ENVIRONMENTAL BIOREMEDIATION AND BIODEGRADATION

Organizers: James J. Valdes, Ananda M. Chakrabarty and Michael Bagdasarian

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Plenary Sessions *Page*

Poster Sessions

Molecular Genetics and Physiology of Microorganisms (Session Sponsored by the U.S. Army Research

X2-001 MOLECULAR GENETICS AND ECOLOGY OF PCB-DEGRADING BACTERIA

Kensuke Furukawa, Dept of Agric. Chem., Kyushu University, Hakozaki, Fukuoka 812, Japan

Environmental contamination of PCBs has become a global concern since their identification in wildlife samples in 1966. A number of biphenyl/PCB-degrading bacteria have been isolated from the environment. They are mostly aerobic gram-negative bacteria which include species of *Pseudomonas, Acinetobacter, Alcaligenes*, and *Moraxella*. Biodegradability and catabolic fate of PCBs by bacteria are greatly influenced by chlorine substitution on biphenyl molecule. It is also evident that biphenyl utilizing strains exibit many different patterns of PCB congenor selectivity for biodegradation. Principal degradation of PCBs to chlorobenzoic acids are catalyzed by four enzymes (biphenyl dioxygenase [encoded by *bphA*], dihydrodiol dehydrogenase [*bphB*], 2,3-dihydroxybiphenyl dioxygenase [*bphC*], and hydrolase [*bphD*]). A gene cluster coding for biphenyl/PCB degrading enzymes was first cloned from *Pseudomonas pseudoalcaligenes* KF707, and more recently from several other *Pseudomonas* strains. 11.3 kb DNA of *P. pseudoalcaligenes* KF707, which included *bphABCXD*, were analyzed by gene specific transposon mutagenesis and totally sequenced. There existed 3.3 kb DNA segment (X) between *bphC* and *bphD*. The function of segment X has not been fully elucidated, but sequence analysis of this segment revealed the presence of highly homologous gene to *xylJ* (encoding 2-hydroxypent-2,4-dienoate hydratase in TOL plasmid pWW0). The *bphABCD* gene cluster (9.4 kb) was cloned from *P. putida* KF715, in which the X segment was absent between *bphC* and *bphD*. Introduction of *bphABCD*(KF715) into benzoate utilizing pseudomonads allowed to utilize biphenyl and degrade many PCB congenors. The chromosomal *bph* DNA of *P. putida* KF715 were highly prone to deletion when it grew on rich media.

Using *bphABC* and *bphD* DNA as the probes, homologies of *bph* genes were examined for various PCB-degraders. Ten out of 32 strains examined possessed very similar, if not identical, *bph* DNAs to KF 707 *bphABCXD*. Six strains possessed homologous *bph* DNAs to that of KF707 with varying extents. Remaining 16 strains show no significant homology. The nucleotide sequences as well as gene order of *bphABC* operon (KF707) were similar to toluene catabolic *todC1C2BADE* operon of *P. putida* F1. The *bphA* region which encodes a multi-components enzyme of biphenyl dioxygenase consist of 5 open reading frames (ORF) in *bphA* region. Of which four ORFs were similar to *todC1C2BA* genes coding for the corresponding enzymes catalizing the initial dioxygenation reaction of toluene. The third open reading frame (ORF3) of *bphA* region is missing in its counterpart in toluene dioxygenase gene-cluster. The products of *bphA1*, *bphA2*, *bphA3*, and *bphA4* are, terminal dioxygenase (large subunit), terminal dioxygnease (small subunit), ferredoxin, and ferredoxin reductase, respectively. The gene organization and homology of their products are very conserved among *bph* and *tod* operon except hydrolase genes, *bphD* and *todF*. The amino acid sequence homologies of the corresponding components between *bphA1A2A3A4BC* and *todC1C2BADE* are 53-65 %. On the other hand, homology of hydrolase is only 35.1 % between *bphD* and *todF*. *bphD* is located downstream of *bphX*, but *todF* is located upstream of *todC1*. Constructuion of hybrid operon of *todC1bphA2A3A4BC* resulted in the relaxation of substrate specificity of dioxygenase. The hybrid dioxygenase gained the novel capability to oxidize benzene and its derivatives such as toluene, phenol, and indole, with reduction of oxidizing activity for biphenyls and biphenyl related compounds.

X2-002 REPORTER GENE ACTIVITY AS A MEANS OF EVALUATING EXOPOLYSACCHARIDES PRODUCTION BY BACTERIA IN REACTOR SYSTEMS, Gill G. Geesey and David G. Davies, Department of Microbiology and Engineering Research Center, Montana State University, Bozeman, MT 59717-0398.

In nature, efficient degradation of a wide variety of organic compounds is achieved through cooperative metabolism by complex assemblages of physiologically-compatable microbial species that reside in surface-associated biofilms. Orientation of the cells with respect to eachother and their surrounding environment is achieved through a matrix of exopolysaccharides and other polymeric products excreted by the microorganisms. An understanding of the conditions that promote exopolymer production by microorganisms used in bioremediation and biodegradation operations is likely to be essential for establishing optimum system performance. Reporter gene activity (lacZ) was used to demonstrate up- regulation of the promoter for algC, a gene involved in alginic acid synthesis in Pseudomonas aeruginosa, when cells of this bacterium became attached to an inert surface. Up-regulation of algC resulted in increased surface accumulation of alginic acid exopolysaccharide based on colorimetric, infrared and ELISA assays. In continuous culture experiments, up-regulation of algC occurred in approximately 30% of the cells that had attached to a surface after a 48 h exposure period. Surface-associated cells produced up to 2-3 times more alginic acid per cell than cells suspended in the bulk aqueous medium. The results demonstrate that reporter genes are a convenient and sensitive means of determining how the environment influences exopolysaccharide production in film-forming bacteria.

X2-003 DEGRADATION OF ALKYL-SUBSTITUTED BENZENES BY BACTERIA FUNCTIONAL UNDER OXYGEN-LIMITED CONDITIONS, Ronald H. Olsen, Jerome J. Kukor, Mark D. Mikesell, and Bryan Kaphammer, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor.

Interest in the development of technology for the *in situ* biodegradation of alkyl-substituted benzenes and benzene prompted us to ascertain the conditions which prevail in subsurface contaminant zones and an estimation of the microorganisms which might function under these environmental conditions . Accordingly, we isolated bacteria from three petroleum contaminated aquifers for their growth on benzene, toluene, ethylbenzene and xylenes (BTEX) both under aerobic and oxygen-limited (hypoxic, i.e., less than 5 mg/L) conditions. We found that many of the BTEX-degrading isolates active under aerobic conditions did not function under hypoxic conditions (1). Some bacterial isolates, however, were active under hypoxic conditions. A bacterial strain typical of such isolates, *Pseudomonas pickettii* PKO1 showed BTEX degradation dependent on the presence of nitrate under hypoxic conditions. The metabolic pathway from PKO1 has been cloned and expressed in *P. aeruginosa* PAO1c wherein the gene arrangement and regulation of the pathway has been determined (2,3,4). This pathway encodes for a novel toluene-3-monooxygenase pathway for BTEX degradation. Benzene, ethylbenzene and xylenes are also substrates for toluene-3-monooxygenase, but only toluene, benzene *meta*-cresol or ethylbenzene are inducers of the enzyme.

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Lignin and Cellulose Degradation

X2-004 BIODEGRADATION OF CHEMICALS BY WHITE ROT FUNGI. Steven D. Aust, Biotechnology Center, Utah State University, Logan, UT 84322-4705.

The mechanism by which white rot fungi degrade a wide variety of chemicals is being investigated with the objective of applying the technology for bioremediation of environmental pollution sites or biotreatment of hazardous waste streams. The white rot fungus Phanerochaete chrysosporium can mineralize both highly oxidized chemicals such as DDT, PCB, PCP and TNT, as well as chemicals that are susceptible to oxidation, such as the polyaromatic hydrocarbons. Recent findings in our laboratory suggest that the fungus can use several mechanisms to effectively reduce highly oxidized chemicals. For examples, TNT and other nitro-containing explosives can be reduced by a fungal plasma membrane potential. Dechlorinations can also be catalyzed by the membrane potential. PCP is first reduced by methylation. Reductions can also be catalyzed by fungal peroxidases. The reactions require a mediator, veratryl alcohol for the lignin peroxidases and manganese for the manganese-dependent peroxidases, and an electron donor, oxalate for the lignin peroxidases and hydroquinones for the manganese-dependent peroxidases. The peroxidases generate intermediate radicals, the oxalic acid anion radical from oxalate or semiquinones radicals from hydroquinones to catalyze subsequent reductions. The reduction of molecular oxygen to form superoxide can also occur by the organic acid anion radicals. Some cation radicals will oxidize with hydrogen peroxide to yield superoxide. Superoxide can also be used to generate the powerful oxidant, the hydroxyl radical, by Haber-Weiss chemistry. (Supported by NIH Grant ES04922.)

Anaerobic Degradation (Session Sponsored by the U.S. Army Research Office)

X2-005 REGULATED GENE EXPRESSION DURING METHANOGENESIS, Aidan N. Hennigan, Diane Stroup, Vanessa J. Steigerwald, Todd D. Pihl, and John N. Reeve, Department of Microbiology, The Ohio State University, Columbus, OH 43210.

Methane biosynthesis is the final step of anaerobic biodegradation in many environments. The enzyme methyl coenzyme M reductase (MR), that generates methane in all methanogens, catalyzes the reduction of a methyl moiety, bound to the methanogen-specific cofactor M. This enzyme constitutes ~10% of the cellular protein in methanogens and evolutionarily-related, MR-encoding genes have been cloned and sequenced from several mesophilic, thermophilic and hyperthermophilic methanogens. In all cases the α, β and γ polypeptide subunits of MR are encoded by *mcr*A, *mcr*B and *mcr*G genes that form part of a single transcriptional unit, the *mcr*BDCGA operon. The precise functions of the polypeptides encoded by the two additional ORFs, *mcr*D and *mcr*C are unknown but evidence has been obtained indicating an involvement with MR activity. Transcription of the *mcr* operon in *Methanococcus vannielii* has been quantitated in cells taken at different stages of growth from batch cultures growing on CO_2+H_2. The numbers of *mcr* transcripts per cell and their half-lives *in vivo* have been determined and related to the amounts and activity of MR present. The effects of substrate depletion and addition of a range of different metabolic inhibitors on *mcr* transcription have also been quantitated. For comparison, the numbers and half-lives of transcripts of a ribosomal protein operon and the housekeeping genes, *arg*G and *sec*Y, have been determined in *M. vannielii* cells at different stages of growth. Some methanogens contain two MR encoding operons that are expressed during different phases of growth in batch culture. The MR encoding genes that are only expressed very early during exponential growth have been cloned from *Methanobacterium thermoautotrophicum* and and *Methanothermus fervidus* and shown to be located, in both methanogens, physically adjacent to a cluster of genes that encode the methyl viologen-reducing hydrogenase and a polyferredoxin. The possibility of coordinated expression of these adjacent transcriptional units during methanogenesis under substrate sufficient conditions will be discussed.

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X2-006 ANAEROBIC BIODEGRADATION OF AROMATIC CHEMICALS OF ENVIRONMENTAL CONCERN, Joseph M. Suflita, University of Oklahoma, Norman, OK 73019.

A wide variety of aromatic pollutants can be biodegraded in anaerobic environments, often by novel mechanisms. Primary anaerobic reactions commonly involve the alteration, removal or addition of aryl substituents prior to the reduction and cleavage of the aromatic ring. However, such transformations are often preceded by long and variable acclimation periods where no substrate transformation is evident. This period is frequently on the order of weeks, months, and sometimes even years. Such time frames are difficult to ignore and likely more important in governing the exposure of recipient ecosystems to pollutants than the rate of transformation once it does occur. Remedial efforts directed toward shortening this phase of "degradation" are considered in light of the environmental, physiological and chemical factors that tend to influence the anaerobic degradation of halogenated, methoxylated, and alkylated aromatic compounds. For instance, the need for co-substrates is critical for the reductive dehalogenation of haloaromatic compounds, the carboxylation of some alkylated pollutants, and the reductive cleavage of ether linkages in lignaceous substrates as well as some pesticidal materials. Examples of how environmental variables, like the availability of alternate electron acceptors, influence the anaerobic biodegradation of selected pollutants will be considered. Pure culture and cell-free extract studies will be used to illustrate the physiological and biochemical basis for such bioconversions. Once the limits to biodegradation are identified, rational strategies can be devised for biotechnological approaches to the remediation of environments tainted with such materials.

Aerobic Degradation

X2-007 CHLORINATED METHANES AS CARBON SOURCES FOR AEROBIC AND ANAEROBIC BACTERIA, Thomas Leisinger, Salome La Roche, Regula Bader, Monika Schmid-Appert, Susanna Braus-Stromeyer, and Alasdair Cook, Mikrobiologisches Institut ETH, ETH Zentrum, CH-8092 Zürich, Switzerland.

Chlorinated methanes are significant environmental pollutants which are subject to a variety of microbially catalyzed substitutive, oxygenative and reductive dehalogenations. Dehalogenation reactions providing the degrading bacteria with a source of carbon and energy have been observed for chloromethane (CH_3Cl) and dichloromethane (CH_2Cl_2) only.

A *Hyphomicrobium* sp. aerobically grows in CH_3Cl-salts medium. The dehalogenation mechanism has not been elucidated. Washed cells suspensions of CH_3Cl-grown cells dechlorinated CH_3Cl in an oxygen-dependent reaction. Methane was not oxidized. This suggests that CH_3Cl is transformed by a monooxygenase, which is not methane monooxygenase, to formaldehyde.
The utilization of CH_3Cl as a bacterial growth substrate under anaerobic conditions has recently been discovered (1). A strictly anaerobic, Gram-positive, coccoid homoacetogenic bacterium grows with CH_3Cl and converts this substrate to acetate. Utilization of CH_3Cl appears to be inducible. CH_2Cl_2 is not utilized by the organism, and $CHCl_3$ and CCl_4 are toxic. The mechanism of dechlorination realized in this new organism is still unknown.

Aerobic utilization of CH_2Cl_2 by facultatively methylotrophic bacteria depends on the synthesis of dichloromethane dehalogenase, which catalyzes a substitutive reaction yielding formaldehyde and inorganic chloride. Nucleophilic displacement of chloride by dichloromethane dehalogenase is not based on the direct attack by hydroxide from water, but on the thiol group of glutathione (GSH) as the initial nucleophile. Characterization of this enzyme, of its structural gene and of the regulatory gene governing its expression, have led to a considerable understanding of aerobic CH_2Cl_2 metabolism. Analysis of the deduced amino acid sequences of two bacterial dichloromethane dehalogenases showed that these enzymes fit into the GSH S-transferase enzyme family. Functional criteria, such as their high activity with CH_2Cl_2 and their lack of activity with the "universal" GSH S-transferase substrate, 1-chloro-2,4-dinitrobenzene, as well as sequence similarities indicate an association of bacterial dichloromethane dehalogenases with the recently defined theta family of eukaryotic GSH S-transferases. The amino acid sequences of the two bacterial enzymes exhibited 56% identity. This supports evolutionary relatedness of the two enzymes but argues against their recent divergence.
CH_2Cl_2 is also used by an acetogenic enrichment culture as the sole source of carbon and energy (2). In cell suspensions of the enrichment culture CH_2Cl_2 was degraded to chloride (1.9 mol/mol CH_2Cl_2) and acetate (0.47 mol/mol CH_2Cl_2). Formic acid was detected as an intermediate but no H_2 was formed. Circumstantial evidence suggests that CH_2Cl_2 in the anaerobic system is mechanistically different from the dichloromethane dehalogenase reaction in aerobic methylotrophs. Isolation and characterization of the organism(s) capable of anaerobic utilization of CH_2Cl_2, as well as establishing the biochemistry of dehalogenation, represent new challenges.

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X2-008 OXIDATION OF SMALL HALOGENATED SOLVENTS BY METHANOTROPHS, Mary Lidstrom[1], Jeremy Semrau[1], Edye Udell[1], Kelly Smith[1], Hoa Nguyen[2] and Sunney Chan[2], [1]Environmental Engineering Science, or [2]Division of Chemistry, California Institute of Technology, Pasadena, CA

Methanotrophs are bacteria capable of growing on methane as sole carbon and energy source. These bacteria also cooxidize small halogenated solvents such as trichloroethylene (TCE) using methane as the primary substrate, and consortia of methanotrophs and heterotrophs can completely mineralize these compounds. In order to utilize the degradative abilities of these bacteria, it is important to understand the biochemistry, genetics and regulation of the enzyme system that carries out the cooxidation reaction. In most methanotrophs, the cooxidation of TCE is effected by the membrane-bound, or particulate methane monooxygenase (pMMO). A few methanotrophs also have the ability to express an alternative MMO, called the soluble MMO (sMMO). The sMMO cooxidizes TCE with a relatively high rate compared to the pMMO. However, the pMMO appears to have a higher affinity for TCE, and is the predominant enzyme in natural populations. This presentation will focus on recent data from our laboratories concerning the pMMO. The pMMO is extremely unstable and has never been reproducibly purified. Most of the current information on the pMMO comes from whole cell studies. We have used spectroscopic and genetic approaches to characterize the pMMO in several methanotrophs. Our data suggest that the pMMO is a copper-containing enzyme, that copper availability is a key variable in controlling the cooxidation abilities of the pMMO, and that the pMMO contains a minimum of three membrane polypeptides, probably in a dimer form. The implications of our results for the utilization of methanotrophs for TCE degradation in situ will be discussed.

X2-009 **STREPTOMYCES** AS HOMOLOGOUS AND RECOMBINANT VEHICLES FOR BIOREMEDIATION OF PESTICIDES, NERVE AGENTS, AND OTHER XENOBIOTICS. Burton M. Pogell, Center for Agricultural Biotechnology, M.B.I, University of Maryland, College Park MD 20742 and Pesticide Degradation Laboratory, ARS, USDA, Beltsville, MD.

The streptomycetes are a diverse group of Gram+ soil microorganisms which undergo the unusual phenomenon of active chromosomal rearrangements, deletions, and amplifications. It has proved possible to isolate species from soil enrichments with unique capabilities to biodegrade numerous xenobiotics. A mutant strain (**S.PSI/5**) of a species first isolated by Liu, Zhang, and Bollag and capable of mineralizing metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide), was also efficient in breaking the recalcitrant carbon-chlorine bond of lindane (h.xachlorocyclohexane) to form water-soluble metabolites and CO_2. The strain also actively N-dealkylated both the N-isopropyl and N-ethyl side chains of atrazine (2-chloro-4-ethylamino-6-isopropylamino-**s**-triazine). **S.PSI/5** grown aerobically in dextrin-tryptone rapidly converted atrazine via 2-chloro-4,6-diamino-**s**-triazine to other water soluble metabolites. Recent experiments show that the strain can also inactivate the nerve agent, VX (O-ethyl-S-2-(diisopropylaminoethyl)methylphosphonothiolate) as measured by loss of inhibition of acetylcholine esterase. It is thought that specific peroxidases are involved in these biodegrative processes.

In addition, streptomycetes have proved to be useful for the heterologous expression of genes coding for the metabolism of pesticides and other foreign compounds: (a)When a **Flavobacterium** gene(**opd**) coding for a membrane associated parathion hydrolase was inserted into plasmid pIJ702 and transformed into **S.lividans** 66, high levels of soluble extra- and intracellular enzyme were produced. N-terminal sequencing of the purified excreted hydrolase showed that the signal peptide was cleaved at amino acid 29, the same cleavage site observed in **Flavobacterium**. Highest levels of production (>30 mg/ml) were achieved in the presence of either the **opd** or a **Streptomyces β-gal** signal sequence when grown in glucose-tryptone under controlled fed-batch conditions with high dissolved oxygen. (b) Similarly, when **Phanerochaete chrysosporium** lignin peroxidase H8 cDNA was inserted on plasmids into **S. lividans**, clones containing upstream **mel** and **opd** promoters and the **opd** signal peptide inserted in place of the H8 signal secreted increased lignin peroxidase enzyme activity. Western blots and ELISA antibody capture assays confirmed the presence of a 38 kD anti-lignin peroxidase-binding protein in broth from clones containing the gene.

Detection and Monitoring

X2-010 MOLECULAR METHODS FOR DETECTING AND MONITORING MICROORGANISMS, Ronald M. Atlas, Dept. of Biology, University of Louisville, Louisville KY 40292

Gene probes are effective for monitoring specific microbial populations. Colony hybridization has proven effective in monitoring specific genotypes within complex microbial communities. Populations capable of using multiple classes of hydrocarbons have been assayed in this manner. The direct use of gene probes with environmental samples depends upon the recovery of nucleic acids with sufficient purity to achieve hybridization. This generally requires the removal of humic substances. Amplification of target nucleotide sequences is generally needed for the analysis of environmental samples in order to compensate for the relatively low proportions of target microorganisms within a microbial community. PCR has proven effective for achieving detection of low levels of target microorganisms and even single cells have been detected in 100 g environmental samples. Pathogens, indicator organisms, and microorganisms with specific functional genes have been detected in this manner. Genetically engineered microorganisms have been monitored in aquatic and soil microcosms using this approach. Several methods have been applied for distinguishing live from dead cells by PCR-gene probe detection. These include the use of large amplicons that rapidly degenerate when a cell dies, the targeting of short half life mRNA, and measuring increases in target nucleic acids after incubation under conditions favoring DNA replication. Using these approaches viable nonculturable bacteria have been detected.

X2-011 MONITORING HORIZONTAL GENE TRANSFER IN ENVIRONMENTAL MICROBIAL COMMUNITIES, Robert V. Miller[1], Tyler A. Kokjohn[2], Dennis J. Saye[3], Steven Ripp[1], S. Kidambi[1], Jean Replicon[4], and O. A. Ogunseitan[5], [1]Oklahoma State Univ., Stillwater, OK, [2]Univ. Nebraska, Lincoln, NB, [3]Nalco Chem. Co., Naperville, IL, [4]Loyola Univ. Chicago, IL, & [5]Univ. California, Irvine, CA.

Three major systems of horizontal gene transfer, conjugation, transduction, and transformation, are recognized in bacteria and are used routinely in the laboratory as tools of genetic analysis. While most environmental studies have focused on conjugation (unidirectional gene transfer requiring cell-to-cell contact), recent reports have documented the potential of both transduction (virus-mediated transfer) and transformation (absorption by the recipient bacterium of cell-free genetic determinants directly from the external milieu) as fertile gene-exchange systems in natural ecosystems (1). Transduction of genetic material has now been verified in wastewater treatment facilities, soils, and freshwater environments. Transformation has been demonstrated in marine and soil environments.

We have been using *Pseudomonas aeruginosa* as a model organism to develop methods for studying horizontal gene transfer in natural habitats. These include methods for the demonstration of conjugation (2) and transduction in freshwater microbial populations (3,4) and in the phyloplane. The majority of our work has dealt with transduction because this mechanism of gene transfer has been virtually ignored in both the design and preliminary testing of genetically-engineered microorganisms for environmental release. Our studies have revealed a significant potential for transduction of both plasmid and chromosomal DNA in these environments. In our model system, transduction was found to be most efficient when the recipient bacterium was a lysogen, probably due to the immunity imparted by the resident prophage. Apparently, environmental lysogens can serve as both efficient sources of transducing phages and as viable recipients for transduced DNA. Our working model predicts a unique sequence of events: (a) Phage virions are produced through induction of an environmental lysogen, (b) These viral particles infect, propagate, and lyse the genetic donor, and (c) Transducing particles produced during this lytic infection transfer DNA to the lysogenic recipient. We observed transduction *in situ* in both the absence and presence of the natural microbial community. Reciprocal chromosomal transduction was observed in chambers inoculated with two lysogens.

The ultimate question that must be answered to determine the effects of gene transfer on natural bacterial populations is whether it acts to affect the equilibrium frequency of an introduced genetic sequence in a natural bacterial community. Therefore, we are now turning our attention to the development of transduction models designed to assess whether this form of gene transfer is effective in altering the makeup of the gene pool available to natural populations of bacteria.

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X2-012 MOLECULAR STRATEGIES IN BIODEGRADATION PROCESS MONITORING AND OPTIMIZATION, Gary S. Sayler, The University of Tennessee, Knoxville.

An array of analytical methods have been applied to developing a quantitative understanding of biodegradative bacterial population structure and activity in microbial communities mediating degradation of contaminants in soils and treatment reactors. DNA/DNA hybridization analysis applied to colony and soil extracted DNA blot hybridization formats has revealed the broad distribution and enrichment of specific plasmids and catabolic genes associated with naphthalene and toluene biodegradation in hydrocarbon contaminated soil; while genes, such as methane monooxygenase and toluene dioxygenase, which are co-metabolically linked to TCE biodegradation are broadly distributed but not enriched in TCE contaminated environments. These relationships have been established for surface, near subsurface and deep subsurface contaminated soils and sediments. Recent applications of mRNA analysis of soil extracted RNA demonstrate the potential for instantaneous quantitation of catabolic gene activity in contaminated soils and, using the NAH plasmid naphthalene catabolic genes, the efficiency of specific metabolic inducers such as salicylate, to enhance biodegradative activity under *in situ* conditions. Correlation ($r > 0.90$) among molecular measures of gene abundance and catabolic activity in soils have been developed for biodegradative population dynamics and measured levels of soil contaminats (for soils contaminated by growth supporting pollutants). Molecular probing methods extend the isolation and development of additional bacterial strains with broader or more robust degradative capability.

Strains recovered from the environment can be developed as bioluminescent reporter bacteria for *in situ* and on-line measurement of the presence, bioavailability and biodegradation of specific pollutants. Using *lux* transcriptional gene fusions with catabolic genes for either naphthalene or toluene catabolism, remote light sensing of catabolic gene expression and induction corresponding to biodegradation has been achieved under simulated *in situ* conditions. Near perfect correlation of bioluminescence from a *nah*A-*lux* reporter strain to naphthalene soil contamination extends over a range of two orders of magnitude with a relative detection limit of biodegradative activity at 50 $\mu g\ l^{-1}$ naphthalene. *Tod-lux* fusions have clearly demonstrated the co-metabolic *tod* mediated degradation of TCE in packed bed treatment reactors exposed to dynamics of toluene concentration. The molecular analytical methods permit more rapid and specific measurement of microbial dynamics in response to chemical contaminants and treatment regimes to enhance biodegradation. In addition, new insight is also possible on the ecological consequence of environmental stresses such as starvation or nutrient limitations and toxicants upon biodegradative functions in microbial communities.

X2-013 A BIOSENSOR FOR MERCURIC ION AND METALLIC MERCURY VAPOR, Jeff McGarvey[1], Chuck Condee[2], Anne O. Summers[1], [1]Univ. of Georgia, Athens, GA, [2]Envirogen, Inc, Lawrenceville, NJ

In many eubacteria transcription of plasmid- and transposon-encoded genes involved in bacterial detoxification of mercuric ion (Hg^{2+}) is controlled by the DNA-binding protein, MerR, which functions as a repressor without Hg^{2+} and as an activator with Hg^{2+}. We have constructed plasmids with the bacterial luciferase genes (*luxAB*) from *Vibrio harveyi* under the control of the *mer* operator-promoter (*merOP*) and MerR protein. In intact cells, the K_{app} for induction of the MerR *merOP-luxAB* fusion by Hg^{2+} was 9 $x10^{-8}$M and the induction follows hyperactivation kinetics comparable to those determined *in vitro*. The response peaked at 1-2 µM Hg^{2+}. As few as 400 induced cells can be detected in an ordinary ATP *luminometer. Luciferase was also inducible in the mer-lux fusions by* cadmium ion (Cd^{2+}) but at concentrations several orders of magnitude higher than for Hg^{2+}. Exposure of cells carrying the *mer-lux* fusion to metallic Hg^0 vapor at ambient air (26°C) concentrations (ca. 0.2 µM) resulted in *luciferase induction approximately equivalent to that effected by ionic* Hg^{2+} at 1µM in solution. Maximum *mer-lux* induction by Hg^0 vapor was dependent on the activity of bacterial oxidative enzymes including products of the *katE* and *katG* genes, indicating that, as in humans, catalases play a role in the oxidation of inert Hg^0 to the reactive Hg^{2+} ionic form. The *mer-lux* fusion is the first biosensor specifically able to detect a toxic metal vapor. We are currently optimizing the sensitivity of this system and examining its *interactions with the oxidative shock regulons.*

X2-014 BIOSENSORS FOR MONITORING CHEMICAL POLLUTANTS IN THE ENVIRONMENT, James J. Valdes[1], Nabil A. Anis[1], and Mohyee A. Eldefrawi[2], [1]U.S. Army Edgewood Chemical Research, and Development Center, Aberdeen Proving Ground, MD 21010-5423, [2]University of Maryland School of Medicine, Baltimore, MD 21201.

Anticholinesterase pesticides are among the hazardous chemicals that pollute our environment. There are several laboratory methods suitable for their detection. The spectrophotometric method of Ellman et al. (1961) is probably the least costly and most widely used. We have developed two alternative detection strategies using biosensor technology. These biosensors utilize acetylocholinesterase (AChE) as their sensing elements and utilize two different transduction modes.

A fiber optic strategy which uses the evanescent waveguide fluorescence principle to detect anticholinesterases (AntiChEs) took advantage of the fact that fluorescein is a pH-sensitive dye and that its fluorescence is quenched by the acid produced from hydrolysis of ACh by AChE. Thus fluorescein-tagged AChE, which retained its catactivity, was immobilized on the surface of quartz fibers and its fluorescence was monitored by a portable fluorimeter (ORD Inc., Salem, Mass.). Hydrolysis of ACh by AChE reduced fluorescence and AntiChEs inhibited the substrate-dependent fluorescence quenching. A single fiber could be used for multiple measurements, even after exposure to irreversible organophosphate AntiChEs, but in this case a 10 min wash with 1 mM 2-PAM was necessary to reactivate the biosensor reuse.

The threshold instrument of Molecular Devices (Palo Alto, CA) which uses a silicon light addressable potentiometric sensor (LAPS) was also used successfully for detecting AntiChEs. In this strategy, AChE was immobilized on nitrocellulose capture membrane using biotin-strepavidin bridges. The immobilized enzyme was exposed to AntiChEs in unknown samples by washing the sample over it at a slow rate (0.13 ml/min). The capture membrane was placed in a compartment containing ACh in buffer and in direct contact with the surface of the silicon sensor. Measurement started by light activation and lasted for 30 sec. Enzyme activity is recorded as potential change (µV/s). The LAPS system was equally sensitive to the optic fiber and the Ellman method. However, the LAPS had many other advantages. It allowed multiple measurements at a time. A single carrier membrane could be used for hundreds of measurements, but required enzyme activation between samples. Both biosensors offer savings in time and material, require little training and offer greater reproducibility over the Ellman et al. method.

Applications-I

X2-015 PCB BIODEGRADATION IN THE LABORATORY AND IN THE ENVIRONMENT, Daniel A. Abramowicz, Bioremediation Laboratory, Environmental Research Center, GE Corporate Resarch and Development Center, Schenectady, NY 12020

Two distinct bacterial systems are known to be involved in biologically-mediated PCB transformations. Both aerobic PCB biodegradation (oxidative attack) and anaerobic PCB dechlorination (reductive attack) have been demonstrated in the laboratory with contaminated soils and sediments. Moreover, widespread PCB dechlorination in anaerobic aquatic sediments has been documented in many freshwater and marine environments. Extensive anaerobic dechlorination of the original Aroclor 1242 contamination has occurred naturally in Hudson River sediments, resulting in the removal of most of the more highly chlorinated PCBs from Upper Hudson River sediments. This dechlorination results in significant benefits, including reduced toxicity, carcinogenicity, bioaccumulation potential, as well as increased aerobic biodegradation of the dechlorination products. Evidence for natural aerobic PCB biodegradation in Upper Hudson River sediments will also be presented.

In the summer of 1991, GE performed a large scale test of ***in situ* aerobic PCB biodegradation** in the Upper Hudson River. The experiments involved six sealed caissons (six feet in diameter) lowered into contaminated sediments that had already undergone extensive anaerobic PCB dechlorination. Results demonstrated approximately 50% biodegradation during the 10 week experiment, with a good correlation observed between laboratory derived expectations and actual field performance. Caissons containing only indigenous microorganisms performed as well as those inoculated with high levels of a broadly-active PCB-degrading bacterium (*Alcaligenes eutrophus* strain H850).

A large scale stimulation of ***in situ* anaerobic PCB dechlorination** in Housatonic River sediments contaminated with Aroclor 1260 was initiated in the summer of 1992. The experiments similarly involve six sealed caissons (six feet in diameter) lowered into contaminated sediments to investigate new methods developed to accelerate PCB dechlorination in the field. Preliminary results from these ongoing experiments demonstrate the potential for this method to stimulate PCB dechlorination *in situ*.

X2-016 BIOCHEMICAL DEMILITARIZATION OF CHEMICAL WARFARE AGENTS, Joseph J. DeFrank* and Steven P. Harvey, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, Maryland.

The United States and the former Soviet Union have agreed by treaty to destroy their stockpiles of chemical warfare agents by the year 2002. High-temperature incineration was the method of destruction selected by the U.S. Army as the best available for its estimated 25,000 tons of agent. In addition to the model installation on Johnston Atoll (JACADS), the Army intends to construct incinerators at the eight sites in the continental U.S. where the chemical agents or munitions are stored. Not only has the estimated costs of the program increased dramatically, but public and political opposition has made it quite unlikely that all the planned facilities will be constructed. In addition to the U.S. difficulties, Russia is currently incapable of carrying out the destruction of its estimated 40,000 tons of chemical agents. Among the possible alternative technologies that could be used for the destruction of the stocks, biological/biochemical methods offer considerable potential. Demilitarization approaches currently under study include: (1) the enzymatic detoxification and microbial degradation of the organophosphorus nerve agents; and (2) the combined chemical/microbial degradation of mustard. Enzymes capable of catalytically detoxifying the highly toxic organophosphorus compounds such as diisopropyl fluorophosphate (DFP), sarin, soman and tabun have been reported in the literature since 1946. In recent years, a number of highly active bacterial enzymes have been identified and characterized. The genes for several of these enzymes have been cloned and the DNA sequences obtained. Enzymes of this type could be used in an initial detoxification step during demilitarization to render the nerve agents harmless and reduce their potential release into the facility or beyond. Once the agents have been detoxified, the material would be processed through one or more biological systems similar to those used for the treatment of industrial wastes. Because of the reactive nature of mustard and its poor solubility, an alkaline hydrolysis step would provide the initial detoxification by converting it to the non-toxic and highly soluble thiodiglycol. Several bacterial strains have been identified that will utilize thiodiglycol as sole carbon and energy source. For both the nerve agents and mustard, the final products of the processes are biomass, carbon dioxide, salts and water (which can be recycled). It is believed that biological/biochemical methods for chemical agent demilitarization will be less expensive than incineration, will reduce the danger of explosion and catastrophic vaporization of agent, and be more acceptable to the residents of nearby communities.

X2-017 PETROLEUM BIOREMEDIATION - A MULTIPHASE PROBLEM, Eugene Rosenberg, Rachel Legmann, Ariel Kushmaro, Ran Taube, and Eliora Z. Ron, Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv, Israel.

Microbial degradation of hydrocarbons is a multiphase reaction, involving oxygen gas, water-insoluble hydrocarbons, water, dissolved salts and microorganisms. The fact that the first step in hydrocarbon catabolism involves a membrane-bound oxygenase makes it essential for microorganisms to come into direct contact with the hydrocarbon substrate. Growth then proceeds on the hydrocarbon/water interface. Bacteria have developed two general strategies for enhancing contact with water-insoluble hydrocarbons: specific adhesion mechanisms and production of extracellular emulsifying agents. Since petroleum is a complex mixture of many different classes of hydrocarbons, of which any particular microorganism has the potential to degrade only part, it follows that the microorganisms must also have a mechanism for desorbing from "used" oil droplets.

The major limitations in bioremediation of hydrocarbon-contaminated water and soil is available sources of nitrogen and phosphorus. The usual sources of these materials, e.g., ammonium sulfate and phosphate salts, have a high water solubility which reduces their effectiveness in open systems because of rapid dilution. We have attempted to overcome this problem by the use of a new controlled-release, hydrophobic fertilizer, F-1, which is a modified urea-formaldehyde polymer containing 18% N and 10% P as P_2O_5. Microorganisms were obtained by enrichment culture that could grow on crude oil as the carbon and energy source and F-1 as the nitrogen and phosphorus source. The microorganisms and the F-1 adhered to the oil/water interace, as observed microscopically and by the fact that degradation proceeded even when the water phase was removed and replaced seven times with unsupplemented water - a simulated open system. Strains which can use F-1 contain a cell-bound, inducible enzyme which depolymerizes F-1.

After optimizing conditions in the laboratory for the use of F-1 and the selected bacteria for degrading crude oil, a field trial was performed on an oil contaminated sandy beach between Haifa and Acre, Israel, in the summer of 1992. The sand was treated with 5 g F-1 per kg sand and inoculated with the selected bacteria; the plot was water with sea water and plowed daily. After 28 days the average hydrocarbon content of the sand decreased from 5.1 mg per g sand to 0.6 mg per g sand. Overall, there was an approx. 86% degradation of pentane extractables as demonstrated by dry weight, I.R. and GLC analyses. An untreated control plot showed only a 15% decrease in hydrocarbons. During the winter of 1992, the entire beach (approx. 200 tons of crude oil) was cleaned using the F-1 bacteria technology. The rate of degradation was 0.06 mg g^{-1} sand day^{-1} (10°C) compared to 0.13 mg g^{-1} sand day^{-1} during the summer (25°C).

X2-018 BIOLOGICAL BIOTREATMENT OF CHLORINATED ORGANICS - LABORATORY AND FIELD STUDIES, Ronald Unterman, Ph.D, Envirogen, Inc., Lawrenceville, New Jersey

As a group, chlorinated organic compounds pose some of the most significant environmental problems confronting us today. These problems include both historical contamination that requires remediation as well as the current production and release of industrial chemicals that require on-line biotreatment. This class of compounds includes many solvents, pesticides, wood preservatives, fire retardant fluids, pulp and paper wastes, and industrial byproducts. Because these contaminants can be found in various matrices (soils, water or air) thus represent significant and different challenges for applications engineering.

This paper will present the status of current studies into the development of new and more effective biocatalysts as well as their application in both traditional and novel biotreatment systems. Specific examples will include the bioremediation of PCB contaminated soils and sediments, and TCE contaminated ground water, as well as bioreactor systems for chlorinated solvents in industrial waste water and chlorinated VOCs in industrial air effluents. To date, field system development has focused on the use of superior, naturally occurring bacterial cultures under optimized process conditions, however, future applications will include the use of genetically engineered microorganisms in bioreactors and eventually in ground water and soil applications.

Engineering Systems

X2-019 FACTORS INFLUENCING THE BIODEGRADATION OF XENOBIOTIC ORGANIC COMPOUNDS IN ENGINEERED SYSTEMS, C. P. Leslie Grady Jr., Environmental Systems Engineering, L. G. Rich Environmental Research Laboratory, Clemson University, Clemson, SC 29634-0919.

Biodegradation of xenobiotic organic compounds in wastewater treatment and groundwater decontamination systems generally is performed by mixed microbial cultures growing on multicomponent organic substrates. This makes it difficult to predict the fate of those compounds, thereby causing engineers to incorporate large factors of safety into their designs, which increases system costs. Changing this situation requires better understanding of the interactions associated with mixed cultures growing on multiple carbon sources.

"Inherent variability" is one result of biodegradation in mixed cultures. Even when operated under stable input and environmental conditions, mixed culture bioreactors exhibit variability with respect to the output concentrations of individual organic compounds. For example, bioreactors degrading 2,4-dichlorophenol in a complex medium were able to achieve an output concentration of approximately 110 µg/L, but the coefficient of variation was 22%. Such variability is thought to be due to microbial interactions, but attempts to influence them through changing the microbial environment have not been successful.

Another important aspect of mixed culture/mixed substrate systems is that only a fraction of the microbial community is capable of degrading any individual xenobiotic constituent. As a consequence, the bulk of the biomass serves as a sorbent for compounds of limited solubility, and this influences their fate. For example, when di-n-butyl phthalate was present in concentrations below its solubility limit, sorption onto nondegrading biomass decreased the rate of biodegradation by reducing the liquid phase concentration. Conversely, when it was present at high concentration, sorption increased the rate of biodegradation because desorption was faster than dissolution.

Prediction of the fate of xenobiotic compounds is complicated by the fact that little is known about the control of enzyme synthesis and function in mixed substrate, carbon-limited environments in which bacteria are growing slowly. For example, when four additional substrates were added to the feed to an axenic continuous culture bioreactor containing a generalist growing on both lysine and 2-chlorophenol (2-CP), the output concentrations of the two original substrates increased even though current models would predict otherwise. Furthermore, when the same experiment was performed with a mixed culture containing specialists as well as the generalist, the output concentration of 2-CP decreased, suggesting that specialists play an important role in mixed culture/mixed substrate systems.

Mixed substrate systems also have an impact on the fate of catabolic plasmids. For example, TOL-containing strains of *P. putida* had much slower growth kinetics on benzoate than TOL-free strains, but had equivalent growth kinetics on five other substrates. This suggests that in the absence of substrates degraded by TOL-encoded enzymes, the nature of the growth substrate will strongly affect the maintenance of the TOL plasmid in the culture, thereby influencing the availability of TOL-encoded enzymes when the substrates degraded by them are reintroduced into the system. We are currently investigating the effects of mixed substrates on plasmid stability under circumstances in which one of the substrates requires plasmid-encoded enzymes.

These and other issues related to mixed culture/mixed substrate systems will be discussed in order to stress the need for more fundamental research on control of enzyme synthesis and function in such systems.

X2-020 BIOREACTOR DESIGN FOR BIOREMEDIATION OF CONTAMINATED SOILS, Yusuf Chisti and Murray Moo-Young, Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Airlift and bubble column types of bioreactors are well established in fermentation and wastewater treatment applications in which reactors up to 1,600 m^3 have been operated [1]; however, airlift devices have not so far been used in soil bioremediation. This paper will identify a niche for airlift bioreactors in soil decontamination and describe the bioreactor design considerations for bioremediation applications.

A particularly attractive treatment method for soils contaminated with hydrocarbons and other organics is soil washing. Washing with high pressure jets of hot water with or without added surfactants is already practised commercially in Europe in Weert at a plant operated by BSN Bodemsanering Nederland. An alternative washing technique utilizes solid-liquid contactors such as mechanically agitated tanks to rub the soil particles against each other in presence of wash solutions. This "agitation scrubbing" procedure has been tested on pilot scale (10-tonne per hour) at the Soil Recycling Demonstration Plant operated by the Toronto Harbour Commission and SNC Inc. in Toronto, Canada. These examples of large-scale soil washing operations are by no means the only ones [2, 3]. While the ex-situ bioremediation of hydrocarbon contaminated soils using washing followed by treatment of the wash water has been proven [2], problems remain. The washing processes clean only the coarser particles (≥ 0.063 mm particle size); smaller particles or fines which retain the bulk of the original contamination are not cleansed. At present, the fines are disposed of in secure landfills. As a result, soil washing technologies are economically applicable only to soils with less than 20-30 % fines by volume.

We believe that the applicability of soil washing operations can be extended to clayey soils (> 30 % fines) by incorporating a fines treatment step based on airlift bioreactors. Airlift reactors are mechanically simple with no moving parts and have proven economics of operation in large scale wastewater treatment applications [1]. In a conceptual fines treatment process an aqueous slurry of the fines could be aerated and pneumatically agitated in airlift devices containing mixed populations of contaminant(s)-degrading microorganisms. Depending on the hydrodynamic conditions, the microbes may grow either as a film on the soil particles or they may remain suspended in the liquid phase. While much design information exists for airlift reactors in wastewater treatment, cell culture and fermentation applications [1, 4], little is known of the hydrodynamic and transport behaviour of soil slurries in those systems. We will attempt to remedy this situation. Questions regarding the effects of solids-loading on circulation of the slurry, the effects of particle size and density on hydrodynamics, and solid-liquid and gas-liquid mass transfer will be addressed with a view to demonstrating the feasibility of airlift bioreactors for decontamination of soil fines. Finally, a case study will be used to illustrate the performance of airlift systems in comparison with bubble columns for degradation of hydrocarbon pollutants.

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X2-021 BIOTECHNOLOGY IN WASTE-WATER TREATMENT SYSTEMS IN MEXICO: NEW DEVELOPMENTS AND TRENDS, Rodolfo Quintero-Ramírez, Biotechnology Institute, National University of Mexico, Cuernavaca, Morelos, Mexico.

In the last few years there has been a growing concern about the environmental problems in Mexico. The present Government has established very strict laws regarding water pollution, it is recognized that water is the limiting factor for the development of the country and also because mexican industry has been a source of water contamination for many years. More recently the Free Trade Agreement with Canada and USA has presented the environmental problem as a new barrier for exchange of products among the three countries.
Most of the research and development work has been carried out by universities and in this presentation several of the new developments will be presented:
a) aerobic systems,
b) anaerobic systems, and
c) combination of these two.
Several projects have reached pilot plant scale and few have been implemented in industry.
In particular it will be described how the oil and petrochemical companies located in Mexico are handling the problem of water pollution after almost seventy years of no action.

Applications-II

X2-022 SUCCESSFUL APPLICATIONS OF BIOLOGICAL TREATMENTS IN THE FIELD

Al W. Bourquin, 1303 Ariola Drive, Pensacola Beach, Florida 32561

Hazardous waste treatment and disposal today is a worldwide environmental problem. Biodegradation has proven to be one of the most promising technologies to solve this enormous problem.

The full scale remediation of contaminated soil and water involves successful interpretation and scale-up of site assessment, material characterization, and laboratory treatability data. Laboratory characterization and treatability data are developed during evaluation of the feasibility of remedial alternatives. Each of these activities can have an enormous impact on the cost of remediation.

Case histories involving TCE, PAH's and oil contaminated soils and groundwater will be discussed to offer a wide range of bioremediation technologies (solid-phase, slurry-phase and in-situ) that can be applied in different situations. These studies will help in developing an understanding of present-day biodegradation potential and range of application for the future.

X2-023 BACTERIAL RESISTANCE MECHANISMS TO ARSENICALS AND ANTIMONIALS. Barry P. Rosen, Department of Biochemistry, Wayne State University, School of Medicine, Detroit, MI 48201.

Resistance to toxic oxyanions of arsenic and antimony in bacteria is conferred by conjugative resistance plasmids which carries *ars* operons (see [1] for a general review). The *ars* operon of resistance factor R773 encodes the *arsA* and *arsB* genes, which produce an oxyanion-translocating ATPase when expressed in *Escherichia coli*. The ArsA protein, an oxyanion-stimulated ATPase, interacts with the ArsB protein to form a multisubunit enzyme complex that can extrude arsenite and antimonite from cells. Solubilized ArsA protein exists in a monomer-dimer equilibrium, where the dimer is the catalytically competent species; binding of the oxyanion substrate stabilizes the dimer and hence catalysis. The 45.5 kDa ArsB protein is an integral membrane protein with 12 membrane-spanning α-helices, generally similar to many secondary porters such as the lactose permease. It is the membrane anchor for the ArsA protein and presumably the ion-conducting pathway.

A similar plasmid, pI258, encodes an *ars* operon and confers resistance in the gram positive bacterium *Staphylococcus aureus*. Strikingly this *ars* operon does not have an *arsA* gene and yet still produces a functional extrusion system. The two ArsB homologues show only 58% identity, but the structural similarity of the two ArsB proteins is more apparent from their superimposible hydropathic profiles. To determine whether that the two ArsB proteins operate similarly, a recombinant gene for a chimeric ArsB protein with the N-terminal 3/5 of the *S. aureus* and C-terminal 2/5 of the *E. coli* ArsB proteins was constructed. When co-expressed with the *arsA* gene, this fusion gene produced higher arsenite resistance than either the native *S. aureus* or *E. coli arsB* genes. Cells expressing the recombinant *arsB* gene showed reduced arsenite uptake, indicative of extrusion.

How can the *ars* operons of gram positive and gram negative organisms be so similar, yet so different? One possibility is that the gram positive and the gram negative systems may both be ATP-driven efflux pumps. In *E. coli* the gene for the catalytic subunit is part of the plasmid operon, while in staphylococci the catalytic subunit might be encoded by a chromosomal gene. Another possibility is that the ArsB protein alone is sufficient for anion conduction, functioning as a secondary anion porter in the absence of a catalytic subunit. Note that the 12 membrane spanning α-helical structure of the ArsB protein is overall very similar to many secondary porters. The membrane potential, which is positive exterior under physiological conditions, would serve as the driving force for anion movement.

We propose a model for the evolution of multisubunit primary pumps. The ArsB protein originated as a secondary anion porter which in stages developed a functional association with a soluble ATPase subunit. Initially secondary porters such as the ArsB protein would evolve physical association with soluble cytosolic ATPases. In subsequent stages the complexes evolved functional ion-translocating ATPase activity. *(This work was supported by United States Public Health Service Grant AI19793).*

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X2-024 TOXIC INORGANICS: RESISTANCES TO TOXIC HEAVY METALS AND MECHANISMS FOR BIO-REMEDIATION,

Simon Silver, University of Illinois College of Medicine, Chicago, IL 60680

Bacterial plasmids contain specific genetic determinants of resistances to a wide range of toxic heavy metals, including AsO_2^-, AsO_4^{3-}, Cd^{2+}, Co^{2+}, CrO_4^{2-}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}. Recent progress on less-familiar resistance systems such as for copper (in Pseudomonas syringae and Escherichia coli), cadmium (and zinc) in Bacillus and in Stephylococcus, cadmium (and zinc, nickel and cobalt) in Alcaligenes, and arsenic (and antimony) in both Gram positive and Gram negative bacteria has provided molecular genetic and biochemical understanding. All of these systems are regulated by cis-acting DNA sequences and trans-acting proteins. Gene fusions of the regulatory regions with reporter genes (mostly lux for bioluminescent light emission) produce highly specific "bio-sensors" for bio-available toxic heavy metals. The copper resistance systems consist of four proteins (one outer membrane, two periplasmic copper-binding, and one inner membrane) whose combined function results in bio-accumulation of Cu^{2+} in the periplasmic space outside the cell. The plasmid Cu^{2+} resistance system interacts with products of chromosomal genes, which are needed for normal levels of Cu^{2+} tolerance. The Cu^{2+} resistance systems are governed by two component (sensor plus DNA-binding transducer proteins, both of which are phosphorylated during Cu^{2+} regulation). The Cd^{2+} (and Zn^{2+}) resistance system of Gram positive bacteria is a member of the P-class cation translocating ATPases and functions to maintain low intracellular cation levels. The arsenic resistance system works similarly as an oxyanion efflux pump and is also an ATPase, but not part of larger ATPase families. Finally, the Cd^{2+} (and other divalent cation) efflux systems of Alcaligenes are also energy dependent, but do not appear to involve ATP. Some of the resistance systems, especially those involving redox chemistry (i.e. chromate and mercury reduction; arsenate reduction and arsenite oxidation) provide the starting materials for potential bioremediation of toxic wastes.

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Policy and Guidelines

X2-025 ENVIRONMENTAL PROGRAMS IN THE U.S. ARMY, Daphne Kamely, Director , Research and Laboratory Management, Headquarters, Department of the Army, Office of the Assistant Secretary of the Army (Research, Development, and Acquisition)

The Department of Defense (DoD) is faced with increasing problems in environmental pollution in the world. Approximately, 10% to 30% of global environmental pollution is generated by the military. Initially, military pollution was left to the regulatory agencies to manage. As the magnitude of the problem became too complex, Congress and the Administration moved to empower the DoD with authority to manage its own programs. As lead agency, the Army focuses on four major programs: remediation, restoration, conservation and pollution prevention. While preliminary efforts concentrated on remediation, the emphasis is now shifting to pollution prevention. Despite increased funding, the military cannot clean up all the hazardous military sites. Moreover, as a result of reduced defense budgets, a significant number of military sites are designated for closure over the next few years. Many of these military sites are contaminated and need to be cleaned before they can be turned over to the civilian sector.

Efforts are now concentrating on pollution prevention and on the development of alternative technologies to minimize waste generation. Technologies include: oxidation, plasma fusion, robotics, supercritical fluids and biodegradation. In the rapidly advancing bioremediation area, methods are being developed for the biodegradation of toxic substances by bacteria, enzymes and biosurfactants. Although promising under laboratory conditions, in the open environment microorganisms require a new set of optimal conditions in order to achieve efficient biodegradation. Furthermore, emphasis must be placed on engineering, packaging, processing and scale-up technologies, in order to be able to carry out bioremediation under optimal field conditions. Finally, once we have overcome the regulatory constraints, microorganisms will be genetically engineered and tested in an effort to achieve more efficient biodegradation.

Molecular Genetics and Physiology of Microorganisms; Lignin and Cellulose Degradation

X2-100 THE MOLECULAR PROPERTIES OF PHTHALATE DIOXYGENASE FROM P. cepacia. Ballou, D. P., Gassner, G., Huang, L., Department of Biological Chemistry, The University of Michigan, Ann Arbor, MI 48109-0606

The phthalate dioxygenase system (PDO) catalyzes the dihydroxylation of phthalate to produce the 4,5-cis-dihydrodiol and is analogous to systems acting on benzene and toluene. It consists of a NADH reductase (PDR), which contains FMN and a [2Fe-2S] ferredoxin center, and the oxygenase (PDO), which consists of a Rieske [2Fe-2S] center and a mononuclear Fe(II) center that is the site of oxygenation. The PDO system has been very amenable to a wide variety of physical studies and is the prototype for such systems that catalyze the first oxygenative step on unactivated aromatic compounds. This step is critical to aerobic bioremediation processes. Data will be presented that characterizes the salient structural and mechanistic features of these dioxygenases and which can lead to a better understanding of this class of enzymes.

The crystal structure of PDR will be shown. The structure of the Rieske center and properties of the mononuclear Fe(II) center will also be shown. Suicide substrates and kinetics of various stages of the reaction mechanism will be reported.

X2-101 STUDY OF METHYLAMINE DEHYDROGENASE AS A MODEL SYSTEM OF PRIMARY AMINE DEGRADATION IN NATURE, Andrei Y. Chistoserdov, Mary E. Lidstrom, Environmental Engineering Science, W. M. Keck Laboratories 138-78, California Institute of Technology, Pasadena, CA 91125.

Several primary, secondary and tertiary amines are classified by the EPA as hazardous and many of these can be degraded by bacteria. One of the most common amine oxidizing systems in bacteria is methylamine dehydrogenase (MADH). MADH is able to oxidize *n*-alkylamines of varying carbon chain length thus providing the cell with carbon, nitrogen and energy and it also necessary for utilization of secondary and tertiary amines. We have initiated mechanistic study of this system in order to understand it in more detail. We have cloned the structural genes (*mau*) for MADH belonging to two different subclasses of the enzyme. The MADH from *Methylobacterium extorquens* AM1 utilizes butylamine as the preferred substrate and it uses amicyanin as an electron acceptor. The MADH from *Methylophilus* sp. W3A1 has methylamine as the preferred substrate and it uses a *c*-type cytochrome as an electron acceptor. Several other genes were found to be linked to the MADH subunit genes, whose products are apparently involved in the assembly of MADH and the synthesis of a unique quinone cofactor from two tryptophans of the MADH small subunit polypeptide chain. A series of *mau* mutants were generated for both bacteria. It appears that *M. extorquens* AM1 but not *Methylophilus* sp. W3A1 has an alternative amine oxidation system which participates in the utilization of amines as sources of nitrogen. We are now conducting a comparative study of amine dehydrogenases with different substrate specificity to elucidate the mechanism of recognition of different polycarbon substrates and the arrangement of the active center of such enzymes. Future work will involve assessing the role of MADH as well as other amine oxidation enzymes in detoxification of amines in the environment.

X2-102 EFFICIENT BIODEGRADATION OF CHLORINATED ALIPHATIC SOLVENTS USING *Methylosinus trichosporium* OB3b MUTANT CONSTITUTIVE IN THE EXPRESSION OF THE SOLUBLE METHANE MONOOXYGENASE, George Georgiou[1], Mark W. Fitch[1], Daniel Weissman[2], Larry E. Linquist[2], Gerald E. Speitel, Jr[2] and Particia Phelps[2], Departments of Chemical Engineering[1] and Civil Engineering[2], University of Texas, Austin TX 78712

The methylotrophic bacterium *Methylosinus trichosporium* OB3b degrades chlorinated aliphatic compounds such as trichloroethylene at rates that are considerably higher than any other organism. Degradation is mediated by the soluble Methane Monooxygenase enzyme (sMMO) which normally catalyzes the oxidation of methane to methanol. The use of *M. trichosporium* OB3b in environmental treatment is complicated by the complex regulation of the sMMO. The most important limitation arises from the fact that the synthesis of sMMO is inhibited by submicromolar concentrations of copper ions. To develop efficient treatment processes we have used a classical genetic approach to isolate mutants that express high levels of sMMO constitutively in the presence of copper concentrations that are inhibitory to wild type cells (Phelps et al. *Appl. Env. Microbiol.* 58 (1992)). The characteristics of the mutants including protein composition, methane monooxygenase activity, growth, copper uptake and genetic stability were characterized in some detail. Strategies for enhancing the "TCE transformation capacity" of the cells, i.e. the ability to to oxidize TCE for extended periods of time were investigated. One strain, *M. trichosporium* OB3b/PP358 which exhibits two-fold higher activities than wild type cells, was employed in batch scale bioreactor experiments. To prevent competition from contaminating microorganisms we isolated streptomycin resistant mutants of PP358 and periodically purged the reactor with an antibiotic solution. Reactors employing wild type *M. trichosporium* OB3b cells immobilized on diatomaceous earth or glass beads gave very low rates of degradation in part because of the inhibitory effect of copper adsorbed on the surface of the immobilization matrix. The use of sMMO constitutive mutants partially alleviated this problem but still the rates of TCE degradation were about one order of magnitude lower than in suspended cultures. In contrast, very high rates (up to 0.6 L TCE/mg dry weight/day) were obtained reproducibly in a hollow fiber membrane reactor unit. These rates represent the highest values reported so far for the *in situ* degradation of trichlorothylene in bioreactors.

X2-103 CLONING AND SEQUENCING OF THE GENE CODING FOR BENZALDEHYDE LYASE, A NOVEL TPP-DEPENDENT ENZYME FROM *Pseudomonas fluorescens* BIOVAR I. **Patricio Hinrichsen and Rafael Vicuña, Department of Molecular and Cellular Biology, Faculty of Biological Sciences, P. Catholic University, Santiago, CHILE.**

Bacterial degradation of lignin-derived compounds has been postulated as a crucial step in the completion of the carbon cycle. At this respect, structures of the diarilpropane(ethane) type have been poorly analyzed, and only two examples are known in which it has been possible to isolate and characterize the enzyme responsible for breakage of the intermonomeric linkage. One of these is benzaldehyde lyase (BL), which in a TPP-dependent reaction transforms benzoin in two molecules of benzaldehyde. In this work, we present the cloning and sequencing of the BL gene and some studies related with its expression, both in *E. coli* and in *Pseudomonas putida* KT2440.

A gene bank from *P. fluorescens* biovar I chromosomal DNA prepared in *E. coli* using the cosmid pLAFRI, was traspassed to *P. putida* KT2440 by triparental mating. Some clones were selected by stable growth in benzoin. Subcloning in pKT230 and pUC19 allowed the isolation of a 2,7-kb fragment that encoded the enzyme, as determined in crude extracts. This fragment was sequenced and found to contain an ORF of 1689 bp. Sequences resembling a conventional promoter were not identified, at least 200 bp 5' upstream from the Shine-Dalgarno signal. Moreover, it was found that BL gene is preceded by a gene coding for a novel benzaldehyde dehydrogenase. Therefore, they could be part of an operon. The BL gene encodes a protein of 58,808 Da, has a codon usage pattern similar to *P. putida* genes and shows two high-homology regions with others TPP-dependent enzymes.

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X2-104 MOLECULAR CLONING AND SEQUENCING OF THE GENES FOR *Pseudomonas paucimobilis* ENZYMES INVOLVED IN THE DEGRADATION OF DIMERIC LIGNIN COMPOUNDS. Yoshihiro Katayama, Eiji Masai, Shinya Kawai, Sachiko Kubota, Seiji Nishikawa and Noriyuki Morohoshi, Department of Applied Bio Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

We isolated *Pseudomonas paucimobilis* SYK-6, which was able to grow on various dimeric lignin compounds as the sole carbon source. The metabolic process is a distinct characteristic of this bacterium, which is equipped with an enzymatic modification system for various dimeric lignin compounds involved in the TCA cycle. The purpose of this study is to determine the function and structure of the genes for the *P. paucimobilis* enzymes involved in the degradation of dimeric lignin compounds. We have already isolated protocatechuate 4,5-dioxygenase genes, *ligA* and *ligB*, which encode a key enzyme in the process. Cleavage of beta-aryl ether is the most important process in lignin degradation because the linkage is the most abundant. The enzyme for the clevage of beta-aryl ether, beta-etherase, was detected in this bacterium. This enzyme catalyzed the unique and reductive cleavage of arylglycerol-beta-aryl ether (Calpha carbonyl type). And beta-etherase genes, *ligE* and *ligF*, were isolated. They were similar to glutathion *S*-transferase. LigE or LigF was activated by glutathion. Furthermore, the Calpha-dehydrogenase gene was isolated. The enzyme catalyzes the Calpha dehydrogenation of arylglycerol-beta-aryl ether (Calpha alcohol type), and therefore this process produces the specific substrate for beta-etherase. This enzyme is a member of ribitol dehydrogenase family. We will discuss about the functions and structures of the genes for degradation of lignin.

X2-105 CLONING AND SEQUENCE ANALYSIS OF LACCASE GENES AND CONSTRUCTION OF A HOST-VECTOR SYSTEM IN *Coriolus versicolor*. Shinya Kawai, Yousuke Iimura, Kenji Takenouchi, Yoshihiro Katayama and Noriyuki Morohoshi, Department of Applied Bio Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo183, Japan

A white-rot basidiomycete, *Coriolus versicolor*, secrets a number of extracellular laccases. Our previous study showed that laccase III, one of these laccases, was concerned in lignin depolymerization. And the genomic laccase III gene has been isolated by using laccase III cDNA fragment as a probe and the nucleotide sequence has been determined. And genes encoding for the homologous laccases were isolated and characterized. Laccase genes construct a multigene family in the *C. versicolor* genome.

We have tried to construct a transformation system for genetic approaches in *C. versicolor*. By these approaches, the lignin degradation mechanism in this fungus will be understood. Trans formation of *C. versicolor* was attempted by using protoplast PEG-$CaCl_2$ method. *C. versicolor* has been transformed to G418 resistant by a plasmid containing APHI gene derived from Tn *903*. The plasmid resulted in the integration into the genome by non-homologous recombination. The gene expression vector was constructed by using the promoter and terminator regions of the cloned laccase III gene. We also tried the heterologous gene expression in *C. versicolor*. The gene encoding for beta-etherase, which catalyzes the unique and reductive cleavage of arylglycerol-beta-aryl ether, from *Pseudomonas paucimobilis* SYK-6 was inserted in the expression vector. Expression of the beta-etherase gene is under investigation.

X2-106 CHARACTERIZATION OF MICROBIAL CATECHOL 2,3-DIOXYGENASES IN HYDROCARBON DEGRADATION : MOLECULAR CLONING, PURIFICATION, AND ACTIVITY. Youngsoo Kim and Kyung Rak Min. Department of Biochemistry, College of Pharmacy, Chungbuk National University, Chungbuk 360-763 KOREA.

Three catechol 2,3-dioxygenases have been cloned from *Achromobacter xylosoxidans* KF701, *Alcaligenes* sp. KF711, and *Pseudomonas putida* KF715 which are biphenyl/polychlorinated biphenyl (PCB)-degrading soil bacteria. These enzymes were purified and compared their physical and enzymic properties with those from naphthalene/salicylate-degrading *P. putida* (NAH7) and toluene/xylene-degrading *Pseudomonas* sp. (pWWO). The catechol 2,3-dioxygenases were identified as yellow bands on native 7.5%-polyacrylamide gel by electrophoresis followed by soaking with 0.5 M catechol. The five enzymes are significantly different from one another in their electrophoretic mobilities on the native PAGE. However three catechol 2,3-dioxygenases cloned from biphenyl/PCB-degrading bacteria have the same electrophoretic mobility on SDS PAGE, in which all of the enzymes have a single band with 39,000 in size. The catechol 2,3-dioxygenases have been purified by acetone cut, DEAE-Sephacyl and immunoaffinity chromatographies. Four catechol 2,3-dioxygenases cloned from NAH7, pWWO, and chromosomal DNA of *A. xylosoxidans* KF701, and *P. putida* KF715 exhibited the highest ring-fission activities to catechol, but the enzyme cloned from *Alcaligenes* sp. KF711 to 4-methylcatechol among catechol, 4-chlorocatechol, 3-methylcatechol, and 4-methylcatechol. Catechol 2,3-dioxygenase cloned from *Alcaligenes* sp. KF711 exhibited higher ring-fission activities to catechol derivatives than those from the other soil bacteria.

X2-107 CONSTRUCTION OF NEW ENZYMATIC ACTIVITY BY CHANGING OF MULTICOMPONENT ENZYME, Kazuhide Kimbara, Satoshi Iuchi, Masao Fukuda and Keiji Yano, *Dept. BioEngineering, Nagaoka Univ. Tech. and JRDC, Niigata 940-21, JAPAN*

Polychlorinated biphenyls (PCBs) are widely distributed environmental pollutants. A bacterial strain *Pseudomonas* sp. KKS102 was isolated from soil using PCBs as a sole source of carbon and energy. Genes involved in degradation of PCBs have been isolated and sequenced. Biphenyl dioxygenase is a multicomponent enzyme which usually consists of four polypeptides, a large (product of *bphA1*) and a small (product of *bphA2*) subunit of iron-sulfur protein, ferredoxin (a product of *bphA3*) and ferredoxin reductase (a product of *bphA4*). Interestingly, biphenyl dioxygenase gene cluster of KKS102 does not contain ferredoxin reductase. A benzene degrading *Pseudomonas putida* Cys 81 was isolated from soil and the gene for benzene dioxygenase was isolated and sequenced. Overall homology of amino acid between biphenyl and benzene dioxygenase is around 60% but these dioxygenases have different specificity. To determine which component is important for substrate specificity, we constructed combinations of each component. Benzene dioxygenase (*bnzA1, A2, A3, A4*) and dihydrodiol dehydrogenase (a product of *bnzB*) were cloned under the *lac* promoter of a multicopy plasmid and each component was replaced by DNA fragments from biphenyl dioxygenase. The resultant plasmids were named pAC3 (*bnzA1, A2, A3, A4, B*), pAC2 (*bphA1, bnzA2, A3, A4, B*), pAC8 (*bphA1, A2, bnzA3, A4, B*) and pAC9 (*bphA1, A2, A3, bnzA4, B*). After induction by IPTG, crude extract was prepared and used for measuring enzymatic activities. Interestingly, pAC2 had lost all enzymatic activities but pAC8 and pAC9 had retained them. To our surprise, though pAC3 did not have enzymatic activity for *p*-chlorobiphenyl, pAC8 and pAC9 acquired the activity. This result strongly suggests that iron-sulfur proteins are responsible for the substrate specificity and formation of active complex between large and small subunit of iron-sulfur protein are very important for the activity.

X2-108 THE BENZENE DEGRADATION SYSTEM IN *PSEUDOMONAS AERUGINOSA* JI104, Atsushi Kitayama, Hajime Nishimura and Yasushi Kawakami*, Department of Chemical Engineering, University of Tokyo, Bunkyo-ku, Tokyo, 113, Japan, *IRI, Kashiwa-shi, Chiba, 277, Japan

Pseudomonas aeruginosa JI104 grows on benzene as a sole source of carbon and energy. From this strain, three different sets of genes responsible for benzene degradation were cloned and expressed in *Escherichia coli*. These sets of genes are quite different from each other in their restriction maps. Southern blotting analysis shows there is little homology among these DNA fragments. One of them encodes enzymes that oxidize benzene to *cis*-benzene dihydrodiol, remaining two encode those oxidizing benzene to phenol as the initial detectable metabolite. DNA sequence analysis reveals the benzene dioxygenase gene in JI104 is identical to the biphenyl dioxygenase gene in *Pseudomonas pseudoalcaligenes* KF707. The two benzene monooxygenases cloned in *E.coli* individually produce different pigments, blue pigment, presumably indigo and unidentified red-brown one.

JI104 adopts two benzene degradation pathways, through either *cis*-benzene dihydrodiol or phenol to catechol. Both of enzymes responsible for initial step of these pathways are expressed inducibly with their substrates in JI104. But the intermediary metabolites analysis reveals that both the level and the lag of their induction are different. Under the particular culture condition, *cis*-benzene dihydrodiol pathway is opened at first. Phenol pathway(s) followed it after some period. It seems that there is some expression control on the JI104's multiple benzene degradation pathways.

X2-109 KINETIC ANALYSIS OF AROMATIC RING-FISSION DIOXYGENASES FROM BTEX-DEGRADING PSEUDOMONADS ISOLATED FROM OXIC AND HYPOXIC ENVIRONMENTS, Jerome J. Kukor, Mark D. Mikesell, and Ronald H. Olsen, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-0620

We have investigated bacterial degradation of benzene, toluene, ethylbenzene, and xylenes (collectively designated BTEX) in petroleum hydrocarbon contaminated hypoxic groundwater environments, i.e., environments in which dissolved oxygen is at a concentration of approximately 5 mg/l, or lower. Bacteria isolated from such environments were screened for their ability to degrade toluene under denitrifying conditions. Three isolates, *Pseudomonas* sp. strain W81, *P. pickettii* PKO1, and *P. fluorescens* CFS215, showed enhanced toluene degradation in hypoxic microcosms when nitrate was present as an alternate electron acceptor. In contrast, transconjugates of archetypal denitrifying bacterial strains, *P. denitrificans* or *P. stutzeri*, carrying the TOL plasmid pWW0, as well as the nitrate-reducing strain *P. cepacia* G4, did not show enhanced, nitrate-dependent toluene degradation in hypoxic microcosms. We have examined kinetic parameters for catechol 2,3-dioxygenase (C23O), a key enzyme of the toluene degradative pathway for all of these strains. C23O purified from *Pseudomonas* sp. strain W31, *P. pickettii* PKO1, and *P. fluorescens* CFS215 had at least 10-fold greater affinity for oxygen and at least 20-fold greater rate of substrate turnover than found for the analogous enzymes from the TOL plasmid, from *P. cepacia* G4, or from *P. putida* PpF1. These differences in kinetic parameters of key degradative enzymes may relate to pathways functional for enhanced bioremediation of BTEX in hypoxic environments.

X2-110 INDUCTION OF TOLUENE NON-DEGRADING MUTANTS BY CATABOLIC INTERMEDIATES OF TOLUENE, Menu Leddy, Donald Phipps, and Harry F. Ridgway, Biotechnology Research Department, Orange County Water District, Fountain Valley, CA 92728-8300

Genetic stability of hydrocarbon-degrading microorganisms is desireable for optimizing bioremediation. A high frequency (>1%) of toluene non-degrading (tol-) mutants was observed in *Pseudomonas putida* 54g grown on gasoline or toluene. Strain 54g contains a 117kb TOL-like plasmid that encodes for the meta-cleavage enzyme catechol-2,3-oxygenase (C23O) and restores toluene utilization when mobilized into tol- cells. Wild-type 54g passaged on benzoate no longer exhibited C23O activity, but retained the ability to grow on toluene, possibly via a chromosomal pathway using a different ring-cleaving enzyme. Thus, tol- mutants possess defects in both the plasmid-encoded meta-cleavage and chromosomal pathways. No tol- mutants reverted to the tol+ phenotype, suggesting loss of genetic material encoding for hydrocarbon degradation. Mutants failed to catabolize toluene or other hydrocarbons, but still grew on glucose, indicating that biosynthetic pathways remained intact. Toluene catabolism was required for tol- mutant production, since no mutants were observed on glucose or R2A medium, even in the presence of toluene. Of the toluene intermediates tested, only benzylalcohol resulted in a substantial increase in tol- mutant production. Much lower numbers of mutants were observed on benzaldehyde. No tol- mutants were observed on benzene, benzoate, or ethylbenzene, suggesting their formation was substrate dependent. A tol- mutant introduced into a log-phase culture of wild-type cells growing on ethylbenzene underwent substantial growth at the expense of carbon released from wild-type cells. Thus, pre-existing (i.e., spontaneous) tol- mutants should have been readily detected in such cultures. That they were not suggests tol- mutants arise via an induction process involving specific intermediates of toluene catabolism, such as benzylalcohol. Though aromatic hydrocarbons provide a source of carbon and energy for bacteria, they may also result in irreversible genetic damage.

X2-111 LOSS OF THE BPH (BIPHENYL) CATABOLIC GENES IS REPORTED FROM THE CATABOLIC PLASMID OF A BIPHENYL-DEGRADING *PSEUDOMONAS* Sp. DURING GROWTH ON BENZOATE AS SOLE CARBON AND ENERGY SOURCE. Gareth Lloyd-Jones, Richard C. Ogden & Peter A. Williams. School of Biological Sciences, University of Wales, Bangor, Gwynedd. LL57 2UN, UK.

A *Pseudomonas* sp. CB406 capable of growing at the expence of biphenyl and the monochlorinated biphenyls was isolated from PCB-contaminated soils provided by Monsanto plc, Newport, UK. The genes required for the catabolism of these compounds by CB406 were located on a large 200Kb non-transmisable plasmid. Growth of this strain on benzoate resulted in large reproducable deletions, of approximatly 100Kb, occuring within this plasmid, with a resulting loss of biphenyl-degrading ability, along with the loss of catechol-2,3-dioxygenase activity which are both present in the wild type strain. Southern hybridizations have enabled mapping of these catabolic genes to the deleted regions of the "benzoate-cured" plasmid.

X2-112 MICROBIAL REDUCTIVE DEHALOGENATION AS MEDIATED BY A GLUTATHIONE *S*-TRANSFERASE GENE PRODUCT IN *FLAVOBACTERIUM*, Cindy S. Orser, James Dutton, Peter Jablonski, and Cleston Lange, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843

We have previously reported the purification of a reductive dehalogenase from cell extracts of *Flavobacterium* sp. strain ATCC 39723 which converts tetrachloro-*p*-hydroquinone (TeCH) to 2,3,6-trichloro-*p*-hydroquinone (TrCH) and then to 2,6-dichloro-*p*-hydroquinone (DiCH) using the reduced form of glutathione (GSH). The protein has a molecular weight of about 30,000 and functions as a dimer. GSH is a common cofactor among eucaryotic detoxication enzymes classified as glutathione *S*-transferases (GSTs; EC 2.5.1.18). Although GSH has recently been reported as a cofactor for microbial dehalogenation, the mechanism of halide removal was not reductive but rather by hydrolytic dechlorination of dichloromethane by *Hyphomicrobium* DM2. Reductive dehalogenation of aromatic compounds has long been recognized as a probable enzymatic mechanism, although biochemical and molecular details are scant. We have cloned and sequenced the gene, *pcpC*, which encodes TeCH reductive dehalogenase from *Flavobacterium*. The gene is constitutively regulated and was functionally expressed in *E. coli*. Cell extracts of the *E. coli pcpC* clone, as well as whole cells, dechlorinated TeCH to TrCH and DiCH. Database searches did not group this GST with any of the eucaryotic subclasses, nor was there significant similarities to the GSH utilizing hydrolytic dechlorinating enzyme from *Hyphomicrobium*. *pcpC* was found to be present in several other microbes reported to dechlorinate pentachlorophenol.

X2-113 DEGRADATION OF AZO DYES BY THE LIGNIN-DEGRADING FUNGUS *Phanerochaete chrysosporium*, V. Renganathan, Jack T. Spadaro, and Michael H. Gold, Department of Chemical and Biological Sciences, Oregon Graduate Institute of Science and Technology, Beaverton, Oregon 97006-1999

Under nitrogen-limiting secondary metabolic conditions, the white-rot basidiomycete *Phanerochaete chrysosporium* extensively mineralized specifically ^{14}C-labeled aromatic rings of the azo dyes 4-phenylazophenol, 4-phenylazo-2-methoxyphenol, Disperse Yellow 3 [2-(4'-acetamidophenylazo)-4-methylphenol], 4-phenylazoaniline, N,N-dimethyl-4-phenylazoaniline, and Disperse Orange 3 [4-(4'-nitrophenylazo)-aniline]. Twelve days after addition, the dyes were mineralized 23.1% to 48.1%. Aromatic rings with substituents such as hydroxyl, amino, acetamido, or nitro functions were mineralized to a greater extent than unsubstituted rings. These results suggest that *P. chrysosporium* may have the potential for textile mill effluent cleanup and for bioremediation of dye-contaminated soil.

X2-114 OXIDATION OF HALIDES BY PEROXIDASES AND THEIR SUBSEQUENT REDUCTIONS, Manish M. Shah and Steven D. Aust, Biotechnology Center, Utah State University, Logan, UT 84322-4705

The white rot fungus Phanerochaete chrysosporium can mineralize highly halogenated organics like PCP, DDT and PCBs. Although lignin peroxidases (LiP) produced by the fungus are proposed to be key components in the degradation of chemicals, in vitro studies have suggested that LiP have haloperoxidase activity. This seems like a conflict to suggest a haloperoxidase activity for LiP as the fungus is mineralizing halogenated chemicals. We therefore investigated the haloperoxidase activity of LiP and other peroxidases. Iodide oxidase was inhibited by EDTA and inhibition was reversed at higher concentrations of iodide. EDTA was decarboxylated in a reaction mixture containing a peroxidase, iodide, H_2O_2 and EDTA. EDTA was also decarboxylated by hypoiodite, a possible intermediate during oxidation of iodide by peroxidases. Iodide dependent pseudocatalatic activity was increased with an increase in the concentration of H_2O_2 and inhibited at higher concentrations of iodide. EDTA was also oxidized by horseradish peroxidase, lactoperoxidase and myeloperoxidase using iodide or bromide as a mediator. However, only myeloperoxidase was able to decarboxylate EDTA using chloride as a mediator. It is proposed that halide is oxidized to hypohalite and then reduced by EDTA, H_2O_2 or halide. It is proposed that H_2O_2 and other fungal reductants might be involved in the inhibition of halogenating activity. (Supported by NIH grant number ES04922.)

X2-115 ISOLATION AND MOLECULAR CHARACTERIZATION OF MICROBIAL STRAINS WITH AROMATIC DIOXYGENASE PATHWAYS, Gerben J. Zylstra, Robert L. Sandoli, Eric J. Basham, Susan D. Biegel, and Varsha A. Didolkar, Center for Agricultural Molecular Biology, Cook College, Rutgers University, New Brunswick, NJ 08903-0231

Aromatic dioxygenase enzymes catalyze the introduction of molecular oxygen into the substrate aromatic nucleus to form *cis*-dihydrodiols. The genes that encode for many of these enzymes have been cloned and their nucleotide sequence determined. Comparisons between the deduced amino acid sequences of distantly related dioxygenases have suggested residues that are involved in catalysis. In order to further define the relationship between amino acid sequence and catalytic activity, especially substrate recognition, it is valuable to compare the amino acid sequences of a number of closely related dioxygenases. Initially, a collection of microorganisms must be obtained that have closely related genes but which do not have totally homologous sequences. A number of microbial strains that could grow at the expense of mononuclear aromatic compounds were isolated from various locations in the United States. The strains were initially screened for their growth substrate range. Further screening involved assays for dioxygenase enzyme activity. Genetic differences between strains were detected by Southern hybridization experiments using the previously cloned genes for various aromatic dioxygenases as probes. Restriction fragment length polymorphisms (or no hybridization) indicated the genetic relatedness of the dioxygenase enzymes between strains. The dioxygenase genes were cloned from a non-hybridizing strain and their nucleotide sequence determined.

Anaerobic Degradation; Aerobic Degradation

X2-200 STERANE BIODEGRADATION BY *MYCOBACTERIUM FORTUITUM*. * Roger A. Acey, **Stephen L. Parker and *Mike L.Bundy, *Department of Chemistry and Biochemistry, **Department of Microbiology, California State University, Long Beach, CA 90840

A field strain of *M. fortuitum* was used as a model biological system to examine the capacity of bacteria to degrade cyclic alkanes such as cholestane. While cholestane did not support measurable bacterial growth by itself, inclusion of either pristane or hexadecane in the culture medium as a primary growth substrate resulted in the biodegradation of the cyclic alkane. However, regardless of the primary growth substrate, the kinetics of cholestane biodegradation appear to be biphasic; the rate of degradation is low during the first three weeks of culture but increases dramatically thereafter. It appears that elevated sterane biodegradation occurs only after a significant amount of the growth substrate has been metabolized. Quantitatively, cholestane biodegradation is a function of the primary growth substrate, e.g., when supplied with pristane, 96% of the cholestane was metabolized within five weeks while with hexadecane less then a third of the cholestane was removed. Subsequently, a mixture of cholestane isomers was substituted for pure cholestane in a culture using pristane as a carbon source. Differential biodegradation of the various isomers comprising this mixture was observed . Preference for individual isomers appears to be a function of the stereochemistry associated with C17 alkyl side chain of the molecule. In fact, androstane, structurally identical to cholestane except that it lacks the C17 alkyl side chain, is not significantly biodegraded under these culture conditions. These observations lend credence to the postulate that the presence and conformation of the alkyl side chain in this class of steranes influences the suitability of these compounds as substrates for microbial biodegradation .

Supported by Chevron Oil Field Research Company

X2-201 COPPER INHIBITION OF *Methylosinus trichosporium* OB3b sMMO^c MUTANTS, David W. Graham[1], and Robert G. Arnold[2]. [1]Department of Civil Engineering, University of Kansas, Lawrence, KS 66049, and [2]Department of Civil Engineering and Engineering Mechanics, University of Arizona, Tucson, AZ 85721.

Methylosinus trichosporium OB3b is a type II methanotrophic bacterium that degrades a variety of halogenated solvents. High rate, broad specificity degradation occurs when the organism expresses soluble methane monoxygenase (sMMO). In the wild-type, sMMO is expressed only at low copper levels (typically <0.25 uM Cu(II)). Recently, five stable mutants, PP311, PP319, PP323, PP333, and PP358, were developed that express sMMO constituitively (sMMO^c), even at elevated copper levels (>10.0 uM Cu(II)). In order to assess the potential utility of the mutants for bioremediation, experiments were performed to determine the viability of the mutants under a variety of copper conditions. Eight copper complexing agents were evaluated with copper concentrations as high as 20.0 uM Cu(II). When copper was provided with strong copper complexing agents such as triethylenetetramine (trien), d-penicillamine, and cysteine, the mutants were chronically inhibited by copper levels as low as 5.0 uM Cu(II). In general, the wild-type strain was more resistant than the mutants to copper complex challenges. For example, "copper-free" grown, wild-type organisms showed no lag phase when challenged with 20 uM Cu(II)/50 uM trien. Under similar conditions, the mutant PP358 showed a 4 day lag phase and had a specific growth rate three times less than in "copper-free" conditions. Results suggest that mutants may be deficient in copper uptake and that they may be poor competitors in some physical environments.

X2-202 METABOLISM OF ARSENIC BY MIXED MICROBIAL CONSORTIA ISOLATED FROM CONTAMINATED LAKE SEDIMENTS NEAR YELLOWKNIFE, NORTHWEST TERRITORIES, CANADA, G.M. Hewitt, J. Jaafar, W.R. Cullen, D.A. Bright and K.J. Reimer, Department of Chemistry, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z1

As part of an ongoing study of geochemical cycling and bioavailability of historically deposited arsenic from gold mining operations in lakes near Yellowknife, N.W.T., enrichment cultures developed from sediment cores from Kam Lake are being examined for their abilities to metabolize arsenate. Inocula were taken from cores at six distinct depths and were added to arsenate amended media selective for aerobic heterotrophs, anaerobic heterotrophs, nitrate dissimilators, iron reducers, manganese reducers, sulfate reducers (lactate), sulfate reducers (acetate), and potential arsenate reducers. Aerobic heterotrophs from a sediment depth of 5 cm continuously depleted inorganic arsenic and actively produced methylarsonic acid and dimethylarsinic acid; however, these compounds were subsequently metabolized to undetected products. Anaerobic enrichments from the 5 cm depth also methylated arsenate. The rate of methylation and the spectrum of metabolites varied among the different physiological groups. Of note is the apparent production of methylated thioarsenicals by sulfate reducers. These preliminary results are important because freshwater sediments have not been examined for the concomitant capacity of different physiological groups of microbes to metabolize arsenic. In addition, identification of previously undetected metabolites will improve future *in situ* mass balance determinations and may improve our understanding of arsenic toxicity in relation to chemical speciation and environmental cycling.

X2-203 A LARGE LINEAR PLASMID FROM *XANTHOBACTER AUTOTROPHICUS* ENCODING CATABOLIC GENES FOR HALOGENATED ALIPHATIC HYDROCARBONS, Peter C.K. Lau, Hélène Bergeron, Diane Labbé, Chantel Turmel and Ginette Tardif, Environmental Genetics Group, Molecular Biology Sector, Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec, Canada H4P 2R2

pXAU1 from *Xanthobacter autotrophicus* GJ10, coding for the haloalkane dehalogenase (*dhl*A) and chloroacetaldehyde dehydrogenase (*xal*A) enzymes in the detoxification pathway of 1,2-dichloroethane, was shown to be a linear plasmid of *ca* 220 kb by zero-integrated field electrophresis (ZIFE) technique. The *xal*A gene as well as its chromosomal homolog, designated *xal*B, were cloned and expressed in *Escherichia coli*. Alignments of the predicted amino acid sequences from xalA/B with known aldehyde dehydrogenase structures revealed the highest degree of identity (62.5%) with the *aco*D-encoded AcDH-II of *Alcaligenes eutrophus*. Both *xal*A/B genes are likely regulated by a transcriptional activator due to the characteristic presence of the -24/-12 promoter sequences. In contrast, the *dhl*A gene, unlinked to the *xal* genes, appears to be regulated by a divergently transcribed gene of 195 codons, the sequence of which resembles the repressor proteins encoded by *tet*R and *bet*I of *E. coli*.

X2-204 BIODEGRADATION OF HOPANES BY *MYCOBACTERIUM FORTUITUM*. *Stephen L. Parker and **Roger A. Acey. **Department of Chemistry and Biochemistry and *Department of Microbiology, California State University, Long Beach, CA 90840.

Hopanes, believed to be resistant to bacterial biodegradation, have been used to gauge the relative degree of biodegradation of petroleum from contaminated environments. Therefore, factors which effect the biodegradation of hopanes impact on the reliability of field measurements assessing environmental clean-up procedures. Laboratory studies on the bacterial biodegradation of these compounds have been prohibitive due to the scarcity of the compounds. We report here that under laboratory conditions, cultures of M. fortuitum using pristane as a primary growth substrate, are capable of degrading hopanes. In cultures containing a mixture of the αα, ββ, and αβ isomers of C29 hopanes, greater than 30 % of the material is degraded within five weeks. Further analysis of the remaining hopane mixture indicates that the relative rates of degradation of the three isomers are concentration dependent, i.e., the more prevalent isomer in the initial mixture is degraded to the greatest degree. Similar levels of biodegradation were also evident with a mixture of the C27αβ, C29 αβ, and C31αβ hopanes. These studies indicate that the hopanes might not be as refractory to microbial biodegradation as was once believed. Finally, since there is no apparent preferential degradation based either on stereochemical considerations or on the chemical nature of the alkyl side chain on the E ring, we postulate that microbial attack is initiated on the common ring system shared by these compounds

Supported by Chevron Oil Field Research Company. We would also like to express our appreciation to Dr. J. M. Moldowan for providing the hopane mixtures.

X2-205 DEGRADATION OF TETRACYCLINE BY A BACTERIAL OXIDOREDUCTASE, Brenda Speer, Department of Biochemistry, Microbiology, and Molecular Biology, University of Maine, Orono, ME 04469-5735.

The tetracycline resistance gene encoded by Tn4351 from the anaerobic bacteria Bacteroides is a novel enzyme that requires oxygen to inactivate tetracycline (Speer and Salyers, J Bacteriol **170**: 1423-1429). Spent media assays have shown that tetracycline* (Tc*, altered tetracycline) cannot inhibit growth of susceptible bacteria (Speer and Salyers, J Bacteriol **171**:148-153). Subsequent experiments and sequence analysis have shown the protein (TetX) to be an NADP-requiring oxidoreductase (Speer et al, J Bacteriol **173**:176-183). Elemental analysis of purified Tc* shows C:N:H ratios are similar to tetracycline. Furthermore, Mass Spectra Analysis and NMR Analysis (carbon and proton) have yielded results that cannot be interpreted suggesting the possibility of aggregates being formed once tetracycline has been altered. UV-Vis analysis of Tc* has shown that the characteristic 354nm peak of tetracycline virtually disappeared while a new peak at 258nm appeared. The loss of the 354nm peak suggests that some alteration in the ring structure is occurring. However, since no data from Mass Spectra analysis was obtained, a molecular weight for Tc* could not be calculated. Since it was not possible to determine the structure of Tc* from these analyses, alternative methods of determining the structure of Tc* are underway in addition to studies on the nature of TetX.

X2-206 PURIFICATION AND CHARACTERIZATION OF 3,4-DIHYDROXYXANTHONE DIOXYGENASE FROM THE XANTHONE-DEGRADING *ARTHROBACTER* SP. STRAIN GFB 100, Paul H. Tomasek and Chun-Ming Chen, Department of Food Science, Rutgers University, New Brunswick, NJ 08903

Arthrobacter sp. strain GFB 100 utilizes an extradiol ring-fission dioxygenase, 3,4-dihydroxyxanthone dioxygenase (DHXD), in the catabolism of xanthone, a three-ring, oxygen-containing heterocyclic compound. DHXD was purified to homogeneity using a procedure which included ammonium sulfate fractionation, DE-53 anion exchange, Phenyl-Sepharose hydrophobic interaction, Q-Sepharose anion exchange, and hydroxyapatite chromatography. This procedure resulted in a 65-fold increase in specific activity with an activity yield of 21%. The specific activity of the purified preparation was 25.8 U/mg. The apparent holoenzyme molecular mass was 111 ± 6.7 kDa and the subunit molecular mass was 28.2 kDa. The enzyme contained 1.75 ± 0.22 mole iron per mole enzyme. These data suggested that the enzyme was a tetramer with a stoichiometry of $Fe^{2+}{}_2\alpha_4$. The isoelectric point of the enzyme was 5.1 - 5.2. DHXD had a optimum pH of 7.5 and was stable in the range of pH 6 to 8. The enzyme exhibited maximum activity at 20 °C with an energy of activation of 6.7 Kcal/mol. The enzyme catalyzed the *meta*-cleavage of 3,4-dihydroxyxanthone to form a *trans*, yellow ring-fission metabolite with a K_m value of 18.3 μM. The substrate analog, 7,8-dihydroxyflavone, was oxidized at about 33% of the rate of 3,4-dihydroxyxanthone with a K_m value of 10.3 μM.

X2-207 COUPLING TRANSPORT AND BIODEGRADATION OF TRICHLOROETHYLENE BY METHANOTROPHS, Edye C. Udell and Mary E. Lidstrom, Environmental Engineering Science, California Institute of Technology, W. M. Keck Laboratories, 138-78, Pasadena, CA 91125

Methanotrophs have previously been shown to degrade trichloroethylene, but most mechanistic studies completed thus far have focused on bacteria present in liquid media. In natural systems up to 90 percent of the biological activity occurs in the particulate and solids fraction of a sample. Thus it is important to determine kinetics of degradation for bacteria attached to a solid phase in order to predict biodegradation capabilities in a natural system.

We have studied the ability of pure cultures of methanotrophs attached to washed sand grains to degrade primary and secondary substrates. We have found that a Type I strain, *Methylomonas albus* BG8, was able to bind to the sand while two Type II strains, *Methylosinus trichosporium* OB3b and *Methylocystis parvus* OBBP, did not bind under the laboratory growth conditions. Using *M. albus* BG8, kinetics of methane consumption and trichloroethylene degradation by attached bacteria are determined and compared to the kinetics of consumption and degradation by free bacteria. Finally, a model is proposed for predicting biodegradation of a chemical contaminant by a single species in an infinite groundwater aquifer. The results of these studies will be useful for further understanding the remediation abilities of bacteria in natural aquifers.

Detection and Monitoring; Applications

X2-300 FIBER OPTIC-BASED BIOSENSOR: APPLICATIONS IN ENVIRONMENTAL MONITORING, George P. Anderson[1], Daya C. Wijesuriya[2], Robert A. Ogert, Lisa C. Shriver-Lake, Joel P. Golden, and Frances S. Ligler, Center for Bio/Molecular Science and Engineering, Code 6900, Naval Research Laboratory, Washington, D.C. 20375

The fiber optic biosensor is a device which measures the formation of fluorescent complexes at the surface of an optical fiber. The fluorimeter utilizes long clad optical fiber with a short section of core exposed near the distal end to form the sensing region. The sensing region is tapered to improve coupling of the evanescent wave generated fluorescence. Antibodies or other binding proteins are immobilized on the core to provide the mechanism for recognizing an analyte of interest and holding the fluorescent complexes on the fiber surface. These features yield a device that is fast, sensitive, and permits analysis of hazardous material remote from the instrumentation.

A laboratory version of the device is being used for assay development and performance characterization while a portable version is under development. Antibodies coated on the fiber are stable for up to two years of lyophilized storage prior to use. The fiber optic biosensor has been used to measure concentrations of toxins in the parts per billion (ng/ml) range in under a minute. Immunoassays for small molecules and whole bacteria are under development. The fiber can be placed in a flow chamber for rapid measurement of multiple samples. While the probe is disposable, antigen can be washed from the antibody to permit probe reuse. The fiber optic biosensor is currently being developed to detect toxins, explosives, and pathogens in environmental samples.

[1]Supported by a NRL-National Research Council Associateship
[2]Supported by a Georgetown Postdoctoral Fellowship

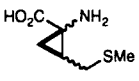
X2-301 ISOLATION AND PRELIMINARY CHARACTERIZATION OF A BACTERIAL STRAIN CAPABLE OF DEGRADING CYANIDE, Peggy J. Arps, Lisa Sporleder, and Michael G. Nelson, Institute of Arctic Biology and the Department of Mining and Geological Engineering, University of Alaska, Fairbanks, AK 99775.

Mining and exploration companies looking for gold in both Alaska and the "lower 48" are becoming more interested in lower-grade ore bodies. One efficient and cost effective way to recover the gold is by cyanide leaching. But wastewater must be treated to remove the cyanide and other chemicals that may be present in order to protect surface and ground water near the mines. Recent work indicates that certain types of naturally-occuring bacteria can be used to purify the water enough to discharge it into the environment. The U. S. Bureau of Mines Salt Lake City Research Center isolated a cyanide-degrading strain of Pseudomonas pseudoalcaligenes from a gold mining site in Nevada and they are currently conducting in situ tests of this strain. In collaborative research efforts with them, we have begun our own laboratory-based studies with this strain to determine the growth conditions for optimal degradation of cyanide and the maximum cyanide concentrations tolerated by these bacteria. Preliminary experiments indicate that sodium cyanide (up to 100 ppm) added to bacterial cell suspensions disappears within a matter of minutes, as determined by the picric acid method of free cyanide measurement. In related experiments, cell free supernatants derived from actively growing cell suspensions were also tested for their ability to lower the concentration of added sodium cyanide. Assays revealed a decrease in free cyanide over time, but with a much slower rate that was over many hours. The disappearance of free cyanide is not due to photooxidation or the production of hydrogen cyanide gas, but is most probably the result of true cyanide degradation. The mechanism(s) by which these microorganisms deal with cyanide and the significance of the project for bioremediation efforts will be discussed.

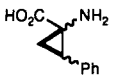
X2-302 ASYMMETRIC SYNTHESES OF 2,3-METHANOLOGS, SOME BIOACTIVITIES OF PEPTIDOMIMETICS FORMED FROM THESE, AND THEIR INFLUENCE ON SECONDARY STRUCTURE

Kevin Burgess,[1] Kwok-Kan Ho, J. Ronald Lake,[2] David H. Malin, [2] and Kamal Payza.[3]
[1] Chemistry, Texas A & M University, College Station, TX 77843; [2] University of Houston - Clear Lake, Houston, TX 77058; [3] NIMH, St Elizabeth's, Washington DC 20032

Asymmetric syntheses of all four **cyclo-Met** stereoisomers have been devised.



cyclo-Met



cyclo-Phe

This amino acid surrogate, and **cyclo-Phe** stereoisomers, have been incorporated into several peptidomimetics to study the influence this type of substitution on secondary structures, and pharmacological activities. For instance, all four isomers of Phe{**cyclo-Met**}ArgPhe-NH_2 have been synthesized and compared with the opiate antagonist PheMetArgPhe-NH_2. The peptidomimetics are all *less* strongly bound to the appropriate receptor, but they are *more* potent than the parent compound *in vivo*. This results can be rationalized in terms of the increased bioavailability of the peptidomimetics due to their enhanced stability with respect to proteases.

Model studies are in progress to access the influence of 2,3-methanologs on peptide secondary structure. The results of NMR, CD, and molecular modeling projects will be presented.

X2-303 METABOLITE DETECTION AS EVIDENCE FOR NATURALLY OCCURRING AEROBIC PCB BIODEGRADATION, William P. Flanagan and Ralph J. May, Chemical/Biological Reactions Laboratory, GE Corporate Research and Development, Schenectady, NY 12301-0008

The existence of naturally occurring microbes capable of degrading or dechlorinating PCBs implies that these processes can occur in the environment. However, additional evidence is required before a conclusive link can be established between laboratory observations and presumed *in situ* degradation. Evidence for ongoing anaerobic PCB-dechlorination in the environment was obtained from the analysis of modified congener distribution patterns at contaminated sites [J.F. Brown Jr. *et al.*, *Science* **236**, 709 (1987)]. Thus far, no direct evidence for the aerobic biodegradation of PCBs in the environment has been reported.

A strong indicator of *in situ* aerobic PCB biodegradation would be the presence of intermediate metabolites such as chlorinated benzoic acids in contaminated sediments. Analysis of sectioned sediment cores taken from PCB-contaminated Hudson River sites revealed that chlorinated benzoic acids were present and that their concentration profiles correlated with PCB depth profiles. From the congener distribution pattern of the chlorobenzoic acids detected, it is extremely unlikely that these compounds were derived from the breakdown of chlorinated herbicides. No chlorobenzoates were detected in sediment samples not contaminated with PCBs. In addition, other metabolites which are less prone to source ambiguity, such as 2-chloro-2',3'-biphenyldihydrodiol and 2-chloro-2',3'-dihydroxybiphenyl, have also been detected. To our knowledge, these results represent the first direct evidence for naturally occurring aerobic PCB biodegradation in the environment.

X2-304 PHYSIOLOGICAL STRESS IN HYDROCARBON-DEGRADING BACTERIA, Harry F. Ridgway, Donald Phipps, Eva Krieger*, and Menu Leddy, Biotechnology Research Department, Orange County Water District, Fountain Valley, CA 92728-8300, *Department of Microbiology, Montana State University, Bozeman, MT 59717

Substantial levels of physiological stress (i.e., injury) have been observed in axenic cultures and native populations of hydrocarbon-degrading bacteria recovered from gasoline-contaminated groundwater. Stress was operationally defined as the inability of viable hydrocarbon-degrading bacteria to grow when plated directly on mineral-salts medium (HCMM2) incubated in hydrocarbon vapor (e.g., gasoline, toluene, benzylalcohol, etc.). Cell viability, in turn, was defined as the ability to form a visible colony (CFU) on a rich 'non-selective' medium, such as R2A. Thus, the degree of physiological stress in a population could be quantitatively expressed as the ratio of CFUs appearing on HCMM2 and R2A media. Stressed bacteria were able to grow on HCMM2 after they had been first 'resusitated' via passage on R2A medium, suggesting that they possessed all genetic information required for hydrocarbon degradation. Often, >90% of native gasoline-degrading bacteria in contaminated groundwater samples were stressed and failed to grow on HCMM2 upon initial subculture. Thus, the true number of hydrocarbon-degrading bacteria could have been greatly underestimated in previous studies in which bacteria were enumerated by direct subculture on mineral-salts media. It is not known whether stressed bacteria are metabolically active *in situ* and capable of hydrocarbon degradation, or whether they can be resusitated to enhance bioremediation. The mechanism of stress induction is not clear, although increased stress appeared to be correlated with laboratory culture age, the presence of certain catabolic intermediates, and other environmental parameters (e.g., starvation).

X2-305 PARAMETERS AFFECTING THE ABILITY OF METHANOTROPHS TO OXIDIZE METHANE AND TRICHLOROETHYLENE, Jeremy D. Semrau, Kelly S. Smith and Mary E. Lidstrom, Environmental Engineering Science, W. M. Keck Labs, 138-78, California Institute of Technology, Pasadena, CA 91125

Pure cultures of methanotrophs representing Type I, Type II, and Type X categories have been examined for their ability to oxidize methane with the particulate form of the methane monooxygenase (pMMO). Methane consumption has been examined separately in whole cell and cell-free extracts to obtain enzymatic kinetic parameters V_{max} (maximal turnover rate), K_S (whole-cell affinity) and apparent K_m (cell-free-extract affinity). Data show that not only does the available copper concentration regulate the expression of the pMMO in cells that can express both the pMMO and the soluble form of the methane monooxygenase (sMMO), but it also affects the kinetics of the pMMO for methane. Electron paramagnetic resonance studies show that as the amount of copper in the growth medium increases, an increasingly large anisotropic copper signal is observed in the membrane fractions. It is possible that under these conditions more copper may be inserted into the pMMO, changing the kinetics of substrate turnover. A Type I strain, *Methylomonas albus* BG8, has been selected to examine the effect of copper on the ability of these cells to cometabolize trichloroethylene (TCE). To obtain estimates of the kinetic parameters of TCE oxidation, experiments must be run which isolate three effects: competition between TCE and methane for active sites, competition for reducing equivalents, and cellular inactivation by TCE-product toxicity. By examining each effect separately, we can generate a predictive model for optimizing TCE degradation by methanotrophs *in situ*. By focusing on pure cultures of methanotrophs first, we can then obtain the necessary information to predict the ability of mixed cultures to degrade TCE.

X2-306 BIODEGRADATION OF STRUCTURALLY RELATED AROMATIC COMPOUNDS IN PLANT RHIZOSPHERES, Jodi R. Shann, and Jay J. Boyle, Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221-0006.

Plant roots harbor large numbers of microorganisms in a soil zone known as the rhizosphere. The high input of plant-derived carbon into this area has led to speculation that rhizosphere microbial communities are characteristically different from those found in non-vegetated soils. This study investigated the potential of these rhizosphere organisms to degrade structurally related aromatic compounds, *ie.* phenol, 2,4-DCP, 2,4-D and 2,4,5-T. Rhizosphere and non-rhizosphere soils were collected from two field sites for a total sampling of seven plant species. Soils were added to serum bottle respirometers and given one of the above aromatic compounds as a radiochemical. Evolution of $^{14}CO_2$ was monitored over time as an indication of full compound mineralization. Data from these field collected soils indicate an increase in degradation by rhizosphere microorganisms relative to non-rhizosphere ones. Although the structurally simple compounds phenol and 2,4-DCP were easily mineralized in both rhizosphere and non-rhizosphere soils, the more recalcitrant 2,4-D and 2,4,5-T were mineralized faster and to a greater extent in rhizosphere soils. Separation of rhizosphere mineralization curves over time suggests a plant species effect. Lipid analysis was used to characterize the microbial communities found in the rhizosphere or non-rhizosphere soils and to assess their relationship to aromatic degradation.

X2-307 ENZYMES FOR THE BIOREMEDIATION OF NITRATE ESTER WASTE. M.K. Speedie[1], L. Geelhaar[1], G.F. Payne[2], W.-Q. Sun[2], and Johnathan Stacy[3]. [1] Department of Biomedicinal Chemistry, University of Maryland at Baltimore, Baltimore, MD 21201; [2] Chemical and Biochemical Engineering and Center for Agricultural Biotechnology, University of Maryland, Baltimore County, Baltimore, MD 21228;[3] Naval Surface Warfare Center, Indian Head, MD 20640.

In the first step of a study designed to examine the potential for enzymatically treating nitrate ester wastes with microbial enzymes, microorganisms were selected from environmental samples from sites known to be previously exposed to nitrate esters. These soil and water samples were screened for microorganisms which were resistant to 750 ppm trinitroglycerin. Additional organisms were selected on the basis of their ability to grow on a medium containing nitroglycerin as a sole nitrogen source. Fifty-six organisms, identified by these screens, were then cultured in media containing nitroglycerin and denitrating ability was assessed using the criteria of loss of trinitroglycerin by HPLC assay of tri-, di- and mono-nitroglycerin combined with a measurement of liberated inorganic nitrite in a resting cell culture. Two isolates which showed the greatest activity by both criteria were selected for further study. One was subsequently identified as *Enterobacter agglomerans* and the second as *Bacillus thuringiensis/cereus*. Cultivation conditions yielding improved denitrating activity were developed. Initial studies for enzyme purification revealed that one organism contains membrane-associated denitrating ability while the other has intracellular enzyme activity. Subsequent studies will be aimed at enzyme purification and characterization.

X2-308 DETERMINATION OF BENZENE USING A *PSEUDOMONAS* - CONTAINING BIOSENSOR, Hai-Meng Tan[1], Thiam-Chye Tan[2] and Shuan-Pei Cheong[2], [1]Department of Microbiology, [2]Department of Chemical Engineering, National University of Singapore, Lower Kent Ridge Road, Singapore 0511, Singapore Benzene and its derivatives are used extensively as industrial products. They are also found in petroleum and petroleum derived compounds. As a group, the alkylbenzenes have, however, been identified by the US EPA as among the 17 chemicals posing the greatest threats to human health. In particular, benzene has been reported to be carcinogenic. The determination of benzene and related compounds is therefore essential in environmental protection and industrial wastes analysis.

Pseudomonas putida ML2 (NCIB 12190) is able to degrade benzene utilizing it as sole carbon and energy source. Benzene is first converted to *cis*-1,2-dihydroxycyclohexa-3,5-diene (*cis*-benzene glycol). This reaction requires the presence of oxygen and is catalysed by the enzyme benzene dioxygenase. The *cis*-benzene glycol formed is dehydrogenated to catechol which then undergoes ring cleavage to *cis, cis*-muconic acid. *Cis, cis*-muconic acid is further degraded via the *ortho* or β-ketoadipate pathway to give tricarboxylic acid (TCA) cycle intermediates.

The aerobic degradation of benzene by *P. putida* ML2 has led us to develop an amperometric biosensor for the determination of this compound and its derivatives. The influence of varying concentrations of benzene on sensitivity was investigated. A linear relationship between the current range and the concentration of benzene was observed up to 9.23 mmol/l in 50 mM PO_4 buffer. The steady state response time ranged from 6 to 12 min, comparable to other biosensors currently in use. These results suggest that the *Pseudomonas*-containing biosensor is of great use for monitoring benzene in natural and industrial waters. The sensor may be further modified to sense toluene and xylenes. Finally, the results on the selectivity and stability of the biosensor will also be presented.

Engineering Systems and Applications

X2-400 ENHANCED ENZYME EXPRESSION AND ACTIVITY IN *E.coli* FOLLOWING THIN FILM IMMOBILIZATION: A MODEL OF STARVATION-INDUCED PROMOTERS FOR EXTENDED BIOCATALYSIS, Michael C. Flickinger[1,3] and Kristi L. Swope[2,3], [1]Dept. of Biochem., [2]Dept. of Chem. Eng. and Mat. Sci. and [3]Inst. for Adv. Studies in Biol. Proc. Technol., Univ. of Minnesota, St. Paul, MN 55108

A model system for extending the enzyme activity of *E.coli* immobilized in thin copolymer films is being studied to extend significantly the activity of immobilized whole cell catalysts. This method has potential for increasing the active life of biocatalysts useful in environmental remediation for several reasons. First, thin film immobilization on nonporous solid supports using a permeable coating results in a catalyst carrier with minimal mass transfer limitations for substrate and oxygen. These films can be multi-layer with an overall film thickness of 10-100 microns. Second, cells can be activated after immobilization facilitating control of catalyst activity after placement at the site where the catalyst will be used. Post-immobilization induction of *lacZ* using the native promoter has been demonstrated. In addition, fusions of the *E.coli* promoters P_{micB} or P_{ompA} to a promoterless *lacZ* gene are being studied. These promoters are induced under conditions of slow growth or nutrient limitation (P, C, N or a single amino acid), probable situations for immobilized cells. Methods have been developed to make uniform multi-layer films, determine *E.coli* viability, distribution, total protein content and enzyme level in films on a polyester carrier. A novel thin film reactor is being developed in order to investigate the respiration rate and periodic induction of *lacZ* using starvation-induction. Periodic induction may be useful for increasing rate-determining enzyme levels in immobilized aerobic bacteria and extending their active life.

X2-401 SORPTION AND METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS USING *SACCHAROMYCES CEREVISIAE* GENE ENGINEERED FOR P450 EXPRESSION AND THE FILAMENTOUS FUNGUS *CUNNINGHAMELLA ELEGANS*, M. Govindaswami, J. B. Reid, D. Warshawsky and J.C. Loper, University of Cincinnati Medical Center, Cincinnati, OH 45267-0524

The filamentous fungus *Cunninghamella elegans* is known to oxidize benzo[a]pyrene (B[a]P) to products similar to those produced by mammalian P450 systems (Cerniglia and Gibson, 1979). Although the yeast *Saccharomyces cerevisiae* encodes relatively few P450 genes, it has been widely used for testing the expression of foreign P450 CDNAS (Guengerich et al., 1991; Urban et al., 1990). In these studies, a Yep based plasmid was constructed containing cDNA for murine *Cyp1a-1* as a galactose inducible gene. *S. cerevisiae* strains with this plasmid or with the control vector were cultured in galactose media; *C. elegans* was cultured in 1/10 Sabouraud dextrose media under conditions for secondary metabolism. High purity 14C-B[a]P labelled at the 7 and 10 positions was delivered in ethylene glycol monomethyl ether. All three cultures accumulated over 90% of the radioactivity in the cells during overnight incubations. The yeast showed *Cyp1-a1* dependent release of water soluble metabolites. In repeat experiments supernatants from several days culture contained 40-70% of the charged radioactivity of which up to 50% was extractable into ethyl acetate. Reverse phase HPLC indicated a very polar metabolite(s) eluting in ca 4 minutes. The cell fraction containing 30-60% of the charged radioactivity was disrupted and extracted with ethyl acetate. HPLC indicated about 50% parent B[a]P plus 5 metabolites. Further studies will identify the metabolites and test for their mineralization in microbial communities. Production of metabolites by this yeast system provides a convenient tool for examination of effects of solvents and matrices on bioavailability of refractive PAHs. The kinetics and effects of solvents related to bioavailability are being further investigated.

X2-402 USAID PROJECT FOR REMEDIATION AND MITIGATION OF AGRICULTURAL BASED ENVIRONMENTAL DEGRADATION AND RESTORATION OF AGRICULTURAL ECOSYSTEMS, Dianne N. Janczewski, Joel I. Cohen and Ray Meyer, Office of Agriculture, U.S. Agency for International Development, Washington, D.C., 20523-1809

R&D/AGR is developing a project to address the need for remediation and mitigation of environmental degradation resulting from agricultural practices. Three broad areas (water systems, soil, and biodiversity) have been identified as targets needing assessment, evaluation, and development of best management strategies (environmentally and economically) within or related to agricultural systems. This project involves evaluation of the impact of agriculture on the environment and development remeditive strategies and alternative agricultural practices based upon applicability of current techniques and practices and on research derived alternatives. Bioremediation will play a major role in addressing the targets of this project in developing countries.

Increased demands for food have increased demand on available land for crop and animal production. Increased crop production has required intensifying land-use often resulting in soil degradation, erosion, and decreased diversity of production systems. Inappropriate pesticide use can contribute to contaminated soils and reduced water quality, and subsequent pollution of natural freshwater and eventually of estuarine and marine systems. Animal production can also contribute to environmental degradation through the disposal of large quantities of animal wastes which contribute to soil, water and air pollution, as do industrial by-products of agricultural processing through the dumping or incineration of "unusable" products. Remediation of agricultural-based environmental degradation requires assessments identifying sources and magnitudes of problems and subsequent research and development of new technologies and environmental/agriculture specialists. The potential use of organisms and systems specifically designed for bioremediation holds promise for the development of environmentally compatible remediation activities. Biodegradation and biorestoration systems are also being explored for their beneficial application to restoration of agricultural ecosystems.

The AID Agricultural Environment Mitigation and Remediation Project is particularly timely in light of revelation of current E. European and Newly Independent States agricultural environmental damage. Experts in environmental assessment and agriculture management are needed to provide technical expertise to facilitate remediation and mitigation activities.

X2-403 DEVELOPMENT OF BioReVOC TECHNOLOGY FOR THE ABATEMENT OF AIR TOXICS. Kailash C. Srivastava, ARCTECH, Inc., 14100 Park Meadow Drive, Chantilly, VA 22021, U.S.A.

Benzene, toluene, ethyl benzene and xylenes, collectively known as BTEX are among the top 189 priority pollutants listed by the EPA. The Clean Air Act Amendment of 1990 (CAAA) limits the emission of volatile organic compounds (VOC) to the atmosphere. Consequently, cost effective technologies are needed to meet the requirements of 1990 CAAA. BioReVOC is a potentially cost effective generic technology for the vapor phase treatment of VOCs by the microorganisms. It can potentially be applied for the abatement of air toxics emitted at temperatures higher than the ambient. Therefore, several microbial consortia were developed. These consortia are capable of growing on BTEX as the sole source of carbon. Based on the growth rates, one of these consortia, NKS_4, obtained from the municipal sludge was studied in detail. The consortium consists of aerobic rods that optimally grow at 50°C. This consortium removed 100% of Benzene, toluene and xylene mixture at 20,000 ppm loading. The consortium survived up to 100,000 ppm of BTX mixture.