium*, *Fusarium* and other imperfect fungal genera among which several melanized *Mycelia sterilia*. Representative bacterial strains were analyzed by 16S rDNA sequencing, the majority of bacteria belonged to the genera *Arthrobacter*, *Bacillus* and *Bacillus*-related genera. Isolated strains (both fungal and bacterial) belong to spore formers and thus could have been potentially stimulated to grow only by the transfer to the growth medium. The results of SEM analysis, cultivation experiments and visualization of microbial activity, confirm the hypothesis that the current microbial community is inactive and represent a stagnant microbial community developed after drastic environmental changes caused by an unfortunate conservation treatment.

Anthony Verdin, Anissa Lounès-Hadj Sahraoui and Roger Durand. (Laboratoire de Mycologie/ Phytopathologie/Environnement, Université du Littoral-Côte d'Opale, 17 avenue Blériot, BP 699, Calais Cedex 62228, France). Degradation of benzo[a]pyrene by mitosporic fungi and extracellular oxidative enzymes. International Biodeterioration & Biodegradation,Volume 53, Issue 2, March 2004, Pages 65-70.

The involvement of extracellular oxidative enzymes (laccase, lignin peroxidase and manganese-dependent peroxidase) in the degradation of benzo[a]pyrene, a high molecular weight polycyclic aromatic hydrocarbon, by three mitosporic fungi (Deuteromycetes) isolated from polluted soils was examined. These fungal strains were found to have different abilities to degrade benzo[a]pyrene: relative degradation percentages per unit biomass for *Trichoderma viride*, *Fusarium solani* and *Fusarium oxysporum* were approximately 39, 17 and 8, respectively. No peroxidase activities were detected in any of the fungal strains. Laccase activities were measured in *F. solani* and *F. oxysporum* cultures, but the specific activities assessed were similar in both

whether in the presence or absence of benzo[a]pyrene. Extracellular laccase and peroxidase activities were not induced by benzo[a]pyrene. Moreover, laccase activities of *F. oxysporum*, a poor benzo[a]pyrene degrader, were fivefold higher than in *F. solani*, a better benzo[a]pyrene degrader. The use of a laccase inhibitor, sodium azide, did not decrease benzo[a]pyrene degradation but the laccase activity was inhibited by 50%. The fact that *T. viride* degrades benzo[a]pyrene more efficiently, without any detectable laccase activity, confirms this result. In conclusion, in these three fungal strains, no apparent correlation between degradation percentage and the tested ligninolytic enzymes production could be shown in our culture conditions.

Ariana Fialová [a], Elke Boschke[b] and Thomas Bley [b]. (a Department of Cultivation Chemistry and Bioengineering, Institute of Chemical Technology in Prague, Technická 5, Prague 6, CZ - 166 28, Czech Republic; b Institute of Food Technology and Bioprocess Engineering, Technische Universität Dresden, Dresden DE-01062, Germany). Rapid monitoring of the biodegradation of phenol-like compounds by the yeast *Candida maltosa* using BOD measurements. International Biodeterioration & Biodegradation Volume 54, Issue 1, July 2004, Pages 69-76.

Since phenol-like compounds can have serious environmental effects, microbiological and biochemical features of their aerobic degradation are of great interest, and monitoring oxygen uptake can give valuable data about the processes involved. It is shown here that the AQUALYTIC® Sensomat System can provide reliable, continuous measurement of biological oxygen demand (BOD), and is suitable for small-scale studies of the degradation of phenol-like compounds by the soil-borne yeast *Candida maltosa*. It was found that *C. maltosa* can use phenol and catechol as sole sources of carbon and energy at concentrations up to 1.7 g l-1 and 1.5 g l-1, respectively, and it is unaffected by resorcinol, even at 2 g l-1. It can also co-metabolise *p*-cresol, but cannot utilise benzoate or salicylate. These results may have practical implications for the use of *C. maltosa* in soil bioremediation, e.g. in bioventing. Enzyme tests were also performed to help interpret the data. Phenol hydroxylase activity reached maximum levels at the beginning of the exponential phase during cultivation on phenol. After the phenol had been completely utilised, the enzyme was slowly degraded.

Benoit Van Aken,[*] Jong Moon Yoon, and Jerald L. Schnoor. (Department of Civil and Environmental Engineering, The University of Iowa, Iowa City, Iowa 52242). Biodegradation of Nitro-Substituted Explosives 2,4,6-Trinitrotoluene, Hexahydro-1,3,5-Trinitro-1,3,5-Triazine, and Octahydro-1,3,5,7-Tetranitro-1,3,5-Tetrazocine by a Phytosymbiotic *Methylobacterium* sp. Associated with Poplar Tissues (*Populus deltoides* x *nigra* DN34). Applied and Environmental Microbiology, January 2004, p. 508-517, Vol. 70, No. 1.

A pink-pigmented symbiotic bacterium was isolated from hybrid poplar tissues (*Populus deltoides* x *nigra* DN34). The bacterium was identified by 16S and 16S-23S intergenic spacer ribosomal DNA analysis as a *Methylobacterium* sp. (strain BJ001). The isolated bacterium was able to use methanol as the sole source of carbon and energy, which is a specific attribute of the genus *Methylobacterium*. The bacterium in pure culture was shown to degrade the toxic explosives 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazene (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine (HMX). [U-ring-^{14}C]TNT (25 mg liter^{-1}) was fully transformed in less than 10 days. Metabolites included the reduction derivatives amino-dinitrotoluenes and diamino-nitrotoluenes. No significant release of $^{14}CO_2$ was recorded from [^{14}C]TNT. In addition, the isolated methylotroph was shown to transform [U-^{14}C]RDX (20 mg liter^{-1}) and [U-^{14}C]HMX (2.5 mg liter^{-1}) in less than 40 days. After 55 days of incubation, 58.0% of initial [^{14}C]RDX and 61.4% of initial [^{14}C]HMX were mineralized into $^{14}CO_2$. The radioactivity remaining in solution accounted for 12.8 and 12.7% of initial [^{14}C]RDX and

[^{14}C]HMX, respectively. Metabolites detected from RDX transformation included a mononitroso RDX derivative and a polar compound tentatively identified as methylenedinitramine. Since members of the genus *Methylobacterium* are distributed in a wide diversity of natural environments and are very often associated with plants, *Methylobacterium* sp. strain BJ001 may be involved in natural attenuation or in situ biodegradation (including phytoremediation) of explosive-contaminated sites.

Bharat Bhushan,[1] Annamaria Halasz,[1] Jim C. Spain,[2] and Jalal Hawari[1*]. (Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada,[1] U.S. Air Force Research Laboratory, Tyndall Air Force Base, Florida 32403[2]). Initial Reaction(s) in Biotransformation of CL-20 Is Catalyzed by Salicylate 1-Monooxygenase from *Pseudomonas* sp. Strain ATCC 29352. Applied and Environmental Microbiology, July 2004, p. 4040-4047, Vol. 70, No. 7.

CL-20 (2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane) ($C_6H_6N_{12}O_{12}$), a future-generation high-energy explosive, is biodegradable by *Pseudomonas* sp. strain FA1 and *Agrobacterium* sp. strain JS71; however, the nature of the enzyme(s) involved in the process was not understood. In the present study, salicylate 1-monooxygenase, a flavin adenine dinucleotide (FAD)-containing purified enzyme from *Pseudomonas* sp. strain ATCC 29352, biotransformed CL-20 at rates of 0.256 ± 0.011 and 0.043 ± 0.003 nmol min^{-1} mg of protein^{-1} under anaerobic and aerobic conditions, respectively. The disappearance of CL-20 was accompanied by the release of nitrite ions. Using liquid chromatography/mass spectrometry in the negative electrospray ionization mode, we detected a metabolite with a deprotonated mass ion $[M - H]^-$ at 345 Da, corresponding to an empirical formula of $C_6H_6N_{10}O_8$, produced as a result of two sequential N denitration steps on the CL- 20 molecule. We also detected two isomeric metabolites with $[M - H]^-$ at 381 Da corresponding to an empirical formula of $C_6H_{10}N_{10}O_{10}$. The latter was a hydrated product of the metabolite $C_6H_6N_{10}O_8$ with addition of two H_2O molecules, as confirmed by tests using ^{18}O-labeled water. The product stoichiometry showed that each reacted CL-20 molecule produced about 1.7 nitrite ions, 3.2 molecules of nitrous oxide, 1.5 molecules of formic acid, and 0.6 ammonium ion. Diphenyliodonium-mediated inhibition of salicylate 1-monooxygenase and a comparative study between native, deflavo, and reconstituted enzyme(s) showed that FAD site of the enzyme was involved in the biotransformation of CL-20 catalyzed by salicylate 1-monooxygenase. The data suggested that salicylate 1-monooxygenase catalyzed two oxygen-sensitive single-electron transfer steps necessary to release two nitrite ions from CL-20 and that this was followed by the secondary decomposition of this energetic chemical.

Brajesh K. Singh,[1,2*] Allan Walker,[1] J. Alun W. Morgan,[1] and Denis J. Wright[2]. (Horticulture Research International, Wellesbourne, Warwick CV35 9EF,[1] Department of Biological Sciences, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, United Kingdom[2]). Biodegradation of Chlorpyrifos by *Enterobacter* Strain B-14 and Its Use in Bioremediation of Contaminated Soils. Applied and Environmental Microbiology, August 2004, p. 4855-4863, Vol. 70, No. 8.

Six chlorpyrifos-degrading bacteria were isolated from an Australian soil and compared by biochemical and molecular methods. The isolates were indistinguishable, and one (strain B-14) was selected for further analysis. This strain showed greatest similarity to members of the order *Enterobacteriales* and was closest to members of the *Enterobacter asburiae* group. The ability of the strain to mineralize chlorpyrifos was investigated under different culture conditions, and the

strain utilized chlorpyrifos as the sole source of carbon and phosphorus. Studies with ring or uniformly labeled [^{14}C]chlorpyrifos in liquid culture demonstrated that the isolate hydrolyzed chlorpyrifos to diethylthiophospshate (DETP) and 3, 5, 6-trichloro-2-pyridinol, and utilized DETP for growth and energy. The isolate was found to possess mono- and diphosphatase activities along with a phosphotriesterase activity. Addition of other sources of carbon (glucose and succinate) resulted in slowing down of the initial rate of degradation of chlorpyrifos. The isolate degraded the DETP-containing organophosphates parathion, diazinon, coumaphos, and isazofos when provided as the sole source of carbon and phosphorus, but not fenamiphos, fonofos, ethoprop, and cadusafos, which have different side chains. Studies of the molecular basis of degradation suggested that the degrading ability could be polygenic and chromosome based. Further studies revealed that the strain possessed a novel phosphotriesterase enzyme system, as the gene coding for this enzyme had a different sequence from the widely studied organophosphate-degrading gene (*opd*). The addition of strain B-14 (10^6 cells g^{-1}) to soil with a low indigenous population of chlorpyrifos-degrading bacteria treated with 35 mg of chlorpyrifos kg^{-1} resulted in a higher degradation rate than was observed in noninoculated soils. These results highlight the potential of this bacterium to be used in the cleanup of contaminated pesticide waste in the environment.

Carla A. Nicholson and Babu Z. Fathepure*. (Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, Oklahoma 74078-3020). Biodegradation of Benzene by Halophilic and Halotolerant Bacteria under Aerobic Conditions. Applied and Environmental Microbiology, February 2004, p. 1222-1225, Vol. 70, No. 2.

A highly enriched halophilic culture was established with benzene as the sole carbon source by using a brine soil obtained from an oil production facility in Oklahoma. The enrichment completely degraded benzene, toluene, ethylbenzene, and xylenes within 1 to 2 weeks. Also, [^{14}C]benzene was converted to $^{14}CO_2$, suggesting the culture's ability to mineralize benzene. Community structure analysis revealed that *Marinobacter* spp. were the dominant members of the enrichment.

Christy A. Smith and Michael R. Hyman*. (Department of Microbiology, North Carolina State University, Raleigh, North Carolina 27695). Oxidation of Methyl *tert*-Butyl Ether by Alkane Hydroxylase in Dicyclopropylketone-Induced and *n*-Octane-Grown *Pseudomonas putida* GPo1. Applied and Environmental Microbiology, August 2004, p. 4544-4550, Vol. 70, No. 8.

The alkane hydroxylase enzyme system in *Pseudomonas putida* GPo1 has previously been reported to be unreactive toward the gasoline oxygenate methyl *tert*-butyl ether (MTBE). We have reexamined this finding by using cells of strain GPo1 grown in rich medium containing dicyclopropylketone (DCPK), a potent gratuitous inducer of alkane hydroxylase activity. Cells grown with DCPK oxidized MTBE and generated stoichiometric quantities of *tert*-butyl alcohol (TBA). Cells grown in the presence of DCPK also oxidized *tert*-amyl methyl ether but did not appear to oxidize either TBA, ethyl *tert*-butyl ether, or *tert*-amyl alcohol. Evidence linking MTBE oxidation to alkane hydroxylase activity was obtained through several approaches. First, no TBA production from MTBE was observed with cells of strain GPo1 grown on rich medium without DCPK. Second, no TBA production from MTBE was observed in DCPK-treated cells of *P. putida* GPo12, a strain that lacks the alkane-hydroxylase-encoding OCT plasmid. Third, all *n*-alkanes that support the growth of strain GPo1 inhibited MTBE oxidation by DCPK-treated cells. Fourth, two non-growth-supporting *n*-alkanes (propane and *n*-butane) inhibited MTBE oxidation in a saturable, concentration-dependent process. Fifth, 1,7-octadiyne, a putative mechanism-

based inactivator of alkane hydroxylase, fully inhibited TBA production from MTBE. Sixth, MTBE-oxidizing activity was also observed in *n*-octane-grown cells. Kinetic studies with strain GPo1 grown on *n*-octane or rich medium with DCPK suggest that MTBE-oxidizing activity may have previously gone undetected in *n*-octane-grown cells because of the unusually high K_s value (20 to 40 mM) for MTBE.

Christel Béra-Maillet,[*] Yves Ribot, and Evelyne Forano[*]. (Unité de Microbiologie, INRA CR de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France). Fiber-Degrading Systems of Different Strains of the Genus *Fibrobacter*. Applied and Environmental Microbiology, April 2004, p. 2172-2179, Vol. 70, No. 4.

The S85 type strain of *Fibrobacter succinogenes*, a major ruminal fibrolytic species, was isolated 49 years ago from a bovine rumen and has been used since then as a model for extensive studies. To assess the validity of this model, we compared the cellulase- and xylanase-degrading activities of several other *F. succinogenes* strains originating from different ruminants, including recently isolated strains, and looked for the presence of 10 glycoside hydrolase genes previously identified in S85. The NR9 *F. intestinalis* type strain, representative of the second species of the genus, was also included in this study. DNA-DNA hybridization and 16S rRNA gene sequencing first classified the strains and provided the phylogenetic positions of isolates of both species. Cellulase and xylanase activity analyses revealed similar activity profiles for all *F. succinogenes* strains. However, the F_E strain, phylogenetically close to S85, presented a poor xylanolytic system and weak specific activities. Furthermore, the HM2 strain, genetically distant from the other *F. succinogenes* isolates, displayed a larger cellulolytic profile on zymograms and higher cellulolytic specific activity. *F. intestinalis* NR9 presented a higher cellulolytic specific activity and a stronger extracellular xylanolytic activity. Almost all glycoside hydrolase genes studied were found in the *F. succinogenes* isolates by PCR, except in the HM2 strain, and few of them were detected in *F. intestinalis* NR9. As expected, the fibrolytic genes of strains of the genus *Fibrobacter* as well as the cellulase and xylanase activities are better conserved in closely related phylogenetic isolates.

C. E. Milliken,[1] G. P. Meier,[2] J. E. M. Watts,[3] K. R. Sowers,[3] and H. D. May[1*]. (Department of Microbiology and Immunology,[1] Department of Pharmaceutical Sciences, Medical University of South Carolina, Charleston, South Carolina,[2] Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, Maryland[3]). Microbial Anaerobic Demethylation and Dechlorination of Chlorinated Hydroquinone Metabolites Synthesized by Basidiomycete Fungi. Applied and Environmental Microbiology, January 2004, p. 385-392, Vol. 70, No. 1.

The synthesis and degradation of anthropogenic and natural organohalides are the basis of a global halogen cycle. Chlorinated hydroquinone metabolites (CHMs) synthesized by basidiomycete fungi and present in wetland and forest soil are constituents of that cycle. Anaerobic dehalogenating bacteria coexist with basidiomycete fungi in soils and sediments, but little is known about the fate of these halogenated fungal compounds. In sediment microcosms, the CHMs 2,3,5,6-tetrachloro-1,4-dimethoxybenzene and 2,3,5,6-tetrachloro-4-methoxyphenol (TCMP) were anaerobically demethylated to tetrachlorohydroquinone (TCHQ). Subsequently, TCHQ was converted to trichlorohydroquinone and 2,5-dichlorohydroquinone (2,5-DCHQ) in freshwater and estuarine enrichment cultures. Screening of several dehalogenating bacteria revealed that *Desulfitobacterium hafniense* strains DCB2 and PCP1, *Desulfitobacterium chlororespirans* strain Co23, and *Desulfitobacterium dehalogenans* JW/DU1 sequentially dechlorinate TCMP to 2,3,5-trichloro-4-methoxyphenol and 3,5-dichloro-4-methoxyphenol (3,5-

DCMP). After a lag, these strains demethylate 3,5-DCMP to 2,6-DCHQ, which is then completely dechlorinated to 1,4-dihydroquinone (HQ). 2,5-DCHQ accumulated as an intermediate during the dechlorination of TCHQ to HQ by the TCMP-degrading desulfitobacteria. HQ accumulation following TCMP or TCHQ dechlorination was transient and became undetectable after 14 days, which suggests mineralization of the fungal compounds. This is the first report on the anaerobic degradation of fungal CHMs, and it establishes a fundamental role for microbial reductive degradation of natural organochlorides in the global halogen cycle.

Daisuke Ishiyama,† Dusica Vujaklija,‡ and Julian Davies*. (Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3). Novel Pathway of Salicylate Degradation by *Streptomyces* sp. Strain WA46. Applied and Environmental Microbiology, March 2004, p. 1297-1306, Vol. 70, No. 3.

A novel salicylate-degrading *Streptomyces* sp., strain WA46, was identified by UV fluorescence on solid minimal medium containing salicylate; trace amounts of gentisate were detected by high-pressure liquid chromatography when strain WA46 was grown with salicylate. PCR amplification of WA46 DNA with degenerate primers for gentisate 1,2-dioxygenase (GDO) genes produced an amplicon of the expected size. Sequential PCR with nested GDO primers was then used to identify a salicylate degradation gene cluster in a plasmid library of WA46 chromosomal DNA. The nucleotide sequence of a 13.5-kb insert in recombinant plasmid pWD1 (which was sufficient for the complete degradation of salicylate) showed that nine putative open reading frames (ORFs) (*sdgABCDEFGHR*) were involved. Plasmid pWD1 derivatives disrupted in each putative gene were transformed into *Streptomyces lividans* TK64. Disruption of either *sdgA* or *sdgC* blocked salicylate degradation; constructs lacking *sdgD* accumulated gentisate. Cell extracts from *Escherichia coli* DH5α transformants harboring pUC19 that expressed each of the *sdg* ORFs showed that conversions of salicylate to salicylyl-coenzyme A (CoA) and salicylyl-CoA to gentisyl-CoA required SdgA and SdgC, respectively. SdgA required CoA and ATP as cofactors, while NADH was required for SdgC activity; SdgC was identified as salicylyl-CoA 5-hydroxylase. Gentisyl-CoA underwent spontaneous cleavage to gentisate and CoA. SdgA behaved as a salicylyl-CoA ligase despite showing amino acid sequence similarity to an AMP-ligase. SdgD was identified as a GDO. These results suggest that *Streptomyces* sp. strain WA46 degrades salicylate by a novel pathway via a CoA derivative. Two-dimensional polyacrylamide gel electrophoresis and reverse transcriptase-PCR studies indicated that salicylate induced expression of the *sdg* cluster.

David L. Freedman,[1*] Meghna Swamy,[2] Nathan C. Bell,[3] and Mathew F. Verce[4]. (Department of Environmental Engineering and Science, Clemson University, Clemson, South Carolina 29634,[1] Shaw Environmental & Infrastructure, Inc., Stoughton, Massachusetts 02072,[2] Westinghouse Savannah River Company, Aiken, South Carolina 29808,[3] Lawrence Livermore National Laboratory, Livermore, California 94550[4]). Biodegradation of Chloromethane by *Pseudomonas aeruginosa* Strain NB1 under Nitrate-Reducing and Aerobic Conditions. Applied and Environmental Microbiology, August 2004, p. 4629-4634, Vol. 70, No. 8.

Pseudomonas aeruginosa strain NB1 uses chloromethane (CM) as its sole source of carbon and energy under nitrate-reducing and aerobic conditions. The observed yield of NB1 was 0.20 (±0.06) (mean ± standard deviation) and 0.28 (±0.01) mg of total suspended solids (TSS) mg of CM^{-1} under anoxic and aerobic conditions, respectively. The stoichiometry of nitrate consumption was 0.75 (±0.10) electron equivalents (eeq) of NO_3^- per eeq of CM, which is consistent with the yield when it is expressed on an eeq basis. Nitrate was stoichiometrically converted to dinitrogen (0.51 ± 0.05 mol of N_2 per mol of NO_3^-). The stoichiometry of oxygen

use with CM (0.85 ± 0.21 eeq of O_2 per eeq of CM) was also consistent with the aerobic yield. Stoichiometric release of chloride and minimal accumulation of soluble metabolic products (measured as chemical oxygen demand) following CM consumption, under anoxic and aerobic conditions, indicated complete biodegradation of CM. Acetylene did not inhibit CM use under aerobic conditions, implying that a monooxygenase was not involved in initiating aerobic CM metabolism. Under anoxic conditions, the maximum specific CM utilization rate (k) for NB1 was 5.01 (±0.06) μmol of CM mg of TSS^{-1} day^{-1}, the maximum specific growth rate (μ_{max}) was 0.0506 day^{-1}, and the Monod half-saturation coefficient (K_s) was 0.067 (±0.004) μM. Under aerobic conditions, the values for k, μ_{max}, and K_s were 10.7 (±0.11) μmol of CM mg of TSS^{-1} day^{-1}, 0.145 day^{-1}, and 0.93 (±0.042) μM, respectively, indicating that NB1 used CM faster under aerobic conditions. Strain NB1 also grew on methanol, ethanol, and acetate under denitrifying and aerobic conditions, but not on methane, formate, or dichloromethane.

Diane Fournier,[1] Annamaria Halasz,[1] Jim Spain,[2] Ronald J. Spanggord,[3] Jeffrey C. Bottaro,[3] and Jalal Hawari[1*]. (Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada,[1] U.S. Air Force Research Laboratory, Tyndall Air Force Base, Florida 32403,[2] SRI International, Menlo Park, California 94025[3]). Biodegradation of the Hexahydro-1,3,5-Trinitro-1,3,5-Triazine Ring Cleavage Product 4-Nitro-2,4-Diazabutanal by *Phanerochaete chrysosporium*. Applied and Environmental Microbiology, February 2004, p. 1123-1128, Vol. 70, No. 2.

Initial denitration of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by *Rhodococcus* sp. strain DN22 produces CO_2 and the dead-end product 4-nitro-2,4-diazabutanal (NDAB), $OHCNHCH_2NHNO_2$, in high yield. Here we describe experiments to determine the biodegradability of NDAB in liquid culture and soils containing *Phanerochaete chrysosporium*. A soil sample taken from an ammunition plant contained RDX (342 μmol kg^{-1}), HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; 3,057 μmol kg^{-1}), MNX (hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine; 155 μmol kg^{-1}), and traces of NDAB (3.8 μmol kg^{-1}). The detection of the last in real soil provided the first experimental evidence for the occurrence of natural attenuation that involved ring cleavage of RDX. When we incubated the soil with strain DN22, both RDX and MNX (but not HMX) degraded and produced NDAB (388 ± 22 μmol kg^{-1}) in 5 days. Subsequent incubation of the soil with the fungus led to the removal of NDAB, with the liberation of nitrous oxide (N_2O). In cultures with the fungus alone NDAB degraded to give a stoichiometric amount of N_2O. To determine C stoichiometry, we first generated [^{14}C]NDAB in situ by incubating [^{14}C]RDX with strain DN22, followed by incubation with the fungus. The production of $^{14}CO_2$ increased from 30 (DN22 only) to 76% (fungus). Experiments with pure enzymes revealed that manganese-dependent peroxidase rather than lignin peroxidase was responsible for NDAB degradation. The detection of NDAB in contaminated soil and its effective mineralization by the fungus *P. chrysosporium* may constitute the basis for the development of bioremediation technologies.

Farinazleen Mohamad Ghazali , Raja Noor Zaliha Abdul Rahman, Abu Bakar Salleh and Mahiran Basri. (Department of Biochemistry & Microbiology, Enzyme and Microbial Technology Research, Faculty of Science and Environmental Studies, Universiti Putra Malaysia, UPM Serdang, Selangor 43400, Malaysia). Biodegradation of hydrocarbons in soil by microbial consortium. International Biodeterioration & Biodegradation Volume 54, Issue1, July 2004, Pages 61-67.

The bioremediation of hydrocarbon in contaminated soils by mixed cultures of hydrocarbon-degrading bacteria was investigated. The mixtures or consortia of bacteria, denoted as Consortium 1 and Consortium 2 consisted of 3 and 6 bacterial strains, respectively. Bacterial

strains used in this study were from the Center for Research in Enzymes and Microbiology (CREAM) collection of strains, at Universiti Putra Malaysia, and were isolated from hydrocarbon-contaminated soil samples by enrichments on either crude oil or individual hydrocarbons as the sole carbon source. The strains were selected based on the criteria that they were able to display good growth in crude oil, individual hydrocarbon compounds or both. Their ability to degrade hydrocarbon contamination in the environment was investigated using soil samples that were contaminated with diesel, crude oil or engine oil. Consortium 2, which consisted of 6 bacterial strains, was more efficient at removing the medium- and long-chain alkanes in the diesel-contaminated soil compared to Consortium 1. Further, Consortium 2 could effectively remove the medium- and long-chain alkanes in the engine oil such that the alkanes were undetectable after a 30-day incubation period. Consortium 2 consisted predominantly of *Bacillus* and *Pseudomonas* spp.

Jadwiga Szostak-Kotowa. (Department of Microbiology, Cracow University of Economics str. Rakowicka 27, Katedra Mikrobiologii, Akademia Ekonomiczna w Krakowie, 31–510, Kraków, Poland). Biodeterioration of textiles. Volume 53, Issue 3 , April 2004, Pages 165-170.

Textiles, particularly those composed of natural organic fibres such as cotton, linen, wool, etc., are readily attacked by microorganisms. Most synthetic fabrics are not readily subject to extensive biodeterioration, but some processing and finishing agents are susceptible to microbial spoilage. Microorganisms can affect all stages of textile processing and storage, with fungi being the most important microorganisms in textile biodeterioration processes. Microbial growth on a textile causes loss of strength and elongation, discolouration and changes in appearance. They follow changes in oxidation state, degree of polymerization and breakdown of molecular structure. There are two main ways of textile protection—control of the environmental physical conditions and treatments with biocides.

Joanna D. Moody,[1] James P. Freeman,[2] Peter P. Fu,[3] and Carl E. Cerniglia[1*]. (Division of Microbiology,[1] Division of Chemistry,[2] Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, Arkansas 72079[3]). Degradation of Benzo[*a*]pyrene by *Mycobacterium vanbaalenii* PYR-1. Applied and Environmental Microbiology, January 2004, p. 340-345, Vol. 70, No. 1.

Metabolism of the environmental pollutant benzo[*a*]pyrene in the bacterium *Mycobacterium vanbaalenii* PYR-1 was examined. This organism initially oxidized benzo[*a*]pyrene with dioxygenases and monooxygenases at C-4,5, C-9,10, and C-11,12. The metabolites were separated by reversed-phase high-performance liquid chromatography (HPLC) and characterized by UV-visible, mass, nuclear magnetic resonance, and circular dichroism spectral analyses. The major intermediates of benzo[*a*]pyrene metabolism that had accumulated in the culture media after 96 h of incubation were *cis*-4,5-dihydro-4,5-dihydroxybenzo[*a*]pyrene (benzo[*a*]pyrene *cis*-4,5-dihydrodiol), *cis*-11,12-dihydro-11,12-dihydroxybenzo[*a*]pyrene (benzo[*a*]pyrene *cis*-11,12-dihydrodiol), *trans*-11,12-dihydro-11,12-dihydroxybenzo[*a*]pyrene (benzo[*a*]pyrene *trans*-11,12-dihydrodiol), 10-oxabenzo[*def*]chrysen-9-one, and hydroxymethoxy and dimethoxy derivatives of benzo[*a*]pyrene. The *ortho*-ring fission products 4-formylchrysene-5-carboxylic acid and 4,5-chrysene-dicarboxylic acid and a monocarboxylated chrysene product were formed when replacement culture experiments were conducted with benzo[*a*]pyrene *cis*-4,5-dihydrodiol. Chiral stationary-phase HPLC analysis of the dihydrodiols indicated that benzo[*a*]pyrene *cis*-4,5-dihydrodiol had 30% 4*S*,5*R* and 70% 4*R*,5*S* absolute stereochemistry. Benzo[*a*]pyrene *cis*-11,12-dihydrodiol adopted an 11*S*,12*R* conformation with 100% optical purity. The enantiomeric

composition of benzo[*a*]pyrene *trans*-11,12-dihydrodiol was an equal mixture of 11*S*,12*S* and 11*R*,12*R* molecules. The results of this study, in conjunction with those of previously reported studies, extend the pathways proposed for the bacterial metabolism of benzo[*a*]pyrene. Our study also provides evidence of the stereo- and regioselectivity of the oxygenases that catalyze the metabolism of benzo[*a*]pyrene in *M. vanbaalenii* PYR-1.

Jang JH, Hirai M, Shoda M. (Chemical Resources Laboratory, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-Ku, 226-8503, Yokohama, Japan). Styrene degradation by Pseudomonas sp. SR-5 in biofilters with organic and inorganic packing materials. Appl Microbiol Biotechnol. 2004 Aug;65(3):349-55.

Pseudomonas sp. SR-5 was isolated as a styrene-degrading bacterium. In liquid culture containing 1% (v/v) styrene, more than 90% styrene was degraded in 53 h and the doubling time of SR-5 was 2 h. The removal of styrene gas was investigated in biofilters for 31 days using an organic packing material of peat and an inorganic packing material of ceramic inoculated with SR-5. The maximum-styrene-elimination capacities for peat and ceramic packing materials were 236 and 81 g m(-3) h(-1), respectively. The percentage of styrene converted to low molecular weight compounds including CO(2) in the peat and ceramic biofilters during a 10-day operation were estimated to be 90.4 and 36.7%, respectively. As the pressure drop in the peat bioflter at the end of experiment was significantly higher than that in ceramic biofilter, a biofilter using a mixture of peat and ceramic was tested. We determined that the maximum elimination capacity was 170 g m(-3) h(-1) and the production of low molecular weight compounds was 95% at a low pressure drop for this mixed packing material filter.

Jody Jellison[a], Jon Connolly[a], Barry Goodell[a], Brian Doyle[a], Barbara Illman[b], Frank Fekete[a] and Andrea Ostrofsky[a]. (a University of Maine, Orono, Maine, USA; b Forest Products Laboratory, Madison, Wisconsin, USA). The role of cations in the biodegradation of wood by the brown rot fungi. International Biodeterioration & Biodegradation Volume 39, Issues 2-3.

This review describes what is presently known about the role of positively charged ions in the colonization and degradation of wood by brown rot fungi. General patterns of cation accumulation and the roles of iron, manganese, calcium and other cations in the fungal environment are discussed. The physiology of brown rot fungi and mechanisms of wood cell wall breakdown are emphasized.

Kramer C, Kreisel G, Fahr K, Kassbohrer J, Schlosser D. (Biogeochemical Processes Research Group, Max Planck Institute for Biogeochemistry, 07745 Jena, Germany). Degradation of 2-fluorophenol by the brown-rot fungus Gloeophyllum striatum: evidence for the involvement of extracellular Fenton chemistry. Appl Microbiol Biotechnol. 2004 Apr;64(3):387-95.

Iron-containing liquid cultures of the brown-rot basidiomycete Gloeophyllum striatum degraded 2-fluorophenol. Two simultaneously appearing degradation products, 3-fluorocatechol and catechol, were identified by gas chromatography and mass spectrometry (GC-MS). Concomitantly, fluoride was produced at approximately 50% of the amount that theoretically could be achieved upon complete dehalogenation. Defluorination was strongly inhibited in the presence of either the hydroxyl radical scavenger mannitol or superoxide dismutase, as well as in the absence of iron. The addition of the natural iron chelator oxalate caused a clear but less extensive inhibition, whereas supplementation with the artificial iron chelator nitrilotriacetic acid increased fluoride production. Extracellular 2-fluorophenol degradation was evidenced by

defluorination, observed upon addition of 2-fluorophenol to cell-free culture supernatants derived from iron-containing fungal cultures. Ultrafiltered culture supernatants oxidized methanol to formaldehyde, known as a product of the reaction of methanol with hydroxyl radical. In addition, G. striatum was found to produce metabolites extractable with ethyl acetate that are capable of reducing Fe3+. GC-MS analysis of such extracts revealed the presence of several compounds. The mass spectrum of a prominent peak matched those previously reported for 2,5-dimethoxyhydroquinone and 4,5-dimethoxycatechol, fungal metabolites implicated to drive hydroxyl radical production in Gloeophyllum. Taken together, these findings further support an extracellular Fenton-type mechanism operative during halophenol degradation by G. striatum.

Leonid S. Pinchuk , Anna V. Makarevich, Galina M. Vlasova , Alexander G. Kravtsov and Vitalyi A. Shapovalov. (Department of Hermetology, V. A. Belyi Metal-Polymer Research Institute of National Academy of Sciences of Belarus, 32a Kirov Street, Gomel 246050, Belarus). Electret-thermal analysis to assess biodegradation of polymer composites. International Biodeterioration & Biodegradation Volume 54, Issue 1 , July 2004, Pages 13-18.

Electret-thermal analysis (ETA) commonly used to study electric polarization of dielectrics has been for the first time applied to assess and examine biodegradation of polymer composites containing low-density polyethylene and cornstarch. Thermally stimulated currents (TSC) spectra for each polymer composite displayed characteristic features. Data showed that TSC spectra varied with the time of composite residence in the soil. Biodegradation of the starch filler particles and oxidation and biodeterioration of polyethylene binder were found to be responsible for the observed TSC spectral changes. ETA results agreed well with other assessment methods, including infrared spectroscopy, differential-thermal analysis, scanning electron microscopy and physico-mechanical testing. Investigation of biodegradation of polymer composites using ETA has proved to be a reliable tool to show biological damage.

Marcio L. B. Da Silva and Pedro J. J. Alvarez[*]. (Department of Civil and Environmental Engineering, University of Iowa, Iowa City, Iowa 52242). Enhanced Anaerobic Biodegradation of Benzene-Toluene-Ethylbenzene-Xylene-Ethanol Mixtures in Bioaugmented Aquifer Columns. Applied and Environmental Microbiology, August 2004, p. 4720-4726, Vol. 70, No. 8.

Methanogenic flowthrough aquifer columns were used to investigate the potential of bioaugmentation to enhance anaerobic benzene-toluene-ethylbenzene-xylene (BTEX) degradation in groundwater contaminated with ethanol-blended gasoline. Two different methanogenic consortia (enriched with benzene or toluene and *o*-xylene) were used as inocula. Toluene was the only hydrocarbon degraded within 3 years in columns that were not bioaugmented, although anaerobic toluene degradation was observed after only 2 years of acclimation. Significant benzene biodegradation (up to 88%) was observed only in a column bioaugmented with the benzene-enriched methanogenic consortium, and this removal efficiency was sustained for 1 year with no significant decrease in permeability due to bioaugmentation. Benzene removal was hindered by the presence of toluene, which is a more labile substrate under anaerobic conditions. Real-time quantitative PCR analysis showed that the highest numbers of *bssA* gene copies (coding for benzylsuccinate synthase) occurred in aquifer samples exhibiting the highest rate of toluene degradation, which suggests that this gene could be a useful biomarker for environmental forensic analysis of anaerobic toluene bioremediation potential. *bssA* continued to be detected in the columns 1 year after column feeding ceased, indicating the robustness of the added catabolic potential. Overall, these results suggest that anaerobic

bioaugmentation might enhance the natural attenuation of BTEX in groundwater contaminated with ethanol-blended gasoline, although field trials would be needed to demonstrate its feasibility. This approach may be especially attractive for removing benzene, which is the most toxic and commonly the most persistent BTEX compound under anaerobic conditions.

Priyangshu Manab Sarma,[1] Dhruva Bhattacharya,[1] S. Krishnan,[1] and Banwari Lal[2*] (Center of Bioresources and Biotechnology, TERI School of Advanced Studies,[1] Microbial Biotechnology, The Energy and Resources Institute, New Delhi, India 110003[2]). Degradation of Polycyclic Aromatic Hydrocarbons by a Newly Discovered Enteric Bacterium, *Leclercia adecarboxylata.* Applied and Environmental Microbiology, May 2004, p. 3163-3166, Vol. 70, No. 5.

A bacterial strain, PS4040, capable of degrading polycyclic aromatic hydrocarbons for use as the sole carbon source was isolated from oily-sludge-contaminated soil. The 16S rRNA gene showed 98.8% homology to that of *Leclercia adecarboxylata*. Comparative molecular typing with the clinical strain of *L. adecarboxylata* revealed that there were few comigrating and few distinct amplimers among them.

R. Borja[a], B. Rincón [a], F. Raposo [a], E. Sánchez [a] and A. Martín [b]. (a Instituto de la Grasa (CSIC), Avda Padre García Tejero 4, 41012, Sevilla, Spain; b Departamento de Ingeniería Química, Facultad de Ciencias, Campus Universitario de Rabanales, Edificio C3, Ctra Madrid-Cádiz, Km 396,14071 Córdoba, Spain). Assessment of kinetic parameters for the mesophilic anaerobic biodegradation of two-phase olive pomace. International Biodeterioration & Biodegradation Volume 53, Issue 2 , March 2004, Pages 71-78.

A kinetic study of the anaerobic biodegradation of two-phase olive pomace (TPOP) was carried out using a laboratory-scale stirred tank reactor. The reactor was operated at 35°C. The influent contained between 20% and 100% TPOP, representing in terms of total chemical oxygen demand (TCOD) 34.5–187.9 g TCOD l-1. The hydraulic retention times (HRTs) were set between 40.0 and 8.3 d. It was found that the increase of influent substrate concentration favoured the process failure reducing the pH and increasing the ratio of the total volatile fatty acid (TVFA) to alkalinity. This ratio was found to be proportional to the substrate concentration (*S*), as follows: TVFA/alkalinity=0.04(*S*). The kinetic model of Andrews was used to describe the relation between anaerobic biodegradation of TCOD and Volatile Solids (VS) and the formation of methane. The values of the kinetic constants for TCOD removal were determined to be 28 g TCOD l-1 d-1, 27 g TCOD l-1 and 352 g TCOD l-1, respectively, for maximum substrate utilization rates (*R*Smax), saturation constant (*K*S) and inhibition constant (*K*i). Process inhibition started at substrate concentrations of around 20 g TCOD l-1. For VS biodegradation the kinetic constant values, *R*Smax, *K*S and *K*i, were 45g VS l-1d-1, 37 and 36 g VS l-1, respectively. Inhibition started at VS concentration of around 18 g l-1. The rates of TCOD removal were lower than those observed for VS removal and inhibition of VS removal occurred at a lower concentration compared to that for TCOD. The *Q*M(max), *K*S and *K*i constants for methane production were approximately 3.1 l CH4 l-1 reactor d-1, 8.7 g TCOD l-1 and 272 g TCOD l-1, respectively. Inhibition of methane formation started at a substrate concentration of around 17 g TCOD l-1. TCOD and VS removal rates were higher than the rate of methane formation and these differences increased when the substrate concentration increased. This fact was underlined by the decrease of pH, the increase of TVFA/alkalinity ratio and the reduction of methane production rate.

S. C. Corgié, T. Beguiristain, and C. Leyval*. LIMOS (Laboratoire des Interactions Microorganismes-Minéraux-Matière Organique dans les Sols, UMR 7137 CNRS-UHP Nancy I, Faculté des Sciences, 54506 Vandoeuvre-les-Nancy Cedex, France). Spatial Distribution of Bacterial Communities and Phenanthrene Degradation in the Rhizosphere of *Lolium perenne* L. Applied and Environmental Microbiology, June 2004, p. 3552-3557, Vol. 70, No. 6.

Rhizodegradation of organic pollutants, such as polycyclic aromatic hydrocarbons, is based on the effect of root-produced compounds, known as exudates. These exudates constitute an important and constant carbon source that selects microbial populations in the plant rhizosphere, modifying global as well as specific microbial activities. We conducted an experiment in two-compartment devices to show the selection of bacterial communities by root exudates and phenanthrene as a function of distance to roots. Using direct DNA extraction, PCR amplification, and thermal gradient gel electrophoresis screening, bacterial population profiles were analyzed in parallel to bacterial counts and quantification of phenanthrene biodegradation in three layers (0 to 3, 3 to 6, and 6 to 9 mm from root mat) of unplanted-polluted (phenanthrene), planted-polluted, and planted-unpolluted treatments. Bacterial community differed as a function of the distance to roots, in both the presence and the absence of phenanthrene. In the planted and polluted treatment, biodegradation rates showed a strong gradient with higher values near the roots. In the nonplanted treatment, bacterial communities were comparable in the three layers and phenanthrene biodegradation was high. Surprisingly, no biodegradation was detected in the section of planted polluted treatment farthest from the roots, where the bacterial community structure was similar to those of the nonplanted treatment. We conclude that root exudates and phenanthrene induce modifications of bacterial communities in polluted environments and spatially modify the activity of degrading bacteria.

Wilfred F. M. Röling,[1,†] Michael G. Milner,[1] D. Martin Jones,[1] Francesco Fratepietro,[1] Richard P. J. Swannell,[2] Fabien Daniel,[2] and Ian M. Head[1*] (School of Civil Engineering and Geosciences and Centre for Molecular Ecology, University of Newcastle, Newcastle upon Tyne NE1 7RU,[1] AEA Technology, Didcot, Oxfordshire OX11 OQJ, United Kingdom[2]). Bacterial Community Dynamics and Hydrocarbon Degradation during a Field-Scale Evaluation of Bioremediation on a Mudflat Beach Contaminated with Buried Oil. Applied and Environmental Microbiology, May 2004, p. 2603-2613, Vol. 70, No. 5.

A field-scale experiment with a complete randomized block design was performed to study the degradation of buried oil on a shoreline over a period of almost 1 year. The following four treatments were examined in three replicate blocks: two levels of fertilizer treatment of oil-treated plots, one receiving a weekly application of liquid fertilizer and the other treated with a slow-release fertilizer; and two controls, one not treated with oil and the other treated with oil but not with fertilizer. Oil degradation was monitored by measuring carbon dioxide evolution and by chemical analysis of the oil. Buried oil was degraded to a significantly greater extent in fertilized plots, but no differences in oil chemistry were observed between the two different fertilizer treatments, although carbon dioxide production was significantly higher in the oil-treated plots that were treated with slow-release fertilizer during the first 14 days of the experiment. Bacterial communities present in the beach sediments were profiled by denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S rRNA gene fragments and 16S rRNA amplified by reverse transcriptase PCR. Similarities between the DGGE profiles were calculated, and similarity matrices were subjected to statistical analysis. These analyses showed that although significant hydrocarbon degradation occurred both in plots treated with oil alone and in the plots treated with oil and liquid fertilizer, the bacterial community structure in these plots was, in general, not significantly different from that in the control plots that were not treated with

oil and did not change over time. In contrast, the bacterial community structure in the plots treated with oil and slow-release fertilizer changed rapidly, and there were significant differences over time, as well as between blocks and even within plots. The differences were probably related to the higher concentrations of nutrients measured in interstitial water from the plots treated with slow-release fertilizer. Bacteria with 16S rRNA sequences closely related (>99.7% identity) to *Alcanivorax borkumensis* and *Pseudomonas stutzeri* sequences dominated during the initial phase of oil degradation in the plots treated with slow-release fertilizer. Field data were compared to the results of previous laboratory microcosm experiments, which revealed significant differences.

Yanzhen Fan[a], Yingying Wang[a], Pei-Yuan Qian[b] and Ji-Dong Gu[a, c]. (a Laboratory of Environmental Toxicology, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, PR China; b Department of Biology, Hong Kong University of Science and Technology, Clearwater Bay, Kowloon, Hong Kong SAR, PR China; c The Swire Institute of Marine Science, The University of Hong Kong, Shek O, Cape d'Aguilar, Hong Kong SAR, PR China). Optimization of phthalic acid batch biodegradation and the use of modified Richards model for modelling degradation. International Biodeterioration & Biodegradation Volume 53, Issue 1_ , January 2004, Pages 57-63.

Microbial degradation of phthalic acid was investigated using cultures of aerobic bacteria enriched from a sewage sludge. The Gompertz function and the Richards function were modified and compared to describe the phthalic acid (PA) degradation process, and both models successfully fitted the biomass growth curve when PA was used as the sole source of growth controlling substrate. However, the modified Richards model was superior in describing the depletion curve of PA. The additional parameter, *m*, in the modified Richards model may be corresponding to the relative importance of the substrate consumption for maintenance. Based on the maximum degradation rates calculated using the modified Richards model, the optimal degradation conditions were determined by an orthogonal test for environmental factors including initial pH, C:N:P ratio and salt concentrations of the culture medium. More than 99% of PA at an initial concentration of 4000 mgl^{-1} was degraded within 5 days under the optimized condition: namely initial pH 6.0, C:N:P=100:5:1, and NaCl concentration 10 gl^{-1}. Our results suggest that both substrate depletion and microbial biomass formation can be modelled and predicted using the modified Richards model and the limitation factor of phthalic acid degradation is the initial pH of the culture.

Yong-Hak Kim[†] and Karl-Heinrich Engesser[*]. (Institut für Siedlungswasserbau, Wassergüte- und Abfallwirtschaft, Universität Stuttgart, D-70569 Stuttgart (Büsnau), Germany). Degradation of Alkyl Ethers, Aralkyl Ethers, and Dibenzyl Ether by *Rhodococcus* sp. Strain DEE5151, Isolated from Diethyl Ether-Containing Enrichment Cultures. Applied and Environmental Microbiology, July 2004, p. 4398-4401, Vol. 70, No. 7.

Twenty strains isolated from sewage sludge were found to degrade various ethers, including alkyl ethers, aralkyl ethers, and dibenzyl ether. In *Rhodococcus* strain DEE5151, induction of ether degradation needed substrates exhibiting at least one unsubstituted C_{α}-methylene moiety as the main structural prerequisite. The cleavage reaction observed with anisole, phenetole, and dibenzyl ether indicates that the initial oxidation occurs at such respective C_{α} positions. Diethyl ether-induced strain DEE5151 degraded dibenzyl ether via intermediately accumulated benzoic acid. Phenetole seems to be subject also to another ether-cleaving enzyme. Other strains of this group showed different enzymatic activities towards the substrate classes investigated.

Yoshifumi Shinoda,[1] Yasuyoshi Sakai,[1] Hiroshi Uenishi,[1] Yasumitsu Uchihashi,[1] Akira Hiraishi,[2] Hideaki Yukawa,[3] Hiroya Yurimoto,[1] and Nobuo Kato[1*]. (Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502,[1] Department of Ecological Engineering, Toyohashi University of Technology, Toyohashi 441-8580,[2] Research Institute of Innovative Technology for the Earth, Soraku-gun, Kyoto 619-0292, Japan[3]). Aerobic and Anaerobic Toluene Degradation by a Newly Isolated Denitrifying Bacterium, *Thauera* sp. Strain DNT-1. Applied and Environmental Microbiology, March 2004, p. 1385-1392, Vol. 70, No. 3.

A newly isolated denitrifying bacterium, *Thauera* sp. strain DNT-1, grew on toluene as the sole carbon and energy source under both aerobic and anaerobic conditions. When this strain was cultivated under oxygen-limiting conditions with nitrate, first toluene was degraded as oxygen was consumed, while later toluene was degraded as nitrate was reduced. Biochemical observations indicated that initial degradation of toluene occurred through a dioxygenase-mediated pathway and the benzylsuccinate pathway under aerobic and denitrifying conditions, respectively. Homologous genes for toluene dioxygenase (*tod*) and benzylsuccinate synthase (*bss*), which are the key enzymes in aerobic and anaerobic toluene degradation, respectively, were cloned from genomic DNA of strain DNT-1. The results of Northern blot analyses and real-time quantitative reverse transcriptase PCR suggested that transcription of both sets of genes was induced by toluene. In addition, the *tod* genes were induced under aerobic conditions, whereas the *bss* genes were induced under both aerobic and anaerobic conditions. On the basis of these results, it is concluded that strain DNT-1 modulates the expression of two different initial pathways of toluene degradation according to the availability of oxygen in the environment.

Yan-Ling Qiu,[1] Yuji Sekiguchi,[2*] Hiroyuki Imachi,[1] Yoichi Kamagata,[2] I-Cheng Tseng,[3] Sheng-Shung Cheng,[4] Akiyoshi Ohashi,[1] and Hideki Harada[1]. (Department of Environmental Systems Engineering, Nagaoka University of Technology, Nagaoka, Niigata 940-2188,[1] Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8566, Japan,[2] Department of Biology,[3] Department of Environmental Engineering, National Cheng Kung University, Tainan 701, Taiwan[4]). Identification and Isolation of Anaerobic, Syntrophic Phthalate Isomer-Degrading Microbes from Methanogenic Sludges Treating Wastewater from Terephthalate Manufacturing. Applied and Environmental Microbiology, March 2004, p. 1617-1626, Vol. 70, No. 3.

The microbial populations responsible for anaerobic degradation of phthalate isomers were investigated by enrichment and isolation of those microbes from anaerobic sludge treating wastewater from the manufacturing of terephthalic acid. Primary enrichments were made with each of three phthalate isomers (*ortho*-, iso-, and terephthalate) as the sole energy source at 37°C with two sources of anaerobic sludge (both had been used to treat wastewater containing high concentrations of phthalate isomers) as the inoculum. Six methanogenic enrichment cultures were obtained which not only degraded the isomer used for the enrichment but also had the potential to degrade part of other phthalate isomers as well as benzoate with concomitant production of methane, presumably involving strictly syntrophic substrate degradation. Our 16S rRNA gene-cloning analysis combined with fluorescence in situ hybridization revealed that the predominant bacteria in the enrichment cultures were affiliated with a recently recognized non-sulfate-reducing subcluster (subcluster Ih) in the group '*Desulfotomaculum* lineage I' or a clone cluster (group TA) in the class delta-*Proteobacteria*. Several attempts were made to isolate these microbes, resulting in the isolation of a terephthalate-degrading bacterium, designated strain JT, in pure culture. A coculture of the strain with the hydrogenotrophic methanogen *Methanospirillum hungatei* converted terephthalate to acetate and methane within 3 months of incubation, whereas strain JT could not degrade terephthalate in pure culture. During the

degradation of terephthalate, a small amount of benzoate was transiently accumulated as an intermediate, indicative of decarboxylation of terephthalate to benzoate as the initial step of the degradation. 16S rRNA gene sequence analysis revealed that the strain was a member of subcluster Ih of the group '*Desulfotomaculum* lineage I', but it was only distantly related to other known species.

Eljko Cokesa, Hans-Joachim Knackmuss, and Paul-Gerhard Rieger*. (Institut für Mikrobiologie, Universität Stuttgart, 70569 Stuttgart, Germany). Biodegradation of All Stereoisomers of the EDTA Substitute Iminodisuccinate by *Agrobacterium tumefaciens* BY6 Requires an Epimerase and a Stereoselective C-N Lyase. Applied and Environmental Microbiology, July 2004, p. 3941-3947, Vol. 70, No. 7.

Biodegradation tests according to Organization for Economic Cooperation and Development standard 301F (manometric respirometry test) with technical iminodisuccinate (IDS) revealed ready biodegradability for all stereoisomers of IDS. The IDS-degrading strain *Agrobacterium tumefaciens* BY6 was isolated from activated sludge. The strain was able to grow on each IDS isomer as well as on Fe^{2+}-, Mg^{2+}-, and Ca^{2+}-IDS complexes as the sole carbon, nitrogen, and energy source. In contrast, biodegradation of and growth on Mn^{2+}-IDS were rather scant and very slow on Cu^{2+}-IDS. Growth and turnover experiments with *A. tumefaciens* BY6 indicated that the isomer R,S-IDS is the preferred substrate. The IDS-degrading enzyme system isolated from this organism consists of an IDS-epimerase and a C-N lyase. The C-N lyase is stereospecific for the cleavage of R,S-IDS, generating D-aspartic acid and fumaric acid. The decisive enzyme for S,S-IDS and R,R-IDS degradation is the epimerase. It transforms S,S-IDS and R,R-IDS into R,S-IDS. Both enzymes do not require any cofactors. The two enzymes were purified and characterized, and the N-termini were sequenced. The purified lyase and also the epimerase catalyzed the transformation of alkaline earth metal-IDS complexes, while heavy metal-IDS complexes were transformed rather slowly or not at all. The observed mechanism for the complete mineralization of all IDS isomers involving an epimerase offers an interesting possibility of funneling all stereoisomers into a catabolic pathway initiated by a stereoselective lyase.

BIOSENSOR

Astier Y, Canters GW, Davis JJ, Hill HA, Verbeet MP, Wijma HJ. (Inorganic Chemistry Laboratory, University of Oxford, UK, Department of Chemistry, Central Research Laboratory University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK, Fax: (+44) 1865-275-914). Sensing Nitrite through a Pseudoazurin-Nitrite Reductase Electron Transfer Relay. Chemphyschem. 2005 May 18.

Nitrite is converted to nitric oxide by haem or copper-containing enzymes in denitrifying bacteria during the process of denitrification. In designing an efficient biosensor, this enzymic turnover must be quantitatively assessed. The enzyme nitrite reductase from Alcaligenes faecalis contains a redox-active blue copper centre and a nonblue enzyme-active copper centre. It can be covalently tethered to modified gold-electrode surfaces in configurations in which direct electron transfer is possible. A surface cysteine mutant of the enzyme can be similarly immobilised on bare electroactive gold substrates. Under such circumstances, however, electron transfer cannot be effectively coupled with substrate catalytic turnover. In using either the natural redox partner, pseudoazurin, or ruthenium hexammine as an "electron-shuttle" or "conduit" between enzyme and a peptide-modified electrode surface, the coupling of electron transfer to catalysis can be utilised in the development of an amperometric nitrite sensor.

Halamek J, Pribyl J, Makower A, Skladal P, Scheller FW. (Analytical Biochemistry, University Potsdam, Karl-Liebknecht Str. 24-25, 14476, Golm, Germany, halamek@rz.uni-potsdam.de) Sensitive detection of organophosphates in river water by means of a piezoelectric biosensor. Anal Bioanal Chem. 2005 May 20.

A highly sensitive piezoelectric biosensor has been developed for detection of cholinesterase inhibitors. The inhibitor benzoylecgonine-1,8-diamino-3,4-dioxaoctane (BZE-DADOO) was immobilized on a monolayer of 11-mercaptomonoundecanoic acid (MUA) self-assembled on the gold surface of the sensor. The binding of high-molecular-weight cholinesterase to the immobilized cocaine derivative was monitored with a mass sensitive piezoelectric quartz crystal (quartz crystal nanobalance; QCN). In the presence of an inhibiting substance in the sample, the binding of cholinesterase to the immobilized inhibitor was reduced. The decrease of the rate of mass change was proportional to the concentration of free inhibitor in the sample. This way the affinity sensor followed anti-cholinesterase toxicity and the enzyme activity of ChE was not addressed. A assay for detection of organophosphates (OP) was optimized. Regeneration of the sensor surface was achieved with 1 mol L(-1) formic acid, which enabled 40 measurements with one sensor. All assays were carried out in a flow-through arrangement. The total measurement time (binding+regeneration) was 25 min and the detection limit for different OP (paraoxon, diisopropylfluorophosphate, chlorpyriphos, and chlorfenvinphos) was down to 10(-10) mol L(-1) (0.02 mug L(-1)). This sensor was used for determination of organophosphate (diisopropylfluorophosphate) levels in river water samples.

Liu A, Honma I, Zhou H. (Energy Technology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Umezono 1-1-1, Tsukuba 305-8568, Japan). Amperometric biosensor based on tyrosinase-conjugated polysacchride hybrid film: Selective determination of nanomolar neurotransmitters metabolite of 3,4-dihydroxyphenylacetic acid (DOPAC) in biological fluid. Biosens Bioelectron. 2005 May 7.

The amperometric detection of neurotransmitters metabolite of 3,4-dihydroxyphenylacetic acid (DOPAC) was achieved at a tyrosinase-chitosan composite film-modified glassy carbon (GC) electrode. The optimal conditions for the preparation of the biosensor were established. This bio-composite film was characterized by scanning electron microscopy (SEM) and Fourier transformed infrared (FT-IR) spectra, suggesting that chitosan covalently connected to chitosan chains. Electrochemical characterization of the bio-hybrid membrane-covered electrodes were also performed in 0.05M phosphate buffer solution (pH 6.52) containing neurotransmitters or their derivatives by using cyclic voltammetry (CV), linear sweep voltammetry (LSV), square wave voltammetry (SWV) and amperometry. This simply-prepared protein-polysaccharide hybrid film provides a microenvironment friendly for enzyme loading. The sensor was operated at -0.15V with a short response time. The current linearly increased with the increasing concentration of DOPAC over the concentration of 6nM-0.2mM. The lower detection limit for DOPAC is 3nM (S/N=3). The sensitivity of the sensor is 40muAmM(-1). A physiological level of neurotransmitters and their derivatives including dopamine, l-dopa, adrenaline, noradrenaline and homovanillic acid as well as ascorbic acid, uric acid and acetaminophen do not affect the determination of DOPAC.

Park JS, Lim SH, Kim BW. (Department of Chemical Engineering, Sungkyunkwan University, Suwon 440-746, South Korea). Interferometric biosensing of DNA-damaging chemicals. Biosens Bioelectron. 2005 May 12.

Recombinant E. coli ACV 1003 (recA::lacZ) was used to measure low concentrations of DNA damaging chemicals, which produce beta-galactosidase via an SOS regulon system. Very low beta-galactosidase activities of less than 0.01unit/ml, corresponding to the 10ng/ml (ppb) of DNA damaging chemicals (e.g. tributyl tin, bisphenol A, etc.) in the environment, can be rapidly determined, by using an alternative interferometric biosensor rather than by the conventional time-consuming enzyme assays. Usually, the conventional enzyme assay as well as the ELISA method requires more than 6h for analysis, which requires expensive biochemicals and much larger cell harvests to achieve the detection limit of the UV-vis spectrometers. Heavily-doped porous silicon destined to be applied to an interferometer was etched to form a Fabry-Perot fringe pattern due to the presence of bound molecules of beta-galactosidase. In order to enhance biolinking efficiency on the porous silicon surface, a calyx crown derivative (ProLinker A) was applied, instead of employing the conventional biomolecular affinity method using biotin, which resulted in a denser linker formation. The change in the effective optical thickness versus beta-galactosidase activity showed a hyperbolic increase up to a concentration of 150 units of beta-galactosidase/ml. When anti beta-galactosidase was bound to the ProLinker A-linked surface, the effective optical thickness was found to be three times as high as that obtained without using anti beta-galactosidase. The resolution obtained was very similar to that afforded by the time-consuming ELISA method; however, the reproducibility was still unsatisfactory below 1 unit beta-galactosidase/ml, due to the non-uniform distribution of the pores in the etched silicon surface.

Name of Journals

1. Acta Microbiol Immunol Hung
2. Anal Bioanal Chem
3. Appl Microbiol Biotechnol
4. Applied and Environmental Microbiology
5. Biomarkers
6. Bioresour Technol
7. Biosens Bioelectron
8. Biotechnol Adv
9. Biotechnology
10. Biotechnology Progress
11. Can J Microbiol
12. Chemosphere
13. Chemphyschem
14. Comp Biochem Physiol C Toxicol Pharmacol
15. Ecotoxicol Environ Safety
16. Environ Health Perspect
17. Environ Int
18. Environ Mol Mutagen
19. Environ Pollut
20. Environ Sci Technol
21. Environ Toxicol
22. International Biodeterioration & Biodegradation
23. J Appl Microbiol
24. J Plant Growth Regul
25. J Radiat Res
26. J Vet Med B Infect Dis Vet Public Health
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30. Sci Total Environ

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