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**Review of scientific literature on microbial  
dechlorination and chlorination of  
key chlorinated compounds**

**11<sup>th</sup> Quarterly Report  
3<sup>rd</sup> Quarter Year 2003**

**Report prepared for EUROCHLOR**

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November 30, 2003

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## ACRONYMS

<b>16S rRNA</b>	16S Ribosomal RNA
<b>CaaD</b>	<i>Trans</i> -3-chloroacrylic Acid Dehalogenase
<b>CB</b>	Chlorobenzene
<b>CBp</b>	Chlorobiphenyl
<b>CDDs</b>	Chlorinated Dibenzo- <i>p</i> -Dioxins
<b>CDFs</b>	Chlorinated Dibenzo- <i>p</i> -Furans
<b>CF</b>	Chloroform
<b>CPO</b>	Chloroperoxidase
<b>CT</b>	Carbon Tetrachloride
<b>2,4-D</b>	2,4-Dichlorophenoxyacetate
<b>1,2-DCA</b>	1,2-Dichloroethane
<b>DCE</b>	Dichloroethene
<b>DCM</b>	Dichloromethane
<b>DDT</b>	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
<b>1,3-DiCB</b>	1,3-Dichlorobenzene
<b>2,7-DiCDD</b>	2,7-Dichlorodibenzo- <i>p</i> -dioxin
<b>DNAPL</b>	Dense Non-Aqueous Phase Liquid
<b>E-acceptor</b>	Electron Acceptor
<b>EDB</b>	Ethylene Dibromide or Dibromoethane
<b>E-donor</b>	Electron Donor
<b>ERs</b>	Enantiomer Ratios
<b>GST</b>	Glutathione S-transferase (GST)
<b>HCH</b>	Hexachlorohexane
<b>PBDEs</b>	Polybrominated Diphenyl Ethers
<b>PCBs</b>	Polychlorinated Biphenyls
<b>PCP</b>	Pentachlorophenol
<b>PCE</b>	Tetrachloroethylene
<b>PCR</b>	Polymerase Chain Reaction
<b>TBF</b>	Trickling Biofilter
<b>TCA</b>	Trichloroacetic acid
<b>2,4,8-TCDF</b>	2,4,8-Trichlorodibenzofuran
<b>TCE</b>	Trichlorethylene
<b>VB</b>	Vinyl Bromide
<b>VC</b>	Vinyl Chloride

# **Review of Scientific Literature on Microbial Dechlorination & Chlorination of Key Chlorinated Compounds**

**11<sup>th</sup> Quarterly Report  
3<sup>rd</sup> Quarter– Year 2003**

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## **1. INTRODUCTION**

This report presents a review of scientific literature published during the second quarter of 2003 (covering August to October 2003) on the microbial halogenation and dehalogenation of the following compounds: vinyl chloride, dichloroethane, chloroform, dichloromethane, hexachlorobenzene, chlorobenzene, 1,2,4-1,2,3-1,3,5-trichlorobenzene, hexachlorobutadiene, octachlorostyrene, dioxins and chlorinated furans. In addition, reports regarding the microbial chlorination of compounds structurally related to those listed above were also reviewed.

## **2. SUMMARY OF MOST IMPORTANT DEVELOPMENTS**

### **2.a. Microbial Dechlorination**

The most important findings in this quarter for microbial dechlorination are as follows: 1) further insights on aerobic VC degradation pathway; 2) new strains of 1,2-dichloroethane halo-respiring bacteria; and 3) new strains of chlorinated dioxin degrading fungi.

A new enzyme in the pathway of aerobic vinyl chloride (VC) metabolism has been discovered (6, 7). The new enzyme is responsible for the second step in the pathway of *Mycobacterium* strains utilizing VC as a sole source of carbon energy. The first step is the monooxygenase reaction yielding VC-epoxide. The newly discovered enzyme, known as

epoxyalkane: coenzyme M (thioethanesulfonate) transferase, degrades the epoxide by forming an 2-hydroxyethyl adduct with coenzyme M.

Two new strains of 1,2-dichloroethane (1,2-DCA) of halorespiring bacteria were discovered (11, 12). *Desulfitobacterium dichloroeliminans* strain DCA1, selectively converts 1,2-DCA and all possible vicinal dichloropropanes and -butanes into completely dechlorinated end products (11). Strain DCA1 exclusively dichloroeliminates its substrates (e.g. producing ethene from 1,2-DCA). The other new strain was a rod shaped, gram positive, non-sporulating acetogenic bacterium strain belonging to the genus *Acetobacterium* which also dechlorinated 1,2-DCA to ethane (12).

Two studies report on new fungal isolates that degrade chlorinated dioxins. *Bjerkandera adusta* strain VH57 was selected from a screening of 500 basidiomycetes from Indonesia as the best degrader of 2,7-dichlorodibenzo-*p*-dioxin. An unidentified fungal strain degraded 2,7-dichlorodibenzo-*p*-dioxin and 2,4,8-trichlorodibenzofuran adsorbed onto activated carbon (58).

## **2.b. Microbial Chlorination**

The most important highlight for microbial chlorination is a publication reporting on the formation of chloromethanes by the abiotic reaction between pectin and chloride (18). This process acting in terrestrial ecosystems and during biomass burning may account for the bulk of atmospheric chloromethane.

## **3. MICROBIAL DECHLORINATION**

### **3.a. General Reviews**

In this quarter, two review articles on biological dechlorination were published (41, 60). The first article provides a comprehensive review of the genes involved in the aerobic dehalogenation of chlorobenzoates, chlorocatechols and 2,4,5-trichlorophenoxyacetic acid (41). This review emphasizes the genetic organization and the regulation of gene expression, as well as evolutionary considerations. The second review article provides a succinct review of microbial dehalogenation of polyhalogenated dioxins (60). The review mainly focuses on

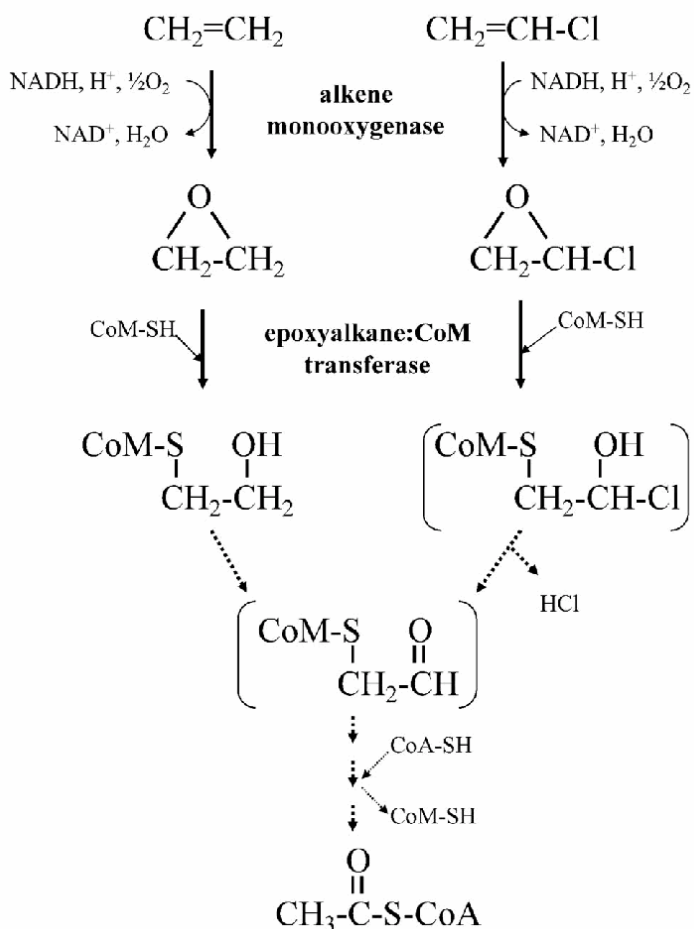
recent developments with respect to the reductive dechlorination of polychlorinated dibenzo-*p*-dioxins by highly enriched cultures and an isolated bacterial culture.

### 3.b. Microbial Dechlorination

#### Vinyl chloride and Other Chlorinated Ethenes

As indicated in each quarterly report, a large number of studies involve research evaluating the degradation of the higher chlorinated ethenes, perchloroethylene (PCE) and trichloroethene (TCE) because these are major groundwater contaminants. Thus information regarding the degradation of lower chlorinated ethenes, vinyl chloride (VC) and dichloroethenes (DCE), are found in these studies. Below the studies are categorized based on parent compound investigated, either lower chloroethenes (VC or DCEs) or higher chloroethenes (PCE or TCE).

**Vinyl Chloride (VC) and Dichloroethenes (DCE).** In this quarter, 6 studies directly investigated the biodegradation of lower chlorinated ethenes. Three studies were concerned with the anaerobic degradation of VC and DCE whereas 3 publications evaluated aerobic degradation of these compounds. The first study reports on a novel enzyme activity in aerobic bacteria that utilize VC as a sole source of carbon and energy (7). Previously it was known that these organisms utilize a monooxygenase to initiate the attack of VC, forming epoxides. However the enzyme involved in the subsequent degradation of VC epoxide was not known. This study reports on a novel enzyme activity responsible for the degradation of VC- or ethane epoxides. A coenzyme M (CoM)-dependent enzyme activity, referred to as epoxyalkane:CoM transferase (EaCoMT), was discovered in extracts from VC and ethene-grown cells of *Mycobacterium* strain JS60. A metabolite identified by mass spectrometry as 2-hydroxyethyl-CoM was produced from epoxyethane. The results indicate that the EaCoMT and monooxygenase enzymes encoded by a single operon (*etnEABCD*) catalyze the initial reactions in both the VC and ethene assimilation pathways. The proposed pathway for the aerobic degradation of VC is shown below in Figure 1.



**Figure 1.** Proposed pathways of VC and ethene assimilation in *Mycobacterium* strains. Intermediates that have not been identified are in brackets, and hypothetical reactions are indicated by dotted lines (6).

In a follow-up study the ubiquity of the EaCoMT pathway among *Mycobacterium* strains was evaluated (6). Ten different mycobacteria isolated on VC or ethene from diverse environmental samples. In all cases, epoxyethane metabolism in cell extracts was dependent on coenzyme M (CoM), with average specific activities of EaCoMT between 380 and 2,910 nmol/min/mg of protein. PCR with primers based on conserved regions of EaCoMT genes from *Mycobacterium* strain JS60 and the propene oxidizers *Xanthobacter* strain Py2 and *Rhodococcus* strain B-276 yielded fragments (834 bp) of EaCoMT genes from all of the VC- and ethene-assimilating isolates. The CoM-mediated pathway of epoxide metabolism appears to be universal in alkene-assimilating mycobacteria, possibly because of plasmid-mediated lateral gene transfer.

Aerobic cooxidation of *cis*- and *trans*DCE by a methane oxidizing bacterium, *Methylosinus trichosporium* OB3b, was explored (23). The authors evaluated both the wild type (wt) strain

and strain PP319, a mutant that expresses soluble methane monooxygenase at elevated copper levels. The study determined the influence of O<sub>2</sub> level and nitrogen-limitation on DCE degradation kinetics and toxicity in both organisms. In general, elevated O<sub>2</sub> conditions reduced DCE degradation rates in OB3b, although the negative effects of O<sub>2</sub> were less in PP319 than in the WT. DCE degradation rates were near zero under nitrogen-limiting conditions and elevated O<sub>2</sub>. Strain PP319 retained moderate DCE degradation rates under most O<sub>2</sub> and copper conditions making it a good candidate for bioremediation applications.

One paper reports on the use of vinyl bromide as a surrogate for measuring anaerobic VC degradation in environmental samples (17). Vinyl bromide (VB) dehalogenation rates and kinetics were studied and compared with those of VC by a methanogenic reductive dechlorinating enrichment culture containing *Dehalococcoides* species and by microcosms from two chloroethene-contaminated sites. The enrichment culture dehalogenated vinyl bromide to ethene at higher rates than VC at similar concentrations. VB was dehalogenated with a higher enzyme affinity than was VC, as indicated by their half-velocity constants, 240 and 21 μM, for VC and VB, respectively. Five to ten times shorter incubation times were required to detect the same level of reductive dehalogenation activity using vinyl bromide as a surrogate.

Anaerobic degradation of both VC and *cis*-DCE was reported for an Fe(III)-reducing enrichment culture, originating from landfill leachate contaminated sediments (20). However, the chlorinated ethene degradation was not linked to the Fe(III) reduction. No reduction products (eg. VC, ethene or ethane) were detected suggesting that the mode of degradation was oxidative, but no proof was provided.

Lastly, acetylene was identified as a potent reversible inhibitor of reductive dechlorination of VC by a mixed dehalogenating anaerobic culture (46). Acetylene at 12 μM caused 90% inhibition of VC reduction.

**Perchloroethylene (PCE) and Trichloroethene (TCE).** In this quarter, there were 10 research reports on the biodegradation of higher chlorinated ethenes. Seven studies evaluated anaerobic degradation; two studies focused on aerobic TCE cooxidation; and one study considered the degradation of PCE and TCE under combined anaerobic-aerobic conditions.

The first anaerobic paper describes the competition for hydrogen (H<sub>2</sub>) among microorganisms in anoxic environments (38). The flux of aqueous hydrogen concentrations in methanogenic sediment microcosms prior to and during reductive dehalogenation of PCE and chlorophenols was measured. Sediment microcosms that were actively methanogenic

maintained a mean H<sub>2</sub> concentration of 9.8 nM. During active PCE dehalogenation, sediment microcosms maintained a mean H<sub>2</sub> concentration of 0.82 nM. These data indicate that the threshold ecosystem H<sub>2</sub> concentration is controlled by microbial populations that couple hydrogen oxidation to thermodynamically favorable electron accepting reactions, including reductive dehalogenation of PCE.

The second anaerobic article presents a model groundwater flowing over a pool of free-product PCE in the presence of an active microbial population converting PCE to *cis*-DCE (5). The model demonstrates that a high biotransformation rate of PCE increases the concentration gradient of PCE at the water-DNAPL interface, enhancing dissolution.

The third anaerobic article, the influence of surfactants on a PCE-dechlorinating mixed culture was investigated in laboratory experiments (39). Many surfactants were evaluated for their effects on the rate and extent of PCE reductive dechlorination and their potential biodegradation by the mixed culture. Based on initial testing, a nonionic surfactant, Tween 80, and an anionic surfactant, Steol CS-330, were selected. Dechlorination of PCE to DCE, VC and ethene (ETH) occurred in all Tween 80-amended microcosms, with a depressed rate of ETH production as the only adverse effect. Steol CS-330, however, inhibited dechlorination beyond DCE at all surfactant concentrations exceeding 25 mg/L.

In the fourth anaerobic study, stable carbon isotope analysis of PCE and TCE was applied to evaluate natural attenuation in the area of a former dry-cleaning plant (61). The <sup>13</sup>C-enrichment in contaminants along the water flow path suggested that both, PCE and TCE were degraded. The enrichment of <sup>13</sup>C in the residual PCE fraction and an isotope fractionation factor from laboratory experiments were used to calculate the extent of biodegradation, which indicated that a major portion of PCE was biodegraded in the course of the plume.

In the fifth anaerobic study, combined isotopic measurements in conjunction with traditional chemical techniques were used to assess *in situ* biodegradation of TCE and CT (24).  $\delta^{13}\text{C}$  of hydrocarbons and  $\delta^{13}\text{C}$  and  $^{14}\text{C}$  of vadose zone CO<sub>2</sub>, sediment organic matter, and groundwater dissolved inorganic carbon (DIC) were measured.  $\delta^{13}\text{C}$  of *cis*-DCE was always heavier than TCE indicating substantial DCE biodegradation.  $^{14}\text{C}$  provided a direct *in situ* measure of complete hydrocarbon mineralization in vadose zone and in groundwater and may improve remediation strategies.

In the sixth report, acetylene was identified as a reversible inhibitor of reductive dechlorination of TCE (as was indicated above for VC) (46). Acetylene at 192  $\mu\text{M}$  caused 90% inhibition of TCE reduction by a mixed dehalogenating anaerobic culture.

The last anaerobic article reports on a PCE reductive dehalogenase of *Dehalobacter restrictus* (34) and is discussed in greater detail in Section 3.c “*In vitro degradation of chlorinated compounds.*”

The first two aerobic TCE degradation articles from this quarter report on TCE cooxidation in biofilters (reactors treating contaminated gases) (29, 30). The first of these tested a trickling biofilter (TBF) system that consists of two parallel operated TBFs units (30). One for biodegradation TCE and the other for reactivation of an inactivated biofilm. The TBF system continuously treated gas-phase TCE by phenol grown cells of *Burkholderia cepacia* G4. The maximum TCE elimination capacity was 17 mg/L<sub>reactor</sub>/day.

The second biofilter publication concerned a pilot scale demonstration project of the treatment of a TCE containing airstream stripped from contaminated wells. The effects of several critical process variables were investigated to evaluate technical and economic feasibility, define operating limits and develop design information for a full-scale biofilter system. Propane was used as the primary substrate necessary to induce the production of a nonspecific oxygenase. Results indicated that the process scheme used to introduce propane into the biofiltration system had a significant impact on the observed TCE removal efficiency. TCE degradation rates were dependent on the inlet contaminant concentration as well as on the loading rate.

Finally one study evaluated a combined anaerobic-aerobic treatment of PCE (57). Anaerobic and aerobic microorganisms were coimmobilized in beads for the simultaneous anaerobic-aerobic treatment of PCE. Two different types of beads were considered which were prepared using alginate and a 0.5% (w/v) CaCl<sub>2</sub> (Ca-alginate beads) or BaCl<sub>2</sub> solution (Ba-alginate beads). It was found that Ba-alginate beads had the highest bacterial growth and provided the most strength. The most effective approach to coimmobilization was to add 200 mL of methanogens into 500 mL of 2% alginate solution and 20 mL of methanotrophs into 500 mL to 2% alginate solution. Ba-alginate beads with coimmobilized methanogens and methanotrophs provided biological PCE dechlorination of 92%.

### **Carbon Tetrachloride (CT) and Chloroform (CF)**

Except for one article concerning isotope fractionation mentioned above in the section on *Perchloroethylene* (24), no other articles on the microbial degradation of carbon tetrachloride and chloroform were found during the period of review.

**Chloromethane (CM) and Dichloromethane (DCM)**

This quarter there were three publications on the dechlorination of dichloromethane. A study on the catalytic mechanism of DCM dehalogenase from *Methylophilus* sp strain DM11 (56) is discussed in Section 3.c “*In vitro degradation of chlorinated compounds*”, while a publication reporting on a rapid method to estimate DCM concentrations in biodegradation assays (26) is dealt with in Section 3.d “*New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds*”.

Evidence for the existence of marine Type II  $\alpha$ -proteobacteria methanotrophs was obtained using PCR amplification of 16S rDNA primers (49). Type II  $\alpha$ -proteobacteria methanotrophs are microorganisms capable of a wide range of cometabolic transformations of chlorinated solvents, which were previously only known from terrestrial environments.

**Dichloroethane (1,2-DCA) and Other Chlorinated Ethanes**

Two studies tested the microbial degradation of chlorinated ethanes this quarter. Isolation of *Desulfitobacterium dichloroeliminans* strain DCA1, a nutritionally defined anaerobic dehalorespiring bacterium that selectively converts 1,2-DCA and all possible vicinal dichloropropanes and -butanes into completely dechlorinated end products was reported (11). This microorganism shows unique dehalorespiratory biochemistry. Unlike known dehalorespiring isolates, strain DCA1 does not carry out reductive hydrogenolysis reactions but rather exclusively dichloroeliminates its substrates. Menaquinone was identified as an essential cofactor for growth of strain DCA1 in pure culture.

Growth-substrate dependent dechlorination of 1,2-DCA by a homoacetogenic bacterium showing 99.7% similarity with *Acetobacterium wieringae* was reported (12). The highest dechlorination rate (2 nmol Cl<sup>-</sup>/min/mg of protein) was determined with formate (40 mM) as the substrate. The *Acetobacterium* sp. strain gradually lost its dechlorination ability during about 10 transfers in pure culture, probably due to undefined nutritional requirements.

**Chlorobenzenes (CB)**

Four reports were found regarding the microbial degradation of chlorobenzenes under aerobic conditions (9, 31, 62, 65).

Biodegradation of 1,3-dichlorobenzene (1,3-DiCB) was reported by a bacterium, *Bacillus cereus* PF-11, isolated from town-gas industrial effluent (62). Degradation rates of up to 32 mg/L/day of the dichlorobenzene were attained in the study. The substrate specificity of the strain PF-11 was relatively low, and the degradation rate for different chlorobenzenes was in the order of monochlorobenzene > 1,3-dichlorobenzene > 1,2-dichlorobenzene.

Treatment of a contaminated gas containing 1,2-dichlorobenzene (1,2-DCB) by biofiltration in laboratory-scale experiments was reported (9). The organic filter medium utilized was composed of peat, maple wood chips, chicken manure and 1,2-DCB-contaminated soil. The biofilter treated air containing 0.30-0.75 g of 1,2-DCB per m<sup>3</sup> and attained a maximum elimination capacity of 9 g 1,2-DCB/m/hour (equivalent to 69% removal efficiency).

Laboratory studies were conducted to investigate the biologically mediated, aerobic mineralization of both freshly added and artificially aged, desorption-resistant 1,4-dichlorobenzene (1,4-DCB) (31). Mineralization of freshly added 1,4-DCB was observed without lag after the addition of a dichlorobenzene degrading culture. In separate experiments, mineralization of artificially aged, desorption-resistant 1,4-DCB was also observed, albeit at much lower rates (0.01 d<sup>-1</sup> in the freshly added treatments vs. 0.002 d<sup>-1</sup> in the desorption-resistant treatments). Mineralization curves in these studies indicated that the microbial population could directly access sorbed 1,4-DCB.

Soil microcosm experiments set up to investigate the fate of [<sup>14</sup>C]-1,2-dichlorobenzene (DCB), [<sup>14</sup>C]-1,2,4-trichlorobenzene (TCB) and [<sup>14</sup>C]-hexachlorobenzene (HCB) in air-soil-plant systems showed that mineralization was <1% for all compounds. (65). The predominant pathway of loss for 1,2-DiCB and 1,2,4-TCB was volatilization. Most of the added label in the HCB-spiked system remained in soil.

### **Chlorinated Dibenzo-*p*-dioxins and -furans (CDDs/CDFs)**

In this quarter, two studies report on the fungal degradation of chlorinated dibenzo-*p*-dioxins (CDDs) (52, 58). Two additional studies, one dealing with the degradation of CDDs by manganese peroxidase from a basidiomycetous fungus (19), and the other with the adsorption of CDDs by a protein from a *Bacillus* sp. (4) are discussed in Section 3.c “*In vitro* degradation of chlorinated compounds”.

*Bjerkandera adusta* strain VH57 was selected as a promising microorganism for dioxin degradation in a screening of 500 strains of basidiomycete fungi from temperate and tropical forests (52). The strain was shown to cause significant biodegradation (40% elimination) of a

dioxin model compound, 2,7-dichlorodibenzo-*p*-dioxin (2,7-DCDD), after 30 days of exposure. Three additional unidentified fungal isolates were also able to degrade 2,7-DiCDD, albeit at a lower rate than VH57.

Degradation of two model CDD/Fs compounds, 2,7-dichlorodibenzo-*p*-dioxin (2,7-DiCDD) and 2,4,8-trichlorodibenzofuran (2,4,8-TCDF), sorbed on activated carbon by an identified fungal strain (fungus 267) was investigated (58). Approximately 70% of the dioxin 2,4,8-TCDF was degraded in 30 days.

### **Hexachlorobutadiene and Octachlorostyrene**

No reports concerning the microbial dechlorination of hexachlorobutadiene and octachlorostyrene were found during the review period.

### **Polychlorinated Biphenyls (PCBs)**

In this quarter, nine publications reported on the microbial degradation of PCBs. Four of these publications address anaerobic degradation of PCBs (15, 28, 40, 44), five publications examine PCB degradation by aerobic bacteria (16, 35, 40, 54, 55), and one study described PCB degradation by yeast (51).

Microbially mediated, reductive dechlorination of Aroclor 1254 in contaminated lake sediments was demonstrated in microcosm studies (44). The average number of total chlorines per biphenyl decreased from 4.8-4.9 to 2.9-3.0 after approximately 260 days of incubation. Dechlorination occurred primarily at the *meta* and *para* positions (58-63% removal). Chlorine atoms in the *ortho* position were not removed. This most closely resembles pattern M, characterized by preferential removal of unflanked and flanked *meta* chlorines. The microcosm results are consistent with sediment cores analyzed from the same locations, which indicate accumulation with depth of the same *ortho*- and *para*-substituted congeners.

Anaerobic biodegradation of weathered PCBs in contaminated sediments was reported in laboratory-scale slurry microcosms (15). The PCB mixture, occurring in the sediments at 1.5-2.5 mg/kg, consisted of mono and di-chlorinated biphenyls along with PCBs partially ascribed to Aroclor 1242 and 1254. Substantial depletion of highly chlorinated PCBs along with the accumulation of low-chlorinated, often *ortho*-substituted biphenyls, was accompanied by significant consumption of sulfate and/or a production of methane. These results suggest that

reductive dechlorination of the weathered PCBs was mediated by indigenous sulfate-reducing bacteria and/or methanogens.

The impact of cobalt (Co) and nickel (Ni) on the reductive dechlorination of PCBs was examined (28). Co and Ni are the central metals of transition-metal cofactors of coenzyme F-430 and vitamin B-12, respectively, on the dechlorination of Aroclor 1248. Enrichment of cultures with Co (0.5-1.0 mM) led to the accumulation of *meta*-rich congeners (process M). In contrast, congener distribution patterns of all cultures supplemented with nickel (0.2-1.0 mM) or low concentrations of Co (0.2 mM) were similar to the pattern produced by the dechlorination process of H'. The authors hypothesize that cobalt in the range 0.5-1.0 mM appears to stimulate the growth of specific populations of *meta*-dechlorinators, which would favor a change in the dechlorination process from H' to M. A shift to M dechlorination is often undesirable since this pattern is known to be less effective on the dechlorination of the more highly chlorinated congeners of PCBs.

Degradation of PCBs in highly contaminated sludges (> 520 ppm) from a groundwater treatment system under aerobic and anaerobic conditions was compared (40). Aerobic experiments utilized by three different inocula (*ie.*, sludge from a poultry processing wastewater treatment plant, and a commercial inoculum (Petrobio<sup>R</sup>) with and without surfactant). The Petrobio<sup>R</sup> inoculum was used in the anaerobic assays. PCB degradation rates were slightly higher in the aerobic than in the anaerobic experiments. For examples, aerobic PCB biodegradation rates for Aroclors 1254 and 1260 were 13.5% and 23%, respectively, faster than the anaerobic biodegradation rates. Overall PCB biodegradation kinetics conformed to first-order, with rate constants ranging from 0.017 d<sup>-1</sup> under anaerobic conditions to 0.022 d<sup>-1</sup> under aerobic conditions.

A newly isolated aerobic bacterium belonging to the genus *Janibacter* sp. was found to cause extensive degradation of PCBs (Aroclor 1242) in liquid medium supplemented with yeast extract (54). High elimination (70-100% of the area of most chromatographic peaks) was observed after seven days of incubation. Degradation was less efficient in soil microcosms. Under the best conditions (sterile soil and 20 weeks of incubation) the strain was able to reduce nine out of the 62 different gas-chromatographic peaks in Aroclor 1242 by 50-100%.

The novel malotriose ester, 6''-O-palmitoylmaltotriose, was shown to enhance the biodegradation of Aroclor 1242 by *Burkholderia cepacia* LB400 (16). Two other related non-ionic surfactants, *ie.*, laurate and stearate malotriose esters, failed to promote microbial degradation, inspite of their positive effect on PCB solubility. Addition of 6''-O-palmitoylmaltotriose (48 mg/l) increased the apparent solubility of Aroclor from 140 to 305

µg/l, and led to 92% biological elimination of PCBs in 24 hours compared to only 50.8% elimination in assays lacking the surfactant. Maltotriose fatty acid monoesters are biodegradable and can be obtained at high yields using a new enzymatic methodology developed by the same authors.

Addition of cosubstrate was shown to enhance the biodegradation of Aroclor 1242 from contaminated sandy soil in slurry-phase reactors (35). Removal of PCB congeners decreased with increasing degree of chlorination: dichlorinated biphenyls (100% removal), followed by trichlorinated (92%) and tetrachlorinated biphenyls (24%) after four months of treatment. The degradability of PCBs in soil was also enhanced by advanced oxidation pretreatment (Fenton reaction) of the soil. Nearly complete elimination of PCBs was attained by the combined chemical-biological process and 72% mineralization of the intermediates generated during the chemical pretreatment.

Experiments with soil contaminated with Aroclor 1242 showed that the plant *Brassica nigra* enhanced PCB removal due to the positive effect of plant roots on gas diffusion into the soil, amendment infiltration, and microbial enrichment (55). Soils containing a single *Brassica nigra* plant achieved 61% PCB removal in the 0-2 cm and 2-6 cm depths after 9 weeks of bioaugmentation, whereas only 43 and 14% PCB removal, respectively, was achieved in unplanted controls. Shifts in the soil microbial community structure were demonstrated by denaturing gradient gel electrophoresis of bacterial 16S ribosomal DNA.

Oxidative stress due to the production of hydrogen peroxide was reported to cause inhibition of cell growth in catalase-deficient yeast cells exposed to PCBs (51). Evidence was obtained using one yeast strain (K601, wild type) that is resistant to the growth-inhibitory effect of PCBs, and a susceptible strain (AA542, PMR1 mutant).

### **Miscellaneous Chlorinated Compounds**

The search query used is specifically designed to review literature on the target compounds listed in the *Introduction* section. Interesting publications concerned with compounds outside of the range list which are found in the search process are briefly discussed below. This quarter our search retrieved six reports on the biodegradation of miscellaneous chlorinated pollutants, including hexachlorocyclohexane (43), technical toxaphene (50), tetrabromobisphenol-A (3), 2,2-dichloropropionic acid (36), 1,3-dichloropropene (63), and the chlorinated warfare agents VX and sulfur mustard (2).

Changes in the enantiomer ratios (ERs) of chiral pollutants in the environment are often considered evidence of biological alteration. However, a recent study shows that racemic environmental ER values for the chiral pesticide  $\alpha$ -hexachlorocyclohexane ( $\alpha$ -HCH) are not necessarily reflective of a lack of biodegradation (43). Periodic measurements in surface waters of the York River (VA, USA) did not show variations in  $\alpha$ -HCH ERs in relation to microbial activity.

Anaerobic degradation of technical toxaphene (Melipax) and several single components of technical toxaphene) by the bacterium *Dehalospirillum multivorans* was investigated (50). After 7 days, more than 50% of technical toxaphene was transformed. In agreement with reports in the literature, reductive dechlorination at geminal chlorine atoms was found to be the major transformation pathway for compounds of technical toxaphene.

Various soil bacteria use 1,3-dichloropropene as a sole source of carbon and energy. One enzyme involved in the catabolism of 1,3-dichloropropene is *trans*-3-chloroacrylic acid dehalogenase (CaaD), which converts the *trans*-isomers of 3-bromo- and 3-chloroacrylate to malonate semialdehyde. A recent paper provides evidence for the hydrolytic nature of the CaaD-catalyzed reaction (63), thereby distinguishing CaaD from a number of dehalogenases whose mechanisms proceed through an alkyl- or aryl-enzyme intermediate.

Dehalogenation of the fire retardant tetrabromobisphenol-A by an anaerobic enrichment culture with formation of tri-, di-, and mono-brominated bisphenol-A was reported (3). 2,2-dibromobisphenol-A was the dominant metabolite formed as result of the reductive debromination of dibromobisphenol-A.

Comparison of the dehalogenase gene pool in bacteria cultivated in  $\alpha$ -halocarboxylic acids, and more specifically 2,2-dichloropropionic acid, and the environmental metagene pool (the collective gene pool of both the culturable and uncultured microbes) from which they were isolated showed that there is a large bias introduced by culturing, not just in the bacteria isolated but also the degradative genes that they contain (36). The dehalogenases in the pure-cultures isolates capable of 2,2-dichloropropionic acid degradation were similar to previously described group I and II dehalogenases. In contrast, the majority of the dehalogenases isolated from activated sludge were not closely related to the dehalogenases in any isolate.

Rapid degradation of sulfur mustard by enzymatic haloperoxidation with chloroperoxidase (CPO) from *Caldariomyces fumago* was reported (2).

### 3.c. *In Vitro* Degradation of Chlorinated Compounds

In this quarter, five articles reported on the *in vitro* degradation of halogenated compounds by enzymes or their cofactors, and/or were devoted to the characterization of dehalogenating enzymes.

In the first of these, a membrane-bound PCE reductive dehalogenase (PceA; EC 1.97.1.8), the terminal component of the respiratory chain of *Dehalobacter restrictus*, was purified 25-fold to apparent homogeneity (34). The enzyme has a molecular weight of 71 kDa. Purified PceA catalyzed the reductive dechlorination of PCE and TCE to *cis*-DCE with a specific activity of 250 nkat/mg of protein with methyl viologen as artificial electron donor. The  $K_m$  values for PCE, TCE, and methyl viologen were 20.4, 23.7, and 47  $\mu\text{M}$ , respectively. Based on the almost identical N-terminal amino acid sequences of PceA of *Dehalobacter restrictus*, *Desulfitobacterium hafniense* strain TCE1 (formerly *Desulfitobacterium frappieri* strain TCE1), and *Desulfitobacterium hafniense* strain PCE-S (formerly *Desulfitobacterium frappieri* strain PCE-S), the *pceA* genes of the first two organisms were cloned and sequenced. Together with the *pceA* genes of *Desulfitobacterium hafniense* strains PCE-S and Y51, the *pceA* genes of *Desulfitobacterium hafniense* strain TCE1 and *Dehalobacter restrictus* form a coherent group of reductive dehalogenases with almost 100% sequence identity.

In the second *in vitro* article, the catalytic mechanism of DCM dehalogenase from the methane oxidizing bacterium, *Methylophilus* sp strain DM11 was elucidated (56). The glutathione (GSH)-dependent dichloromethane (DCM) dehalogenase from *Methylophilus* sp strain DM11 catalyzes the dechlorination of  $\text{CH}_2\text{Cl}_2$  to formaldehyde via a highly reactive, genotoxic intermediate, S-(chloromethyl)glutathione (GS- $\text{CH}_2\text{Cl}$ ). The catalytic mechanism of the enzyme toward a series of dihalomethane and monohaloethane substrates suggests that the initial addition of GSH to the alkylhalides is fast and that the rate-limiting step in turnover is the release of either the peptide product or formaldehyde. The turnover numbers for a series of dihalomethanes fall in a very narrow range (1 to 3  $\text{s}^{-1}$ ). The pre-steady-state kinetics of the DM11-catalyzed addition of GSH to  $\text{CH}_3\text{CH}_2\text{Br}$  exhibits a burst of S-(ethyl)-glutathione ( $k_b = 96 \text{ s}^{-1}$ ) followed by a steady state with  $k_{at} = 0.13 \text{ s}^{-1}$ . The rate constants for solvolysis of  $\text{CH}_3\text{SCH}_2\text{Cl}$ , a model for GS- $\text{CH}_2\text{Cl}$ , range between 1  $\text{s}^{-1}$  (1:1 dioxane/water) and 64  $\text{s}^{-1}$  (1:10 dioxane/water). Solvolysis of the S-(halomethyl)glutathione intermediates may also occur in the active site of the enzyme preventing the release of the genotoxic species. Together, the results indicate that dissociation of the GS- $\text{CH}_2\text{X}$  or GS- $\text{CH}_2\text{OH}$  intermediates from the enzyme may be a relatively rare event.

In the third *in vitro* article, the dioxin model compound, 2,7-dichlorodibenzo-*p*-dioxin (2,7-DiCDD), was shown to be degraded by manganese peroxidase from white rot basidiomycete *Phanerochaete sordida* YK-624 in the presence of polyunsaturated fatty acids (19). After incubation with MnP for 24 hours in the presence of highly polyunsaturated long chain fatty acids (4 to 6 double bonds) from 60 to 90% of the DiCDD was eliminated. In contrast, complete recovery of the unaltered 2,7-DiCDD was obtained in enzymatic treatments supplemented with fatty acids having 3 or less double bonds. The results suggest involvement of lipid peroxidation in the mechanisms. Reaction with lipoxidase (lipoxygenase), a dioxygenase which catalyzes peroxidation of unsaturated fatty acids, was carried out in the presence of 2,7-DiCDD and a fatty acid with 6 double bonds for 24 hours. Like the MnP reaction, the lipoxidase reaction also exhibited DCDD conversion. The authors hypothesized that the DCDD forms an adduct with a compound formed from unsaturated fatty acids via peroxidation.

In the fourth *in vitro* article, adsorption of halogenated aromatic pollutants (*i.e.*, polychlorinated dibenzo-*p*-dioxins and dibenzofurans (CDD/Fs), polychlorinated biphenyls (PCBs), chlorinated benzenes, and chlorinated naphthalenes) by a protein released from *Bacillus pumilus* was demonstrated (4). These halogenated aromatics were adsorbed considerably not only by cells but also by the released protein. Compound sorption generally increased with increasing degree of chlorination.

4-Chlorobenzoyl-coenzyme A (4-CBA-CoA) dehalogenase catalyzes the hydrolytic dehalogenation of 4-CBA-CoA to 4-hydroxybenzoyl-CoA by using an active site aspartate as the nucleophile. Formation of the corresponding Meisenheimer complex is followed by chloride ion expulsion which forms the arylated intermediate. This is then hydrolyzed to the product. In the fifth *in vitro* article, the strength of dehalogenase-substrate hydrogen bonding was shown to correlate with the rate of Meisenheimer intermediate formation (14).

### **3.d. New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds**

#### **Tools for Biodegradation Monitoring**

A simple spectrophotometric method was described to estimate the concentrations of dichloromethane (DCM) in biodegradation experiments (26). DCM concentration was estimated from its rate of degradation using a coupled enzymatic assay of two reactions: one

catalyzed by DCM dehalogenase in the presence of glutathione (Eq. (1)), and a second reaction catalyzed by formaldehyde dehydrogenase with  $\text{NAD}^+$  (Eq. (2)).



## 4. MICROBIAL CHLORINATION

### 4.a. General Reviews

The reactivity of haloperoxidases, particularly the mechanisms of oxidation, epoxidation and sulfoxidation reactions catalysed by chloro- and bromoperoxidases was reviewed in a recent report (13). Almost all the reactions discussed do not involve halogenation. The authors do review, however, the distribution of haloperoxidases in nature.

### 4.b. Microbial Chlorination in the Environment

#### Chloromethanes

One publication observed natural formation of chloromethane (CM) Abiotic conversion of chloride to CM occurs readily in plant material, with the widespread plant component pectin acting as a methyl donor (18). Significant CM emissions from senescent and dead leaves were observed at ambient temperatures. The emissions increase when temperatures are increased. Annual CM emissions from pectin were estimated to be in the range 0.03-2.5 Tg. This ubiquitous process acting in terrestrial ecosystems and during biomass burning could contribute the bulk of atmospheric CM. The mechanism of abiotic  $\text{Cl}^-$  methylation from methyl esters in pectin is not fully understood.

#### Chlorinated Compounds

Only one publication was found during this quarter on the formation of organohalogens by a bacterium (21).

Six novel halogenated soraphen analogues (polyketide metabolites) were isolated from the bacterium *Sorangium cellulosum* using precursor directed biosyntheses. More specifically, the new metabolites were obtained by feeding the strain substituted *meta* and *para*-fluorophenylalanine and cinnamate (21). The best precursor for the novel starter acids was cinnamate but *ortho* substituents were not tolerated by the further biosynthetic pathways.

### **Chlorinated Natural Organic Matter**

No publications on the microbial formation of chlorinated natural organic matter in the terrestrial environment were found during the third quarter of 2003.

## **4.c. Chlorination by Marine and Freshwater Organisms**

### **Chloromethanes**

This quarter there was only one report on the natural formation of chloromethanes, chloroethanes and chloroethenes by marine microorganisms (1).

The formation of volatile halogenated compounds by alga is thought to be related to oxidative stress, involving  $H_2O_2$  and algal peroxidases. Strong correlations between the releases of  $H_2O_2$  and brominated and some iodinated compounds by brackish-water algae to the seawater medium were observed in a recent study, which is in agreement with the latter hypothesis. In contrast, no such correlation was found for  $CHCl_3$ , suggesting the involvement of other formation mechanisms as well. The authors also investigated the effect of temperature changes on the production of hydrogen peroxide and volatile halocarbons by six brackish-water algal species (the diatom *Pleurosira laevis*, the brown alga *Fucus vesiculosus* and four filamentous green algae, *Cladophora glomerata*, *Enteromorpha ahlneriana*, *E. flexuosa* and *E. intestinalis*). The algae were exposed to temperature changes of 0-11°C under high irradiation promoting oxidative stress. Sixteen volatile halocarbons were detected in seawater after incubations of 66 algal samples. These were: trichloromethane ( $CHCl_3$ = chloroform), trichloroethene ( $C_2HCl_3$ = trichloroethylene), tetrachloroethane ( $C_2Cl_4$ = perchloroethylene), tribromomethane ( $CHBr_3$ = bromoform), dibromomethane ( $CH_2Br_2$ ), chlorodibromomethane ( $CHClBr_2$ ), bromochloromethane ( $CH_2BrCl$ ), bromodichloromethane ( $CHCl_2Br$ ), diiodomethane ( $CH_2I_2$ ), iodomethane ( $CH_3I$ ), iodoethane ( $C_2H_5I$ ), chloriodomethane ( $CH_2ClI$ ), 1-iodopropane ( $C_3H_7I$ ), 2-iodopropane (*iso* $C_3H_7I$ ), 1-iodobutane ( $C_4H_9I$ ) and 2-iodobutane (*sec* $C_4H_9I$ ). All 16 compounds occurred in 70–100% of the 66 samples, except for

$C_2HCl_3$  which was only produced by two out of six species and  $CH_2ClI$ . The production rates, as well as the quantitative composition of 16 volatile halocarbons, were strongly species-dependent and different types of responses to temperature were recorded. However, no response patterns to temperature change were found that were consistent for all species or for all halocarbons. The overall highest production rates were found for  $CHCl_3$ ,  $CHBr_3$ ,  $CHBr_2Cl$ ,  $CH_2Br_2$  and  $CH_2I_2$ . As an example, the maximum production rate of  $CHCl_3$  and  $CHBr_3$  found in the study were 405 and 74 pmol (g dry weight -DW)/hour, respectively. The authors hypothesize that the differences in the production of volatile halocarbons between algal species are related to the types and activities of the haloperoxidases in the different species, as well as the algae's different production levels of  $H_2O_2$ .

### Other Chlorinated Compounds

Several studies report on the identification and characterization of new halogenated metabolites from marine organisms, including: algae (10, 22, 25, 27, 53, 59), corals (64, 48, 47), sponges (13, 32, 33, 45), bacteria (21). In addition, one study reports on the synthesis of hydroxylated and methoxylated polybrominated diphenyl ethers (PBDEs) for use as reference standard for analytical and toxicological studies (37). Hydroxylated and methoxylated PBDEs may be natural products or they may be formed as metabolites of PBDEs, frequently used as flame retardants.

Nine novel halogenated oxylipin metabolites were isolated from the brown alga *Eisenia bicyclis* (25). The derivatives contained chlorine or iodine substituents. The structures of these oxylipin metabolites were confirmed by NMR and mass spectroscopy and compared with spectral data in the literature. Several studies report on the isolation of new halogenated metabolites from red alga, including: five new halogenated octodane monoterpenes having a cyclohexadienone from the red alga *Portieria hornemanni* (27); a new brominated sesquiterpene derivative, aldingenin A, from *Laurencia aldingensis* (10); four new brominated sesquiterpenes (59), three chlorinated C12 fatty acid metabolites from *Gracilaria verrucosa* (53), and two labdane type brominated diterpenes containing unprecedented eight- and seven-membered ether rings from the red alga *Laurencia obtusa* (22). The structures of the isolated compounds were elucidated by means of spectral data analysis.

Three publications report on the isolation of new halogenated metabolites from corals. Fifteen new halogenated prostanoids were isolated as minor constituents from the Okinawan soft coral *Clavularia viridis* (64). The metabolites contained chloro-, iodo-, or bromo-substituents. The structures of these compounds were determined on the basis of spectroscopic analysis and chemical conversion. Furthermore, eight new brominated oxylipins and oxylipin

glycosides were isolated from red sea corals (*Dendrophyllia* and *Dendronephthya*) sp (47). Their structures were elucidated mainly on the basis of NMR spectroscopic data. The compounds showed toxic activity in assays with a brine shrimp, sea urchin eggs (*Paracentrotus lividus*), and a crown gall tumor (*Agrobacterium tumefaciens*).

Three publications reported on the isolation of new halogenated compounds from sponges. Five novel brominated polyacetylenic diols, diplynes A-E, were isolated from the Philippines sponge *Diplastrella* sp. by employing bioassay-guided fractionation using the HIV-1 integrase inhibition assay (32, 33). The novel metabolites were characterized by spectroscopic analysis. Three new bromotyrosine derivatives, along with the previously described (3-bromo-4-hydroxyphenyl)acetonitrile, were isolated from an association of two sponges, *Jaspis wondoensis* and *Poecillastra wondoensis* (45). The structures of the new compounds were established on the basis of NMR and MS spectroscopic analysis. Several metabolites displayed significant cytotoxicity against human lung, ovarian, skin and colon cancer cell lines, and/or toxicity against antibiotic-resistant *Staphylococcus* strains.

#### 4.d. Chlorinating Enzymes

Except for the aforementioned review article (13) on haloperoxidases, there were two articles on the enzymatic formation of organohalogens this quarter (42).

Two fungal chloroperoxidases (CPOs), the heme enzyme from *Caldariomyces fumago* and the vanadium enzyme from *Curvularia inaequalis*, were shown to chlorinate a dimeric model compound, 1-(4-ethoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-dihydroxypropane (42). The model compound represents the major nonphenolic structure in lignin. Both enzymes also cleaved this dimer to give 1-chloro-4-ethoxy-3-methoxybenzene and 1,2-dichloro-4-ethoxy-5-methoxybenzene. The authors hypothesized that CPOs may have roles in the natural production of high-molecular-weight chloroaromatics and in lignin breakdown.

A novel, S-adenosyl-L-methionine (SAM)-dependent methyltransferase from the fungus *Trichoderma longibrachiatum* was characterized (8). This enzyme catalyzes the *ortho* methylation of several chlorophenols and other halogenated phenols, and it is thought to be involved in the formation of chloroanisoles in cork taint of wines. The methyltransferase was specifically induced by several chlorophenols, particularly those containing three or more chlorine atoms. The enzyme activity was also specific for halogenated phenols containing fluoro, chloro, or bromo substituents.

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## 5. REFERENCES CITED

1. Abrahamsson, K., K.-S. Choo, M. Pedersen, G. Johansson, and P. Snoeijs. 2003. Effects of temperature on the production of hydrogen peroxide and volatile halocarbons by brackish-water algae. *Phytochemistry* 64:725-734.
2. Amitai, G., R. Adani, M. Hershkovitz, P. Bel, I. Rabinovitz, and H. Meshulam. 2003. Degradation of VX and sulfur mustard by enzymatic haloperoxidation. *Journal of Applied Toxicology* 23:225-233.
3. Arbeli, Z., and Z. Ronen. 2003. Enrichment of a Microbial Culture Capable of Reductive Debromination of the Flame Retardant Tetrabromobisphenol-A, and Identification of the Intermediate Metabolites Produced in the Process. *Biodegradation* 14:385-395.
4. Choi, S. D., H. B. Hong, and Y. S. Chang. 2003. Adsorption of halogenated aromatic pollutants by a protein released from *Bacillus pumilus*. *Water Research* 37:4004-4010.
5. Chu, M., P. K. Kitaradis, and P. L. McCarty. 2003. Effects of biomass accumulation on microbially enhanced dissolution of a PCE pool: a numerical simulation. *Journal of Contaminant Hydrology* 65:79-100.
6. Coleman, N. V., and J. C. Spain. 2003. Distribution of the coenzyme m pathway of epoxide metabolism among ethene- and vinyl chloride-degrading *Mycobacterium* strains. *Applied and Environmental Microbiology* 69:6041-6046.
7. Coleman, N. V., and J. C. Spain. 2003. Epoxyalkane: Coenzyme M transferase in the ethene and vinyl chloride biodegradation pathways of *Mycobacterium* strain JS60. *Journal of Bacteriology* 185:5536-5545.
8. Coque, J. J. R., M. L. Alvarez-Rodriguez, and G. Larriba. 2003. Characterization of an inducible chlorophenol O-methyltransferase from *Trichoderma longibrachiatum* involved in the formation of chloroanisoles and determination of its role in cork taint of wines. *Applied and Environmental Microbiology* 69:5089-5095.
9. Coutu, C., G. Martineau, C. Guy, and R. Samson. 2003. Characterization of an organic filter medium for the bilofiltration treatment of air contaminated with 1,2-dichlorobenzene. *Journal of Chemical Technology and Biotechnology* 78:907-917.
10. de Carvalho, L. R., M. T. Fujii, N. F. Roque, M. J. Kato, and J. H. G. Lago. 2003. Aldingenin A, new brominated sesquiterpene from red algae *Laurencia aldingensis*. *Tetrahedron Letters* 44:2637-2640.

11. De Wildeman, S., G. Diekert, H. Van Langenhove, and W. Verstraete. 2003. Stereoselective microbial dehalorespiration with vicinal dichlorinated alkanes. *Applied and Environmental Microbiology* 69:5643-5647.
12. De Wildeman, S., A. Neumann, G. Diekert, and W. Verstraete. 2003. Growth-substrate dependent dechlorination of 1,2-dichloroethane by a homoacetogenic bacterium. *Biodegradation* 14:241-247.
13. Dembitsky, V. M. 2003. Oxidation, epoxidation and sulfoxidation reactions catalysed by haloperoxidases. *Tetrahedron* 59:4701-4720.
14. Dong, J., X. F. Lu, Y. S. Wei, L. S. Luo, D. Dunaway-Mariano, and P. R. Carey. 2003. The strength of dehalogenase-substrate hydrogen bonding correlates with the rate of Meisenheimer intermediate formation. *Biochemistry* 42:9482-9490.
15. Fava, F., S. Gentilucci, and G. Zanaroli. 2003. Anaerobic biodegradation of weathered polychlorinated biphenyls (PCBs) in contaminated sediments of Porto Marghera (Venice Lagoon, Italy). *Chemosphere* 53:101-109.
16. Ferrer, M., P. Golyshin, and K. N. Timmis. 2003. Novel maltotriose esters enhance biodegradation of Aroclor 1242 by *Burkholderia cepacia* LB400. *World Journal of Microbiology & Biotechnology* 19:637-643.
17. Gu, A. Z., H. D. Stensel, J. M. H. Pietari, and S. E. Strand. 2003. Vinyl bromide as a surrogate for determining vinyl chloride reductive dechlorination potential. *Environmental Science & Technology* 37:4410-4416.
18. Hamilton, J. T. G., W. C. McRoberts, F. Keppler, R. M. Kalin, and D. B. Harper. 2003. Chloride methylation by plant pectin: An efficient environmentally significant process. *Science* 301:206-209.
19. Harazono, K., Y. Watanabe, T. Fukatsu, and R. Kurane. 2003. Trapping of 2,7-dichlorodibenzo-p-dioxin in aqueous solution by enzymatic reaction of fungal manganese peroxidase in the presence of polyunsaturated fatty acids. *Current Microbiology* 47:250-254.
20. Hata, J., K. Takamizawa, N. Miyata, and K. Iwahori. 2003. Biodegradation of cis-1,2-dichloroethylene and vinyl chloride in anaerobic cultures enriched from landfill leachate sediment under Fe(III)-reducing conditions. *Biodegradation* 14:275-283.
21. Hill, A. M., and B. L. Thompson. 2003. Novel soraphens from precursor directed biosynthesis in *Sorangium cellulosum*. *Chem. Commun.* 12:1360-1361.
22. Iliopoulou, D., N. Mihopoulos, V. Roussis, and C. Vagias. 2003. New brominated labdane diterpenes from the red alga *Laurencia obtusa*. *Journal of Natural Products* 66:1225-1228.

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23. Kim, H. J., and D. W. Graham. 2003. Effects of oxygen and nitrogen conditions on the transformation kinetics of 1,2-dichloroethenes by *Methylosinus trichosporium* OB3b and its sMMOC mutant. *Biodegradation* 14:407-414.
  24. Kirtland, B. C., C. M. Aelion, P. A. Stone, and D. Hunkeler. 2003. Isotopic and geochemical assessment of in situ biodegradation of chlorinated hydrocarbons. *Environmental Science & Technology* 37:4205-4212.
  25. Kousaka, K., N. Ogi, Y. Akazawa, M. Fujieda, Y. Yamamoto, Y. Takada, and J. Kimura. 2003. Novel Oxylipin Metabolites from the Brown Alga *Eisenia bicyclis*. *J. Nat. Prod.* 66:1318-1323.
  26. Krausova, V. I., F. T. Robb, and J. M. Gonzalez. 2003. Bacterial degradation of dichloromethane in cultures and natural environments. *Journal of Microbiological Methods* 54:419-422.
  27. Kuniyoshi, M., N. Oshiro, T. Miono, and T. Higa. 2003. Five new halogenated octodane monoterpenes having a cyclohexadienone from the red alga *Portieria hornemanni*. *J. Chinese Chem. Soc. (Taipei)* 50:167-170.
  28. Kwon, O. S., Y. J. Kim, K. J. Cho, J. A. Lee, Y. E. Kim, I. Y. Hwang, and J. H. Kwon. 2003. Influence of transition-metal cofactors on the reductive dechlorination of polychlorinated biphenyls (PCBs). *Journal of Microbiology* 41:189-195.
  29. Lackey, L. W., J. R. Gamble, and J. L. Boles. 2003. Biofiltration of trichloroethylene-contaminated air: A pilot study. *Journal of the Air & Waste Management Association* 53:1248-1255.
  30. Lee, E. Y., B. D. Ye, and S. Park. 2003. Development and operation of a trickling biofilter system for continuous treatment of gas-phase trichloroethylene. *Biotechnology Letters* 25:1757-1761.
  31. Lee, S. J., J. H. Pardue, W. M. Moe, and K. T. Valsaraj. 2003. Mineralization of desorption-resistant 1,4-dichlorobenzene in wetland soils. *Environmental Toxicology and Chemistry* 22:2312-2322.
  32. Lerch, M. L., M. K. Harper, and D. J. Faulkner. 2003. Brominated polyacetylenes from the Philippines sponge *Diplastrella* sp. *Journal of Natural Products* 66:667-670.
  33. Lerch, M. L., M. K. Harper, and D. J. Faulkner. 2003. Diplynes A-E, five novel brominated polyacetylenes and three sulfated analogues from the sponge *Diplastrella* sp. (1.88 g). *J. Nat. Prod.* (ASAP Article, in press) 66:667-670.
  34. Maillard, J., W. Schumacher, F. Vazquez, C. Regeard, W. R. Hagen, and C. Holliger. 2003. Characterization of the corrinoid iron-sulfur protein tetrachloroethene reductive

- dehalogenase of *Dehalobacter restrictus*. *Applied and Environmental Microbiology* 69:4628-4638.
35. Manzano, M. A., J. A. Perales, D. Sales, and J. M. Quiroga. 2003. Enhancement of aerobic microbial degradation of polychlorinated biphenyl in soil microcosms. *Environmental Toxicology and Chemistry* 22:699-705.
  36. Marchesi, J. R., and A. J. Weightman. 2003. Comparing the dehalogenase gene pool in cultivated alpha-halocarboxylic acid-degrading bacteria with the environmental metagene pool. *Applied and Environmental Microbiology* 69:4375-4382.
  37. Marsh, G., R. Stenutz, and A. Bergman. 2003. Synthesis of hydroxylated and methoxylated polybrominated diphenyl ethers - Natural products and potential polybrominated diphenyl ether metabolites. *European Journal of Organic Chemistry*:2566-2576.
  38. Mazur, C. S., W. J. Jones, and C. Tebes-Stevens. 2003. H-2 consumption during the microbial reductive dehalogenation of chlorinated phenols and tetrachloroethene. *Biodegradation* 14:285-295.
  39. McGuire, T., and J. B. Hughes. 2003. Effects of surfactants on the dechlorination of chlorinated ethenes. *Environmental Toxicology and Chemistry* 22:2630-2638.
  40. Nakhla, G., J. Kochany, and A. Lugowski. 2002. Evaluation of PCBs biodegradability in sludges by various microbial cultures. *Environmental Progress* 21:85-93.
  41. Ogawa, N., K. Miyashita, and A. M. Chakrabarty. 2003. Microbial genes and enzymes in the degradation of chlorinated compounds. *Chemical Record* 3:158-171.
  42. Ortiz-Bermudez, P., E. Srebotnik, and K. E. Hammel. 2003. Chlorination and cleavage of lignin structures by fungal chloroperoxidases. *Applied and Environmental Microbiology* 69:5015-5018.
  43. Padma, T. V., R. M. Dickhut, and H. Ducklow. 2003. Variations in alpha-hexachlorocyclohexane enantiomer ratios in relation to microbial activity in a temperate estuary. *Environmental Toxicology and Chemistry* 22:1421-1427.
  44. Pakdeesusuk, U., D. L. Freedman, C. M. Lee, and J. T. Coates. 2003. Reductive dechlorination of polychlorinated biphenyls in sediment from the Twelve Mile Creek arm of Lake Hartwell, South Carolina, USA. *Environmental Toxicology and Chemistry* 22:1214-1220.
  45. Park, Y., Y. Liu, J. Hong, C.-O. Lee, H. Cho, D.-K. Kim, K. S. Im, and J. H. Jung. 2003. New Bromotyrosine Derivatives from an Association of Two Sponges, *Jaspis wondoensis* and *Poecillastra wondoensis*. *J. Nat. Prod.* (ASAP Article, in press).

46. Pon, G., M. R. Hyman, and L. Semprini. 2003. Acetylene inhibition of trichloroethene and vinyl chloride reductive dechlorination. *Environmental Science & Technology* 37:3181-3188.
47. Rezanka, T., and V. M. Dembitsky. 2003. Brominated oxylipins and oxylipin glycosides from Red Sea corals. *European Journal of Organic Chemistry*:309-316.
48. Rezanka, T., and V. M. Dembitsky. 2003. Eight new brominated oxylipins and oxylipin glycosides from red sea corals (*Dendrophyllia* and *Dendronephthya*) sp. *Eur. J. Org. Chem.* 2:309-316.
49. Rockne, K. J., and S. E. Strand. 2003. Amplification of marine methanotrophic enrichment DNA with 16S rDNA PCR primers for Type II alpha proteobacteria methanotrophs. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* 38:1877-1887.
50. Ruppe, S., A. Neumann, and W. Vetter. 2003. Anaerobic transformation of compounds of technical toxaphene. I. Regiospecific reaction of chlorobornanes with geminal chlorine atoms. *Environmental Toxicology and Chemistry* 22:2614-2621.
51. Ryu, J. H., Y. Lee, S. K. Han, and H. Y. Kim. 2003. The role of hydrogen peroxide produced by polychlorinated biphenyls in PMR1-deficient yeast cells. *Journal of Biochemistry* 134:137-142.
52. Sato, A., Y. Watanabe, N. B. Nugroho, E. Chrisnayanti, U. J. Natusion, Koesnandar, and H. Nishida. 2003. Screening for dioxin-degrading basidiomycetes from temperate and tropical forests. *World Journal of Microbiology & Biotechnology* 19:763-766.
53. Shoeb, M., and M. Jaspars. 2003. Chlorinated C12 Fatty Acid Metabolites from the Red Alga *Gracilaria verrucosa*. *J. Nat. Prod.* (ASAP article, in press).
54. Sierra, I., J. L. Valera, M. L. Marina, and F. Laborda. 2003. Study of the biodegradation process of polychlorinated biphenyls in liquid medium and soil by a new isolated aerobic bacterium (*Janibacter* sp.). *Chemosphere* 53:609-618.
55. Singer, A. C., D. Smith, W. A. Jury, K. Hathuc, and D. E. Crowley. 2003. Impact of the plant rhizosphere and augmentation on remediation of polychlorinated biphenyl contaminated soil. *Environmental Toxicology and Chemistry* 22:1998-2004.
56. Stourman, N. V., J. H. Rose, S. Vuilleumier, and R. N. Armstrong. 2003. Catalytic mechanism of dichloromethane dehalogenase from *Methylophilus* sp strain DM11. *Biochemistry* 42:11048-11056.

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57. Sung-In, Y., C. Youn-Kyoo, and L. Byung-Chan. 2003. Effective bead preparation of coimmobilized methanogenic and methanotrophic bacteria for tetrachloroethene degradation. *Biodegradation* 14:347-355.
  58. Tachibana, S., S. Tokuoka, and K. Itoh. 2003. Adsorption of dioxins by activated carbon and their degradation by a white-rot fungus. *Mokuzai Gakkaishi* 49:227-232.
  59. Topcu, G., Z. Aydogmus, S. Imre, A. C. Goren, J. M. Pezzuto, J. A. Clement, and D. G. I. Kingston. 2003. Brominated Sesquiterpenes from the Red Alga *Laurencia obtusa*. *J. Nat. Prod.* (ASAP Article, in press).
  60. van Pee, K. H. 2003. Dehalogenation of polyhalogenated dioxins. *Angewandte Chemie-International Edition* 42:3718-3720.
  61. Vieth, A., J. Muller, G. Strauch, M. Kastner, M. Gehre, R. U. Meckenstock, and H. H. Richnow. 2003. In-situ biodegradation of tetrachloroethene and trichloroethene in contaminated aquifers monitored by stable isotope fractionation. *Isotopes in Environmental and Health Studies* 39:113-124.
  62. Wang, L., Q. Zhou, B. S. Zhang, Z. L. Li, H. Chua, and D. M. Ren. 2003. The biodegradation of 1,3-dichlorobenzene by an adapted strain *Bacillus cereus* PF-11 derived from town-gas industrial effluent. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* 38:1837-1848.
  63. Wang, S. C., M. D. Person, W. H. Johnson, and C. P. Whitman. 2003. Reactions of trans-3-chloroacrylic acid dehalogenase with acetylene substrates: Consequences of and evidence for a hydration reaction. *Biochemistry* 42:8762-8773.
  64. Watanabe, K., M. Sekine, and K. Iguchi. 2003. Isolation and Structures of New Halogenated Prostanoids from the Okinawan Soft Coral *Clavularia viridis*. *J. Nat. Prod.* (ASAP Article, in press).
  65. Wilson, S. C., and A. A. Meharg. 2003. Investigation of organic xenobiotic transfers, partitioning and processing in air-soil-plant systems using a microcosm apparatus. Part II: comparing the fate of chlorobenzenes in grass planted soil. *Chemosphere* 53:583-591.

## 6. ANNEX

Abrahamsson, K., K.-S. Choo, et al. (2003). "Effects of temperature on the production of hydrogen peroxide and volatile halocarbons by brackish-water algae." *Phytochemistry* 64(3): 725-734.

Marine algae produce volatile halocarbons, which have an ozone-depleting potential. The formation of these compounds is thought to be related to oxidative stress, involving H<sub>2</sub>O<sub>2</sub> and algal peroxidases. In our study we found strong correlations between the releases of H<sub>2</sub>O<sub>2</sub> and brominated and some iodinated compounds to the seawater medium, but no such correlation was found for CHCl<sub>3</sub>, suggesting the involvement of other formation mechanisms as well. Little is known about the effects of environmental factors on the production of volatile halocarbons by algae and in the present study we focused on the influence of temperature. Algae were sampled in an area of the brackish Baltic Sea that receives thermal discharge, allowing us to collect specimens of the same species that were adapted to different field temperature regimes. We exposed six algal species (the diatom *Pleurosira laevis*, the brown alga *Fucus vesiculosus* and four filamentous green algae, *Cladophora glomerata*, *Enteromorpha ahlneriana*, *E. flexuosa* and *E. intestinalis*) to temperature changes of 0-11 [deg]C under high irradiation to invoke oxidative stress. The production rates, as well as the quantitative composition of 16 volatile halocarbons, were strongly species-dependent and different types of responses to temperature were recorded. However, no response patterns to temperature change were found that were consistent for all species or for all halocarbons. We conclude that the production of certain halocarbons may increase with temperature in certain algal species, but that the amount and composition of the volatile halocarbons released by algal communities are probably more affected by temperature-associated species shifts. These results may have implications for climatic change scenarios.

Amitai, G., R. Adani, et al. (2003). "Degradation of VX and sulfur mustard by enzymatic haloperoxidation." *Journal of Applied Toxicology* 23(4): 225-233.

Chloroperoxidase (CPO) isolated from *Caldariomyces fumago* (20 U ml<sup>-1</sup>) together with urea hydrogenperoxide (UPER, 0.5 mM) and sodium chloride as co-substrate (NaCl, 0.5 M) caused rapid breakdown of VX (10 μM) (t<sub>1/2</sub>) 8 s, 25 degreesC, 50 mM tartarate, pH 2.75). Glucose oxidase (GOX, *Aspergillus niger*) and glucose were used as an alternative source for H<sub>2</sub>O<sub>2</sub>. A mixture of GOX (20 U ml<sup>-1</sup>), glucose (GLU 0.45 M), CPO (20 U ml<sup>-1</sup>) and NaCl (0.5 M) caused a 3.8-fold slower degradation of VX (10 μM) (t<sub>1/2</sub>) = 30 s, 25 degreesC, 50 mM tartarate, pH 2.75). The concentrations of H<sub>2</sub>O<sub>2</sub> and chlorine produced by this enzyme/substrate mixture depended mainly on the GLU concentration. Horseradish peroxidase (HRP) together with UPER (1 mM) and sodium iodide (NaI, 0.05 M) caused progressive degradation of VX that was more than 400-fold slower than with CPO (20 U ml<sup>-1</sup>), UPER (0.5 mM) and NaCl (0.5 M) (t<sub>1/2</sub>) = 55 min, 25 degreesC, pH 8). Skin decontamination of VX by CPO was tested in pig-ear skin in vitro. The chemical agent VX (0.01 M, 100 μl) was degraded by 98% within 3 h of skin diffusion when a mixture of UPER/NaCl/CPO was applied 60 min prior to VX application. A mixture of UPER/NaCl without CPO also caused significant VX degradation (94%) during skin diffusion whereas it did not cause any VX degradation in solution. Degradation of VX in skin, obtained without exogenous CPO, may indicate involvement of endogenous intradermal haloperoxidase-like enzyme. Reagent UPER (1 mM) did not cause any degradation of VX in solution or during its skin diffusion. Furthermore, a mixture of CPO, UPER and NaCl caused rapid degradation of sulfur mustard (HD). Sulfur mustard (50 μM) incubated in the presence of CPO (4 U ml<sup>-1</sup>), UPER (0.05 M) and NaCl (0.5 M) at pH 2.75 and 30 degreesC was oxidized by 97% and 99% within 5 and 10 min, respectively. The oxidation products HD sulfoxide, HD sulfone and HD sulfoxidevinyl were identified by GC/MS in the enzymatic chloroperoxidation mixture. Copyright (C) 2003 John Wiley Sons, Ltd.

Anon. (2003). "Potential explanation for fluorinated compounds' persistence." *Environmental Science & Technology* 37(17): 312A-313A.

Arbeli, Z. and Z. Ronen (2003). "Enrichment of a Microbial Culture Capable of Reductive Debromination of the Flame Retardant Tetrabromobisphenol-A, and Identification of the Intermediate Metabolites Produced in the Process." *Biodegradation* 14(6): 385-395.

Choi, S. D., H. B. Hong, et al. (2003). "Adsorption of halogenated aromatic pollutants by a protein released from *Bacillus pumilus*." *Water Research* 37(16): 4004-4010.

Previous studies of the biosorption of halogenated aromatic pollutants (HAPs) have focused on the sorption of these compounds by cell bodies. However, in this study we investigated the adsorption of HAPs by biocompounds released from a bacterium, *Bacillus pumilus*. When *B. pumilus* was exposed to high temperature, it released a protein and carbohydrates, exclusively. After determining experimental conditions using 1,2,3,4-tetrachlorinated dibenzofuran (1,2,3,4-TCDF), the adsorption characteristics of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), chlorinated benzenes, and chlorinated naphthalenes were investigated. These HAPs were adsorbed considerably not only by cells but also by the released protein. In general, highly chlorinated congeners were adsorbed to a greater extent on the protein than lowly chlorinated ones, and the amount adsorbed differed between isomers. The present results are consistent with adsorption occurring via a passive physico-chemical mechanism. Finally, the importance of biocompounds released or excreted from microorganisms for the removal of HAPs is discussed. (C) 2003 Elsevier Ltd. All rights reserved.

Chu, M., P. K. Kitaradis, et al. (2003). "Effects of biomass accumulation on microbially enhanced dissolution of a PCE pool: a numerical simulation." *Journal of Contaminant Hydrology* 65(1-2): 79-100.

Recent studies have shown that dechlorinating bacteria can accelerate the dissolution rate of dense, nonaqueous phase liquids (DNAPLs) containing tetrachloroethene (PCE). We present an advection-dispersion-reaction model for a two-dimensional domain, with groundwater flowing over a pool of free-product PCE. PCE is converted to *cis*-1,2-dichloroethene (cDCE) and toxicity due to PCE or cDCE is neglected. We adopt previously published correlations relating biomass concentrations and hydraulic conductivity, accounting for biofilm growth and plug-like growth. The system of coupled equations is solved numerically. The high biotransformation rate of PCE increases the concentration gradient of PCE at the water-DNAPL interface, enhancing dissolution. The higher the electron donor (ED) concentration, the larger the dissolution enhancement. Based on the values of maximum specific rate we used, when the electron donor is unlimited, the active biomass accumulates adjacent to the water-DNAPL interface and microbial reactions can significantly enhance the pool dissolution. The resulting steady-state dissolution rate can be approximated by a half-order solution when zero-order kinetics are suitable for representing the microbial reaction. However, bioclogging may significantly reduce local hydraulic conductivity; thus, it decreases the flow near the water-DNAPL interface, decreasing dissolution. When the ED is the limiting factor, active biomass accumulates away from the interface. This creates a no-flow zone between the active biomass and the interface. The enlargement of the no-flow zone, due to the donor limitation, diminishes the concentration gradient and the flushing around the water-DNAPL interface. Such adverse impacts may significantly decrease the enhancement predicted by models that do not consider the effects of bioclogging. (C) 2002 Elsevier Science B.V All rights reserved.

Coleman, N. V. and J. C. Spain (2003). "Distribution of the coenzyme m pathway of epoxide metabolism among ethene- and vinyl chloride-degrading Mycobacterium strains." *Applied and Environmental Microbiology* 69(10): 6041-6046.

An epoxyalkane:coenzyme M (CoM) transferase (EaCoMT) enzyme was recently found to be active in the aerobic vinyl chloride (VC) and ethene assimilation pathways of Mycobacterium strain JS60. In the present study, EaCoMT activity and genes were investigated in 10 different mycobacteria isolated on VC or ethene from diverse environmental samples. In all cases, epoxyethane metabolism in cell extracts was dependent on CoM, with average specific activities of EaCoMT between 380 and 2,910 nmol/min/mg of protein. PCR with primers based on conserved regions of EaCoMT genes from Mycobacterium strain JS60 and the propene oxidizers Xanthobacter strain Py2 and Rhodococcus strain B-276 yielded fragments (834 bp) of EaCoMT genes from all of the VC- and ethene-assimilating isolates. The Mycobacterium EaCoMT genes form a distinct cluster and are more closely related to the EaCoMT of Rhodococcus strain B-276 than that of Xanthobacter strain Py2. The incongruence of the EaCoMT and 16S rRNA gene trees and the fact that isolates from geographically distant locations possessed almost identical EaCoMT genes suggest that lateral transfer of EaCoMT among the Mycobacterium strains has occurred. Pulsed-field gel electrophoresis revealed large linear plasmids (110 to 330 kb) in all of the VC-degrading strains. In Southern blotting experiments, the strain JS60 EaCoMT gene hybridized to many of the plasmids. The CoM-mediated pathway of epoxide metabolism appears to be universal in alkene-assimilating mycobacteria, possibly because of plasmid-mediated lateral gene transfer.

Coleman, N. V. and J. C. Spain (2003). "Epoxyalkane: Coenzyme M transferase in the ethene and vinyl chloride biodegradation pathways of Mycobacterium strain JS60." *Journal of Bacteriology* 185(18): 5536-5545.

Mycobacterium strains that grow on ethene and vinyl chloride (VC) are widely distributed in the environment and are potentially useful for biocatalysis and bioremediation. The catabolic pathway of alkene assimilation in mycobacteria is not well characterized. It is clear that the initial step is a monooxygenase-mediated epoxidation that produces epoxyethane from ethene and chlorooxirane from VC, but the enzymes involved in subsequent transformation of the epoxides have not been identified. We investigated epoxyethane metabolism in Mycobacterium strain JS60 and discovered a coenzyme M (CoM)-dependent enzyme activity in extracts from VC- and ethene-grown cells. PCR amplifications using primers targeted at epoxyalkane:CoM transferase (EaCoMT) genes yielded part of the JS60 EaCoMT gene, which was used to clone an 8.4-kb genomic DNA fragment. The complete EaCoMT gene (etnE) was recovered, along with genes (etnABCD) encoding a four-component monooxygenase and two genes possibly involved in acyl-CoA ester metabolism. Reverse transcription-PCR indicated that the etnE and etnA genes were cotranscribed and inducible by ethene and VC. Heterologous expression of the etnE gene in Mycobacterium smegmatis mc(2) 155 using the pMV261 vector gave a recombinant strain capable of transforming epoxyethane, epoxypropane, and chlorooxirane. A metabolite identified by mass spectrometry as 2-hydroxyethyl-CoM was produced from epoxyethane. The results indicate that the EaCoMT and monooxygenase enzymes encoded by a single operon (etnEABCD) catalyze the initial reactions in both the VC and ethene assimilation pathways. CoM-mediated reactions appear to be more widespread in bacteria than was previously believed.

Coque, J. J. R., M. L. Alvarez-Rodriguez, et al. (2003). "Characterization of an inducible chlorophenol O-methyltransferase from Trichoderma longibrachiatum involved in the formation of chloroanisoles and determination of its role in cork taint of wines." *Applied and Environmental Microbiology* 69(9): 5089-5095.

A novel S-adenosyl-L-methionine (SAM)-dependent methyltransferase catalyzing the O-methylation of several chlorophenols and other halogenated phenols was purified 220-fold to apparent homogeneity from mycelia of Trichoderma longibrachiatum CECT 20431. The enzyme could be identified in partially purified protein preparations by direct photolabeling with [methyl-<sup>3</sup>H]SAM, and this reaction was prevented by previous incubation with S-adenosylhomocysteine. Gel filtration indicated that the M<sub>r</sub> was 112,000, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that the enzyme was composed of two subunits with molecular weights of approximately 52,500. The enzyme had a pH optimum between 8.2 and 8.5 and an optimum temperature of 28°C, with a pI of 4.9. The K<sub>m</sub> values for 2,4,6-trichlorophenol and SAM were 135.9 ± 12.8 and 284.1 ± 35.1 μM, respectively. S-Adenosylhomocysteine acted as a competitive inhibitor, with a K<sub>i</sub>

of 378.9 +/- 45.4  $\mu\text{M}$ . The methyltransferase was also strongly inhibited by low concentrations of several metal ions, such as  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Ag}^{+}$ , and to a lesser extent by p-chloromercuribenzoic acid, but it was not significantly affected by several thiols or other thiol reagents. The methyltransferase was specifically induced by several chlorophenols, especially if they contained three or more chlorine atoms in their structures. Substrate specificity studies showed that the activity was also specific for halogenated phenols containing fluoro, chloro, or bromo substituents, whereas other hydroxylated compounds, such as hydroxylated benzoic acids, hydroxybenzaldehydes, phenol, 2-methoxyphenol, and dihydroxybenzene, were not methylated.

Coutu, C., G. Martineau, et al. (2003). "Characterization of an organic filter medium for the biofiltration treatment of air contaminated with 1,2-dichlorobenzene." *Journal of Chemical Technology and Biotechnology* 78(8): 907-917.

Laboratory experiments were conducted to determine the potential for removing 1,2-dichlorobenzene (1,2-DCB) in gaseous phase by biofiltration. Experiments were carried out over 8 months in a steel tank (0.45  $\text{m}^3$ ) using an organic filter medium composed of peat, maple wood chips, chicken manure and 1,2-DCB-contaminated soil. During the first 6 months, the biofilter was operated without injecting 1,2-DCB in order to characterize the physicochemical, mechanical and microbiological properties of the filter bed. The results revealed that it is an excellent medium for both microbial development (up to  $10^9$  cells  $\text{g}(\text{dry soil})^{-1}$ ) for heterotrophic bacteria and long-term stability with a limited drop dry of pressure (30 cm of water) and no clogging. Over the final 2 months, the biofilter treated air laden with 1,2-DCB (0.30 and 0.75  $\text{g m}^{-3}$ ) and the maximum elimination capacity reached was 9  $\text{g m}^{-3} \text{h}^{-1}$  (inlet load of 13  $\text{g m}^{-3} \text{h}^{-1}$ ), which represented 69% efficiency. Elimination performance was strongly dependent upon inlet concentration, sorption/desorption and biodegradation phenomena occurring in the filter medium. Sorption/desorption and biodegradation mechanisms during the start-up period were characterized using the elimination efficiency (%). At the beginning of the 1,2-DCB injection, the microorganisms were strongly impacted and sorption/desorption phenomena prevailed. With the decrease of the inlet concentration, biodegradation progressively increased to become the most important mechanism. It was concluded that biofiltration possesses an excellent potential for treating volatile chlorinated benzene, known to be recalcitrant to biodegradation. (C) 2003 Society of Chemical Industry.

de Carvalho, L. R., M. T. Fujii, et al. (2003). "Aldingenin A, new brominated sesquiterpene from red algae *Laurencia aldingensis*." *Tetrahedron Letters* 44(13): 2637-2640.

A new brominated bisabolene derivative, aldingenin A, was isolated from red alga *Laurencia aldingensis* Saito et Womersley (Ceramiiales, Rodophyta). Its structure was determined by analysis of spectroscopic data ( $^1\text{H}$ - and  $^{13}\text{C}$ -NMR, IR, MS), including bidimensional NMR ( $^1\text{H}$ - $^1\text{H}$ -COSY, HMQC, HMBC and NOESY) and biogenetic considerations. (C) 2003 Elsevier Science Ltd. All rights reserved.

de Jong, R. M., J. J. W. Tiesinga, et al. (2003). "Structure and mechanism of a bacterial haloalcohol dehalogenase: a new variation of the short-chain dehydrogenase/reductase fold without an NAD(P)H binding site." *Embo Journal* 22(19): 4933-4944.

Haloalcohol dehalogenases are bacterial enzymes that catalyze the cofactor-independent dehalogenation of vicinal haloalcohols such as the genotoxic environmental pollutant 1,3-dichloro-2-propanol, thereby producing an epoxide, a chloride ion and a proton. Here we present X-ray structures of the haloalcohol dehalogenase HheC from *Agrobacterium radiobacter* AD1, and complexes of the enzyme with an epoxide product and chloride ion, and with a bound haloalcohol substrate mimic. These structures support a catalytic mechanism in which Tyr145 of a Ser-Tyr-Arg catalytic triad deprotonates the haloalcohol hydroxyl function to generate an intramolecular nucleophile that substitutes the vicinal halogen. Haloalcohol dehalogenases are related to the widespread family of NAD(P)H-dependent short-chain dehydrogenases/reductases (SDR family), which use a similar Ser-Tyr-Lys/Arg catalytic triad to catalyze reductive or oxidative conversions of various secondary alcohols and ketones. Our results reveal the first structural details of an SDR-related enzyme that catalyzes a substitutive dehalogenation reaction rather than a redox reaction, in which a halide-binding site is found at the location of the NAD(P)H

binding site. Structure-based sequence analysis reveals that the various haloalcohol dehalogenases have likely originated from at least two different NAD-binding SDR precursors.

De Wildeman, S., G. Diekert, et al. (2003). "Stereoselective microbial dehalorespiration with vicinal dichlorinated alkanes." *Applied and Environmental Microbiology* 69(9): 5643-5647.

The suspected carcinogen 1,2-dichloroethane (1,2-DCA) is the most abundant chlorinated C-2 groundwater pollutant on earth. However, a reductive in situ detoxification technology for this compound does not exist. Although anaerobic dehalorespiring bacteria are known to catalyze several dechlorination steps in the reductive-degradation pathway of chlorinated ethenes and ethanes, no appropriate isolates that selectively and metabolically convert them into completely dechlorinated end products in defined growth media have been reported. Here we report on the isolation of *Desulfitobacterium dichloroeliminans* strain DCA1, a nutritionally defined anaerobic dehalorespiring bacterium that selectively converts 1,2-dichloroethane and all possible vicinal dichloropropanes and -butanes into completely dechlorinated end products. Menaquinone was identified as an essential cofactor for growth of strain DCA1 in pure culture. Strain DCA1 converts chiral chlorosubstrates, revealing the presence of a stereoselective dehalogenase that exclusively catalyzes an energy-conserving anti mechanistic dichloroelimination. Unlike any known dehalorespiring isolate, strain DCA1 does not carry out reductive hydrogenolysis reactions but rather exclusively dichloroeliminates its substrates. This unique dehalorespiratory biochemistry has shown promising application possibilities for bioremediation purposes and fine-chemical synthesis.

De Wildeman, S., A. Neumann, et al. (2003). "Growth-substrate dependent dechlorination of 1,2-dichloroethane by a homoacetogenic bacterium." *Biodegradation* 14(4): 241-247.

A rod shaped, gram positive, non sporulating *Acetobacterium* strain was isolated that dechlorinated 1,2-dichloroethane (1,2-DCA) to ethene at a dechlorination rate of up to 2 nmol Cl<sup>-</sup> min<sup>-1</sup> mg<sup>-1</sup> of protein in the exponential growth phase with formate (40 mM) as the substrate. Although with other growth substrates such as pyruvate, lactate, H<sub>2</sub>/CO<sub>2</sub>, and ethanol higher biomass productions were obtained, the dechlorination rate with these substrates was more than 10-fold lower compared with formate growing cells. Neither cell extracts nor autoclaved cells of the isolated *Acetobacterium* strain mediated the dechlorination of 1,2-DCA at significant rates. The addition of 1,2-DCA to the media did not result in increased cell production. No significant differences in corrinoid concentrations could be measured in cells growing on several growth-substrates. However, these measurements indicated that differences in corrinoid structure might cause the different dechlorination activity. The *Acetobacterium* sp. strain gradually lost its dechlorination ability during about 10 transfers in pure culture, probably due to undefined nutritional requirements. 16S rDNA analysis of the isolate revealed a 99.7% similarity with *Acetobacterium wieringae*. However, the type strains of *A. wieringae* and *A. woodii* did not dechlorinate 1,2-DCA.

Dembitsky, V. M. (2003). "Oxidation, epoxidation and sulfoxidation reactions catalysed by haloperoxidases." *Tetrahedron* 59(26): 4701-4720.

This review will examine the reactivity of the haloperoxidases, particularly the mechanism of oxidation by hydrogen peroxide, and the mechanism of oxidation, epoxidation and sulfoxidation, including the newly reported regioselectivity and enantioselectivity of the vanadium haloperoxidases. This is the first review which combines oxidation, epoxidation, and sulfoxidation catalysed by haloperoxidases isolated from different natural sources.

Dong, J., X. F. Lu, et al. (2003). "The strength of dehalogenase-substrate hydrogen bonding correlates with the rate of Meisenheimer intermediate formation." *Biochemistry* 42(31): 9482-9490.

4-Chlorobenzoyl-coenzyme A (4-CBA-CoA) dehalogenase catalyzes the hydrolytic dehalogenation of 4-CBA-CoA to 4-hydroxybenzoyl-CoA by using an active site aspartate as the nucleophile. Formation of the corresponding Meisenheimer complex (EMc) is followed by chloride ion expulsion which forms the arylated intermediate (EAr). This is then hydrolyzed to the product. In this paper, we explore the relationship between active site polarizing forces acting on the benzoyl carbonyl and the rate of formation of the Meisenheimer complex. The polarizing forces at the C=O group were modulated by introducing site-selected mutations

(A112V, Y65D, G113A, G113S, G113N, and F64P), near the C=O binding site. Using either the substrate, 4-CBA-CoA, or the substrate analogue, 4-methylbenzoyl-CoA (4-MBA-CoA), Raman difference spectroscopy provided the position of the C=O stretching frequency ( $\nu(\text{C}=\text{O})$ ) for a total of 10 enzyme-ligand complexes. In turn, the values of the C=O frequencies could be converted to differences in effective hydrogen bonding strengths between members of the series, based on earlier model studies [Clarkson, J., Tonge, P. J., Taylor, K. L., Dunaway-Mariano, D., and Carey, P. (1997) *Biochemistry* 36, 10192-10199]. Catalysis in the F64P, G113A, G113S, and G113N dehalogenase mutants was very slow with  $k(\text{cat})$  values ranging from  $8 \times 10^{-3}$  to  $7.6 \times 10^{-6}$  s<sup>-1</sup>. The EAr intermediate did not accumulate to a detectable level on these enzymes during a single turnover. Catalysis in the Y65D and A112V dehalogenase mutants were almost as efficient as catalysis in wild-type dehalogenase with  $k(\text{cat})$  values of 0.1-0.6 s<sup>-1</sup>. In wild-type dehalogenase, 22% of the bound substrate accumulated as the EAr intermediate during a single turnover ( $k(\text{obs})$  for EAr formation = 24 s<sup>-1</sup>); in the Y65D mutant, the level of accumulation is 17% ( $k(\text{obs})$  for EAr formation = 3 s<sup>-1</sup>), and in the A112V mutant, the level is 23% ( $k(\text{obs})$  for EAr formation = 17 s<sup>-1</sup>). The  $k(\text{obs})$  for EAr formation in wild-type dehalogenase and the more active dehalogenase mutants (Y65D and A112V) was taken to be an estimate of the  $k$  for EMc formation, and the  $k(\text{obs})$  for EP formation in a single turnover was taken to be an estimate of the  $k$  for EMc formation in the severely impaired mutants (F64P, G113A, G113S, and G113N). A plot of the log  $k(\text{obs})$  for EMc formation versus the C=O stretching frequency of bound 4-CBA-CoA (or 4-MBA-CoA) is a straight line ( $R^2 = 0.9584$ ). Throughout the series,  $\nu(\text{C}=\text{O})$  varied by 61 cm<sup>-1</sup>, corresponding to the change in hydrogen bonding enthalpy of 67 kJ/mol. The results show that changes in polarizing forces at the benzoyl carbonyl are transmitted to the benzoyl (4) position and correlate with the rate of aromatic nucleophilic addition five chemical bonds away. Interestingly, the relationship between effective polarizing forces and reactivity seen here for dehalogenase is similar to that reported for the addition-elimination reaction involving the hydrolysis of a series of acyl serine proteases.

Fava, F., S. Gentilucci, et al. (2003). "Anaerobic biodegradation of weathered polychlorinated biphenyls (PCBs) in contaminated sediments of Porto Marghera (Venice Lagoon, Italy)." *Chemosphere* 53(2): 101-109.

The biodegradation of weathered polychlorinated biphenyls (PCBs) (mono and di-chlorinated biphenyls along with PCBs partially ascribed to Aroclor 1242 and 1254) occurring at 1.5-2.5 mg/kg in three different sediments collected from the Porto Marghera contaminated area of Venice Lagoon (Italy) was reported in this study. Strictly anaerobic, slurry microcosms consisting of sediments suspended (at 25% v/v) in a marine salt medium, lagoon water or lagoon water supplemented with NaHCO<sub>3</sub> and Na<sub>2</sub>S were developed and monitored for PCB transformation, sulfate consumption and methane (CH<sub>4</sub>) production for 6 months. A marked depletion of highly chlorinated biphenyls along with the accumulation of low-chlorinated, often ortho-substituted biphenyls was observed in the biologically active microcosms, where a remarkable consumption of sulfate and/or a significant production of CH<sub>4</sub> were also detected. Notably, a more extensive PCB transformation was observed in the microcosms developed with site water (both without or with NaHCO<sub>3</sub> plus Na<sub>2</sub>S), where both the initial concentration of sulfate and sulfate consumption were five fold-higher than in the corresponding microcosms with salt medium. These data indicate that weathered PCBs of the three contaminated sediments of Porto Marghera utilized in this study can undergo reductive dechlorination, probably mediated by indigenous sulfate-reducing and/or methanogenic bacteria. (C) 2003 Elsevier Ltd. All rights reserved.

Ferrer, M., P. Golyshin, et al. (2003). "Novel maltotriose esters enhance biodegradation of Aroclor 1242 by *Burkholderia cepacia* LB400." *World Journal of Microbiology & Biotechnology* 19(6): 637-643.

The objective of this research was to evaluate the effect of enzymatically synthesized maltotriose fatty acid monoesters (Ferrer, M., et al. 2000 *Tetrahedron* 56, 4053 - 4061) on Aroclor 1242 solubilization and biodegradation. Three forms of the surfactant, laurate, palmitate and stearate monoester, were tested. Potential enhancement of solubilization of hydrophobic substances mediated by these non-ionic surfactants was exploited in this study. A polychlorinated biphenyl (PCB) degrading organism, *Burkholderia cepacia* LB400, was also selected. It was found that all surfactants were effective in solubilizing Aroclor 1242 but the rate of Aroclor 1242

biodegradation proceeded rapidly only in the presence of 6"-O-palmitoylmaltotriose. For example, the addition of 48 mg 6"-O-palmitoylmaltotriose/l increased the apparent solubility from 140 to 305 µg/l. As a result, only 8% of the Aroclor remained at the end of 24 h incubation. In contrast, 49.2% of the Aroclor 1242 remained in the absence of surfactant. It appears that maltotriose fatty acid monoesters can significantly increase the bioavailability, and thereby accelerate the biodegradation of highly chlorinated PCBs, particularly Aroclor 1242, by *Burkholderia cepacia* LB400. The possibility of obtaining these biodegradable surfactants with high yield, easy recovery and high purity by using a new enzymatic methodology, makes maltotriose esters available for bioremediation purposes.

Gu, A. Z., H. D. Stensel, et al. (2003). "Vinyl bromide as a surrogate for determining vinyl chloride reductive dechlorination potential." *Environmental Science & Technology* 37(19): 4410-4416.

Site evaluation for bioremediation of chlorinated ethenes may need treatability studies to assess the reductive dechlorination potential of vinyl chloride (VC). Dehalogenation of vinyl bromide (VB) was investigated as a surrogate measurement for the dechlorination potential of VC. VB dehalogenation rates and kinetics were studied and compared with those of VC by a methanogenic reductive dechlorinating enrichment culture that was dominated by *Dehalococcoides* species and by microcosms from two chloroethene-contaminated sites. The enrichment culture dehalogenated VB to ethene at higher rates than VC at similar concentrations. VB was dehalogenated with a higher enzyme affinity than was VC, as indicated by their half-velocity constants, 240±150 and 21±8 µM, for VC and VB, respectively. Cross-inhibition study exhibited some evidence for competitive inhibition between VC and VB, suggesting that their degradation might be catalyzed by the same enzyme in the culture. Laboratory microcosm studies using subsurface soil and groundwater from two contaminated sites demonstrated that the production of the reductive dehalogenation product (ethene) could be detected faster with VB as a substrate than with VC. As a result, a substantially shorter (up to 5-10 times) incubation time would be required to detect the same level of reductive dehalogenation activity using VB as a surrogate for VC in treatability assessments.

Hamilton, J. T. G., W. C. McRoberts, et al. (2003). "Chloride methylation by plant pectin: An efficient environmentally significant process." *Science* 301(5630): 206-209.

Atmospheric chloromethane (CH<sub>3</sub>Cl) plays an important role in stratospheric ozone destruction, but many uncertainties exist regarding the strengths of its sources and sinks and particularly regarding the processes generating this naturally occurring gas. Evidence is presented here that CH<sub>3</sub>Cl is produced in many terrestrial environments by a common mechanism. Abiotic conversion of chloride to CH<sub>3</sub>Cl occurs readily in plant material, with the widespread plant component pectin acting as a methyl donor. Significant CH<sub>3</sub>Cl emissions from senescent and dead leaves were observed at ambient temperatures; those emissions rose dramatically when temperatures increased. This ubiquitous process acting in terrestrial ecosystems and during biomass burning could contribute the bulk of atmospheric CH<sub>3</sub>Cl.

Harazono, K., Y. Watanabe, et al. (2003). "Trapping of 2,7-dichlorodibenzo-p-dioxin in aqueous solution by enzymatic reaction of fungal manganese peroxidase in the presence of polyunsaturated fatty acids." *Current Microbiology* 47(3): 250-254.

In the presence of polyunsaturated fatty acids, including *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA), 2,7-dichlorodibenzo-p-dioxin (DCDD) was treated with manganese peroxidase (MnP) from white rot basidiomycete *Phanerochaete sordida* YK-624. After incubation with MnP, DCDD could not be extracted from the reaction mixture with *n*-hexane and was trapped in the water layer. DCDD was released by alkalification of the water layer. DCDD was also trapped after treatment with lipoxidase, which produces hydroperoxides from unsaturated lipids. The amounts of thiobarbituric acid-reactive substances produced in the MnP reactions with three highly unsaturated fatty acids were higher than the amounts produced with three fatty acids with a lower

degree of unsaturation. These results suggest that a DCDD-trapping compound may be produced by peroxidation of the polyunsaturated fatty acids.

Hata, J., K. Takamizawa, et al. (2003). "Biodegradation of cis-1,2-dichloroethylene and vinyl chloride in anaerobic cultures enriched from landfill leachate sediment under Fe(III)-reducing conditions." *Biodegradation* 14(4): 275-283.

An anaerobic, Fe(III)-reducing enrichment culture, which originated from a sediment sample collected at a landfill in Nanji-do, Seoul, Korea, was capable of degrading cis-1,2-dichloroethylene (cis-DCE) and vinyl chloride (VC). Although it exhibited the ability under Fe(III)-reducing conditions, the chlorinated ethenes degradation was not linked to the Fe(III) reduction. During cis-DCE degradation, no VC, ethene, or ethane was detected through the experimental period. Also, this culture did not accumulate ethene and ethane during the VC degradation. It was unlikely that cis-DCE was reductively dechlorinated to VC and then the VC formed was dechlorinated fast enough. Because the kinetic data showed that the rate of cis-DCE degradation was 3.5 times higher than that of VC. Whereas glucose supported the culture growth and the degradation, formate, acetate, butyrate, propionate, lactate, pyruvate, and yeast extract did not. The results appeared consistent with the involvement of oxidative degradation mechanism rather than reductive dechlorination mechanism. The traits of the culture described here are unusual in the anaerobic degradation of chlorinated ethenes and may be useful for searching an effective organism and mechanism regarding anaerobic cis-DCE and VC degradation.

Hill, A. M. and B. L. Thompson (2003). "Novel soraphens from precursor directed biosynthesis in *Sorangium cellulosum*." *Chem. Commun.* 12: 1360-1361.

Six novel halogenated soraphen analogues have been isolated from the wild-type producing organism using precursor directed biosyntheses; the best 'delivery vehicle' for the novel starter acids was cinnamate but ortho substituents were not tolerated by the soraphen PKS.

Iliopoulou, D., N. Mihopoulos, et al. (2003). "New brominated labdane diterpenes from the red alga *Laurencia obtusa*." *Journal of Natural Products* 66(9): 1225-1228.

Two labdane type brominated diterpenes (1 and 2) containing unprecedented eight- and seven-membered ether rings have been isolated from the organic extract of the red alga *Laurencia obtusa*, collected from Mitikas Bay in the Ionean Sea, Greece. The structures of the new natural products, as well as their relative stereochemistry, were established by means of spectral data analysis, including 2D NMR experiments.

Kim, H. J. and D. W. Graham (2003). "Effects of oxygen and nitrogen conditions on the transformation kinetics of 1,2-dichloroethenes by *Methylosinus trichosporium* OB3b and its sMMOC mutant." *Biodegradation* 14(6): 407-414.

Transformation kinetics of trans- and cis-dichloroethylenes (DCE) by *Methylosinus trichosporium* OB3b wild type (WT) and PP319, a mutant that expresses soluble methane monooxygenase at copper levels up to  $12 \mu\text{M}$  Cu (sMMOC), were determined to assess the effects of O<sub>2</sub> level and N<sub>2</sub>-fixation on degradation capabilities. Two issues were examined: (1) the influence of O<sub>2</sub> level and nitrogen-limitation on DCE degradation kinetics and toxicity in both organisms, and (2) the relative utility of PP319 for contaminant degradation in bioreactors. When both organisms were grown under high O<sub>2</sub> conditions (80% saturation in air), maximum transformation rates (V<sub>max</sub>) and apparent first-order rate constants (V<sub>max</sub>/K<sub>M</sub>) were lower compared with organisms grown under low

O<sub>2</sub> conditions (10% saturation in air) regardless of nitrogen level. Further, V<sub>max</sub> values were near zero in nitrogen-limited

WT cultures when O<sub>2</sub> was high (as expected), whereas PP319 retained moderate V<sub>max</sub> levels even at high O<sub>2</sub> levels. In general, elevated O<sub>2</sub> conditions reduced DCE degradation rates in OB3b, although the negative effects

of O<sub>2</sub> were less in PP319 than in the WT. Given that PP319 retained moderate DCE degradation rates under most O<sub>2</sub> and copper conditions, the mutant appears to have some utility for biodegradation applications.

Kirtland, B. C., C. M. Aelion, et al. (2003). "Isotopic and geochemical assessment of in situ biodegradation of chlorinated hydrocarbons." *Environmental Science & Technology* 37(18): 4205-4212.

Currently there is no in situ method to detect and quantify complete mineralization of chlorinated hydrocarbons (CHCs) to CO<sub>2</sub>. Combined isotopic measurements in conjunction with traditional chemical techniques were used to assess in situ biodegradation of trichloroethylene (TCE) and carbon tetrachloride (CT). Vadose zone CHC, ethene, ethane, methane, O<sub>2</sub>, and CO<sub>2</sub> concentrations were analyzed using gas chromatography over 114 days at the Savannah River Site.  $\delta(13)C$  of CHC and  $\delta(13)C$  and C-14 of vadose zone CO<sub>2</sub>, sediment organic matter, and groundwater dissolved inorganic carbon (DIC) were measured. Intermediate metabolites of TCE and CT accounted for less than or equal to 10% of total CHCs.  $\delta(13)C$  of cis-1,2-dichloroethylene (DCE) was always heavier than TCE indicating substantial DCE biodegradation. C-14-CO<sub>2</sub> values ranged from 84 to 128 percent modern carbon (pMC), suggesting that plant root-respired CO<sub>2</sub> was dominant. C-14-CO<sub>2</sub> values decreased over time (up to 12 pMC), and contaminated groundwater C-14-DIC (76 pMC) was substantially depleted relative to the control (121 pMC). C-14 provided a direct measure of complete CHC mineralization in vadose zone and groundwater in situ and may improve remediation strategies.

Kousaka, K., N. Ogi, et al. (2003). "Novel Oxylipin Metabolites from the Brown Alga *Eisenia bicyclis*." *J. Nat. Prod.* 66(10): 1318-1323.

Nine novel oxylipin metabolites together with several known ones were isolated from the brown alga *Eisenia bicyclis*. Five (1-5) of them are ecklonialactone derivatives containing a chlorine or an iodine atom, and two (6 and 7) are cymathere type oxylipins with a lactone ring or a chlorine atom. The structures of these oxylipin metabolites were confirmed by NMR and mass spectroscopy and compared with spectral data in the literature. The postulated biosynthetic pathway of these metabolites is discussed.

Krausova, V. I., F. T. Robb, et al. (2003). "Bacterial degradation of dichloromethane in cultures and natural environments." *Journal of Microbiological Methods* 54(3): 419-422.

Dichloromethane (DCM) is a toxic pollutant showing prolonged persistence in water. DCM biodegradation is usually determined from increases in Cl ions, gas chromatography, or by using radioisotopes. Herein, we present an original and easy spectrophotometric method to estimate DCM concentrations in cultures and environmental samples during DCM biodegradation experiments. (C) 2003 Elsevier Science B.V. All rights reserved.

Kuniyoshi, M., N. Oshiro, et al. (2003). "Five new halogenated octadane monoterpenes having a cyclohexadienone from the red alga *Portieria hornemanni*." *J. Chinese Chem. Soc. (Taipei)* 50(1): 167-170.

Kwon, O. S., Y. J. Kim, et al. (2003). "Influence of transition-metal cofactors on the reductive dechlorination of polychlorinated biphenyls (PCBs)." *Journal of Microbiology* 41(3): 189-195.

To enhance the reductive dechlorination of polychlorinated biphenyls (PCBs) under anaerobic conditions, we examined the adjunctive effects of cobalt (Co) and nickel (Ni), which are the central metals of transition-metal cofactors of coenzyme F-430 and vitamin B-12, respectively, on the dechlorination of Aroclor 1248. After 32 weeks of incubation, the average numbers of chlorines per biphenyl in culture vials supplemented with 0.2, 0.5, and 1.0 mM of Co reduced from 3.88 to 3.39, 2.92, and 3.28, respectively. However, the numbers of chlorine after supplementing with Ni decreased from 3.88 to 3.43, regardless of the Ni concentrations. The observed congener distribution patterns of all vials with different conditions were similar to the pattern produced by the dechlorination process of H' after 21 weeks of incubation, and these patterns were unchanged up to week 32, except for vials supplemented with 0.5 and 1.0 mM of Co. In vials containing 0.5 mM of Co, meta-rich congeners, such as 25/25-, 24/25-, and 25/23-chlorobiphenyls (CBPs), which were found as accumulated products of dechlorination in other conditions, were further dechlorinated, and 25/2-, 24/2-, and 2/2-CBPs were concomitantly increased after 32 weeks of incubation. In this case, the congener distribution was similar to the dechlorination pattern of process M. From these results, we suggested that the enrichment of cultures with Co might stimulate the growth of specific populations of meta-dechlorinators, and that populations might promote a change in the dechlorination process from H' to M, which is known to be less effective on the dechlorination of the more highly chlorinated congeners of PCBs.

Lackey, L. W., J. R. Gamble, et al. (2003). "Biofiltration of trichloroethylene-contaminated air: A pilot study." *Journal of the Air & Waste Management Association* 53(10): 1248-1255.

This project demonstrated the biofiltration of a trichloroethylene (TCE)-contaminated airstream generated by air stripping groundwater obtained from several wells located at the Anniston Army Depot, Anniston, AL. The effects of several critical process variables were investigated to evaluate technical and economic feasibility, define operating limits and preferred operating conditions, and develop design information for a full-scale biofilter system. Long-term operation of the demonstration biofilter system was conducted to evaluate the performance and reliability of the system under variable weather conditions. Propane was used as the primary substrate necessary to induce the production of a nonspecific oxygenase. Results indicated that the process scheme used to introduce propane into the biofiltration system had a significant impact on the observed TCE removal efficiency. TCE degradation rates were dependent on the inlet contaminant concentration as well as on the loading rate. No microbial inhibition was observed at inlet TCE concentrations as high as 87 parts per million on a volume basis.

Lee, E. Y., B. D. Ye, et al. (2003). "Development and operation of a trickling biofilter system for continuous treatment of gas-phase trichloroethylene." *Biotechnology Letters* 25(20): 1757-1761.

A parallel trickling biofilter (TBF) system that consists of two TBFs units in parallel, one for biodegradation of trichloroethylene (TCE) and the other for reactivation of an inactivated biofilm, was developed and operated for continuous treatment of gas-phase TCE by *Burkholderia cepacia* G4. For inlet loadings below 8.6 mg TCE l(-1) d(-1), complete removal of TCE was achieved. The maximal TCE elimination capacity was 17 mg l(-1) d(-1).

Lee, S. J., J. H. Pardue, et al. (2003). "Mineralization of desorption-resistant 1,4-dichlorobenzene in wetland soils." *Environmental Toxicology and Chemistry* 22(10): 2312-2322.

Laboratory studies were conducted to investigate the biologically mediated, aerobic mineralization of both freshly added and artificially aged, desorption-resistant 1,4-dichlorobenzene (1,4-DCB). The adsorption and desorption of 1,4-DCB isotherms were established in three wetland soils using decant-refill batch techniques. Significant nonlinearity and hysteresis were observed in the isotherms with a hysteresis index ranging from 0.11 (relatively low hysteresis) in a marsh soil to 2.26 (relatively high hysteresis) in a bottomland hardwood soil from the Petro Processor (PPI) Superfund site. Mineralization of freshly added 1,4-DCB was observed in all three soils without lag after the addition of a 1,4-DCB degrading culture. Mineralization curves were plotted above theoretical lines predicted from a first-order model assuming instantaneous desorption, indicating that the

microbial population had access to sorbed 1,4-DCB. In separate experiments, mineralization of artificially aged, desorption-resistant 1,4-DCB was also observed. Mineralization curves in these studies also indicated that the microbial population could directly access sorbed 1,4-DCB. The extent and rate of mineralization of desorption-resistant 1,4-DCB decreased significantly, including rate constants decreasing from approximately 0.01 d<sup>-1</sup> in the freshly added treatments to approximately 0.002 d<sup>-1</sup> in the desorption-resistant treatments. Although sorption/desorption partitioning helped explain mineralization patterns in the treatments with freshly added 1,4-DCB, no differences were observed in mineralization curves in the desorption-resistant treatments between soils with widely varying sorption/desorption properties.

Lerch, M. L., M. K. Harper, et al. (2003). "Brominated polyacetylenes from the Philippines sponge *Diplastrella* sp." *Journal of Natural Products* 66(5): 667-670.

Five novel brominated polyacetylenic diols, diplynes A-E (2-6), and three sulfated analogues, diplyne A 1-sulfate (7), diplyne C 1-sulfate (8), and 2-deoxydiplyne D sulfate (9), were isolated from the Philippines sponge *Diplastrella* sp. by employing bioassay-guided fractionation using the HIV-1 integrase inhibition assay. The novel metabolites were characterized by interpretation of spectroscopic data.

Lerch, M. L., M. K. Harper, et al. (2003). "Diplynes A-E, five novel brominated polyacetylenes and three sulfated analogues from the sponge *Diplastrella* sp. (1.88 g)." *J. Nat. Prod.* ( ASAP Article, in press) 66(5): 667-670.

Maillard, J., W. Schumacher, et al. (2003). "Characterization of the corrinoid iron-sulfur protein tetrachloroethene reductive dehalogenase of *Dehalobacter restrictus*." *Applied and Environmental Microbiology* 69(8): 4628-4638.

The membrane-bound tetrachloroethene reductive dehalogenase (PCE-RDase) (PceA; EC 1.97.1.8), the terminal component of the respiratory chain of *Dehalobacter restrictus*, was purified 25-fold to apparent electrophoretic homogeneity. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed a single band with an apparent molecular mass of 60 +/- 1 kDa, whereas the native molecular mass was 71 +/- 8 kDa according to size exclusion chromatography in the presence of the detergent octyl-beta-D-glucopyranoside. The monomeric enzyme contained (per mol of the 60-kDa subunit) 1.0 +/- 0.1 mol of cobalamin, 0.6 +/- 0.02 mol of cobalt, 7.1 +/- 0.6 mol of iron, and 5.8 +/- 0.5 mol of acid-labile sulfur. Purified PceA catalyzed the reductive dechlorination of tetrachloroethene and trichloroethene to cis-1,2-dichloroethene with a specific activity of 250 +/- 12 nkat/mg of protein. In addition, several chloroethanes and tetrachloromethane caused methyl viologen oxidation in the presence of PceA. The K<sub>m</sub> values for tetrachloroethene, trichloroethene, and methyl viologen were 20.4 +/- 3.2, 23.7 +/- 5.2, and 47 +/- 10 μM, respectively. The PceA exhibited the highest activity at pH 8.1 and was oxygen sensitive, with a half-life of activity of 280 min upon exposure to air. Based on the almost identical N-terminal amino acid sequences of PceA of *Dehalobacter restrictus*, *Desulfitobacterium hafniense* strain TCE1 (formerly *Desulfitobacterium frappieri* strain TCE1), and *Desulfitobacterium hafniense* strain PCE-S (formerly *Desulfitobacterium frappieri* strain PCE-S), the *pceA* genes of the first two organisms were cloned and sequenced. Together with the *pceA* genes of *Desulfitobacterium hafniense* strains PCE-S and Y51, the *pceA* genes of *Desulfitobacterium hafniense* strain TCE1 and *Dehalobacter restrictus* form a coherent group of reductive dehalogenases with almost 100% sequence identity. Also, the *pceB* genes, which may code for a membrane anchor protein of PceA, and the intergenic regions of *Dehalobacter restrictus* and the three *desulfitobacteria* had identical sequences. Whereas the *cprB* (chlorophenol reductive dehalogenase) genes of chlorophenol-dehalorespiring bacteria are always located upstream of *cprA*, all *pceB* genes known so far are located downstream of *pceA*. The possible consequences of this feature for the annotation of putative reductive dehalogenase genes are discussed, as are the sequence around the iron-sulfur cluster binding motifs and the type of iron-sulfur clusters of the reductive dehalogenases of *Dehalobacter restrictus* and *Desulfitobacterium dehalogenans* identified by electron paramagnetic resonance spectroscopy.

Manzano, M. A., J. A. Perales, et al. (2003). "Enhancement of aerobic microbial degradation of polychlorinated biphenyl in soil microcosms." *Environmental Toxicology and Chemistry* 22(4): 699-705.

This article reports the results of various biodegradation experiments on polychlorinated biphenyl (PCB)-contaminated sandy soil employing a mixed culture of acclimatized bacteria. Following the optimization of different variables without chemical pretreatment, the elimination rate achieved of Aroclor(R) 1242 in Slurry-phase reactors was 61 % after four months of treatment, with the presence of biphenyl as cosubstrate being the most important factor affecting PCB biodegradation. The biodegradation occurred as a first-order process, and it proved most effective in respect to dichlorinated biphenyls (100% removal), followed by trichlorinated (92%) and tetrachlorinated biphenyls (24%). The results also showed that the degradability of PCBs in soil may be enhanced by an advanced oxidation pretreatment (Fenton reaction), producing almost 100% elimination of PCBs at the end of the integrated chemical-biological process and 72% mineralization of the intermediates generated during the chemical pretreatment.

Marchesi, J. R. and A. J. Weightman (2003). "Comparing the dehalogenase gene pool in cultivated alpha-halocarboxylic acid-degrading bacteria with the environmental metagene pool." *Applied and Environmental Microbiology* 69(8): 4375-4382.

Culture-dependent and culture-independent approaches were used to determine the relationship between the dehalogenase gene pool in bacteria enriched and isolated on 2,2-dichloropropionic acid (22DCPA) and the environmental metagene pool (the collective gene pool of both the culturable and uncultured microbes) from which they were isolated. The dehalogenases in the pure-cultures isolates, which were able to degrade 22DCPA, were similar to previously described group I and II dehalogenases. Significantly, the majority of the

dehalogenases isolated from activated sludge by degenerate PCR with primers specific for alpha-halocarboxylic acid dehalogenases were not closely related to the dehalogenases in any isolate. Furthermore, the dehalogenases found in the pure cultures predominated in the enrichments but were a minor component of the community used to inoculate the batch cultures. Phylogenetic analysis of the dehalogenase sequences isolated by degenerate PCR showed that the diversity of the group II deh gene was greater than that of the group I deh gene. Direct plating of the activated sludge onto minimal media supplemented with 22DCPA resulted in biomass and DNA from which dehalogenases were amplified. Analysis of the sequences revealed that they were much more closely related to the sequences found in the community used to start the enrichments. However, no pure cultures were obtained with this isolation method, and thus no pure cultures were available for identification. In this study we examined the link between genes found in pure cultures with the metagene pool from which they were isolated. The results show that there is a large bias introduced by culturing, not just in the bacteria isolated but also the degradative genes that they contain. Moreover, our findings serve as a caveat for studies involving the culturing of pure cultures of bacteria and conclusions which are drawn from analysis of these organisms.

Marsh, G., R. Stenutz, et al. (2003). "Synthesis of hydroxylated and methoxylated polybrominated diphenyl ethers - Natural products and potential polybrominated diphenyl ether metabolites." *European Journal of Organic Chemistry*(14): 2566-2576.

Hydroxylated and methoxylated polybrominated diphenyl ethers (OH-PBDEs and MeO-PBDEs) may be natural products or they may be formed as metabolites of polybrominated diphenyl ethers (PBDEs), frequently used as flame retardants. The aim of this work was to synthesize authentic OH- and MeO-PBDE reference standards for analytical and toxicological studies. Brominated phenoxybenzaldehydes were prepared either by coupling of 2,4-dibromophenol with various fluorobenzaldehydes or by coupling of brominated hydroxybenzaldehydes with 2,2',4,4'-tetrabromodiphenyliodonium chloride. OH-PBDEs were synthesized via the brominated phenoxybenzaldehydes by Baeyer-Villiger oxidation and acid-catalyzed hydrolysis. These OH-PBDEs were ortho- and para-brominated (relative to the hydroxy group) with benzyltrimethylammonium tribromide and/or ortho- brominated with bromine/tert-butylamine, and were also brominated with bromine in one case. MeO-PBDEs were obtained by methylation of the prepared OH-PBDEs. MeO-PBDEs were also prepared through the coupling of brominated methoxyphenols with 2,2',4,4'-tetrabromodiphenyliodonium salts, the corresponding OH-PBDEs being obtained after demethylation. A majority of the OH-/MeO-PBDEs prepared have the hydroxy/methoxy group in the ortho position relative to the diphenyl ether bond. All OH-/MeO-PBDEs prepared have 2,4-dibromo substitution patterns (relative to the diphenyl ether bond) in the non-hydroxy-/non-methoxy-containing ring. ((C) Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2003).

Mazur, C. S., W. J. Jones, et al. (2003). "H<sub>2</sub> consumption during the microbial reductive dehalogenation of chlorinated phenols and tetrachloroethene." *Biodegradation* 14(4): 285-295.

Competition for molecular hydrogen exists among hydrogen-utilizing microorganisms in anoxic environments, and evidence suggests that lower hydrogen concentrations are observed with more energetically favorable electron-accepting processes. The transfer of electrons to organochlorines via reductive dehalogenation reactions plays an important role in hydrogen dynamics in impacted systems. We studied the flux of aqueous hydrogen concentrations in methanogenic sediment microcosms prior to and during reductive dehalogenation of a variety of substituted chlorophenols (CP) and tetrachloroethene (perchloroethylene, PCE). Mean hydrogen concentrations during reductive dehalogenation of 2,4-CP, 2,3,4-CP, and PCP were 3.6 nM, 4.1 nM, and 0.34 nM, respectively. Sediment microcosms that were not dosed with chlorophenols yet were actively methanogenic maintained a significantly higher mean hydrogen concentration of 9.8 nM. During active PCE dehalogenation, sediment microcosms maintained a mean hydrogen concentration of 0.82 nM. These data indicate that during limiting hydrogen production, the threshold ecosystem hydrogen concentration is controlled by microbial populations that couple hydrogen oxidation to thermodynamically favorable electron accepting reactions, including reductive dehalogenation of chloroaromatic and chloroaliphatic compounds. We also present revised estimates for the Gibbs free energy available from the reductive dehalogenation of a variety of substituted

chlorophenols based on recently published values of vapor pressure, solubility, and pK(a) for these compounds.

McGuire, T. and J. B. Hughes (2003). "Effects of surfactants on the dechlorination of chlorinated ethenes." *Environmental Toxicology and Chemistry* 22(11): 2630-2638.

The influence of surfactants on a perchloroethene (PCE) dechlorinating mixed culture was investigated in laboratory experiments. Surfactants (Steol CS-330, Aerosol MA 80-I, alpha olefin sulfonate 14 to 16, Neodol 25-7, Tween 80, alkyl polyglycoside, C(16)TAB [trimethylammonium bromide], and sodium dodecyl sulfate) were evaluated for their effects on the rate and extent of PCE reductive dechlorination and their potential biodegradation by the mixed culture. Limited, if any, surfactant biodegradation was observed for the surfactants tested, and all surfactants impaired dechlorination in either the rate of PCE dechlorination or the terminal dechlorination products observed. Based on initial testing, a nonionic surfactant, Tween 80, and an anionic surfactant, Steol CS-330, were selected for additional investigation. Dechlorination of PCE to dichloroethene (DCE), vinyl chloride (VC), and ethene (ETH) occurred in all Tween 80-amended microcosms, with a depressed rate of ETH production as the only adverse effect. Steol CS-330, however, inhibited dechlorination beyond DCE at all surfactant concentrations exceeding 25 mg/L. Attempts to acclimate a culture to Steol CS-330 were unsuccessful. Inhibition of VC and ETH production was reversible on dilution of the surfactant to a concentration of 10 mg/L or less, indicating that surfactant interactions with the enzyme system responsible for reductive dechlorination of DCE may be the cause of inhibition.

Min, J., Y. S. Chang, et al. (2003). "Bacterial detection of the toxicity of dioxins, polychlorinated biphenyls, and polybrominated diphenyl ethers." *Environmental Toxicology and Chemistry* 22(10): 2238-2242.

Polychlorinated dibenzo-p-dioxins (PCDDs), biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) were found to induce several specific stresses within bacterial cells. Four different recombinant *Escherichia coli* with specific stress promoters (i.e., the *recA* responsive to DNA damage, *fabA* responsive to membrane damage, *katG* responsive to oxidative damage, and *grpE* responsive to protein damage) that were fused to the *lux* operon from *Vibrio fischeri* showed very unique specificities in terms of their stress responses in the presence of PCDD and PCBs. In addition, a recombinant bacterium with the *lac* promoter fused to the *lux* operon from *Xenorhabdus luminescens* also showed dose-dependent responses via a loss of bioluminescence because of the addition of the PCDDs and PCBs. Brominated diphenyl ethers (BDE)-47, -99, and -153 congeners, however, were not found to induce any stress within the bacterial cells, indicating that these chemicals do not stimulate any cellular stresses related to those tested. These three congeners, however, did result in different levels of general cellular toxicity, which was found to be dependent on the position of the bromine. Finally, the cellular toxicity within the bacteria was found to increase when exposed to mixtures of dioxins, PCBs, and PBDEs, possibly from synergistic effects.

Nakhla, G., J. Kochany, et al. (2002). "Evaluation of PCBs biodegradability in sludges by various microbial cultures." *Environmental Progress* 21(2): 85-93.

The application of aerobic and anaerobic treatment for removal of PCBs in sludges generated from a groundwater treatment system was investigated. The sludge was characterized by PCB concentrations of > 520 ppm, with Aroclors 1254 and 1260 accounting for 26 and 74%, respectively, as well as total organic carbon (TOC), and oil and grease (O&G) concentrations of 108,500 and 18,600 mg/L, respectively. Four treatments were explored. three aerobic and one anaerobic. Two types of bacterial inocula were aerobically tested: sludge from a poultry processing wastewater treatment plant, and a commercial seed (Petrobio(R)). Petrobio was tested aerobically with and without a biosurfactant/enzymes product (BOD-Balance(TM)), and was also tested anaerobically. Over a period of 60 days, PCB concentrations on a volumetric and solids basis were reduced by 73.4 to 88.0% and 64.7 to 80%, respectively. Aroclors 1254 and 1260 concentrations decreased by about 76 to

89%, and 73 to 89%, respectively. TOC and O&G removal efficiencies ranged from 77 to 93%, and from 52 to 86%, respectively. Generally, sludge from the poultry processing facility achieved the best results and exhibited the most rapid biodegradation kinetics. Aerobic PCB biodegradation rates for Aroclors 1254 and 1260 were 13.5% and 23%, respectively, faster than the anaerobic biodegradation rates. Overall PCB biodegradation kinetics conformed to first-order, with rate constants ranging from 0.017 d(-1) under anaerobic conditions to 0.022 d(-1) under aerobic conditions, while both TOC and O&G biodegradation kinetics followed first-order kinetics, with rate coefficients in the ranges of 0.025-0.047 and 0.014 and 0.04 d(-1), respectively.

Ogawa, N., K. Miyashita, et al. (2003). "Microbial genes and enzymes in the degradation of chlorinated compounds." *Chemical Record* 3(3): 158-171.

Microorganisms are well known for degrading numerous natural compounds. The synthesis of a multitude of chlorinated compounds by the chemical industry and their release into the natural environment have created major pollution problems. Part of the cause of such pollution is the inability of natural microorganisms to efficiently degrade synthetic chlorinated compounds. Microorganisms are, however, highly adaptable to changes in the environment and have consequently evolved the genes that specify the degradation of chlorinated compounds to varying degrees. Highly selective laboratory techniques have also enabled the isolation of microbial strains capable of utilizing normally recalcitrant highly chlorinated compounds as their sole source of carbon and energy. The evolution and role of microbial genes and enzymes, as well as their mode of regulation and genetic interrelationships, have therefore been the subjects of intense study. This review emphasizes the genetic organization and the regulation of gene expression, as well as evolutionary considerations, regarding the microbial degradation of chlorobenzoates, chlorocatechols, and chlorophenoxyacetic acids. (C) 2003 The Japan Chemical journal Forum and Wiley Periodicals, Inc.

Ortiz-Bermudez, P., E. Srebotnik, et al. (2003). "Chlorination and cleavage of lignin structures by fungal chloroperoxidases." *Applied and Environmental Microbiology* 69(8): 5015-5018.

Two fungal chloroperoxidases (CPOs), the heme enzyme from *Caldariomyces fumago* and the vanadium enzyme from *Curvularia inaequalis*, chlorinated 1-(4-ethoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-dihydroxypropane, a dimeric model compound that represents the major nonphenolic structure in lignin. Both enzymes also cleaved this dimer to give 1-chloro-4-ethoxy-3-methoxybenzene and 1,2-dichloro-4-ethoxy-5-methoxybenzene, and they depolymerized a synthetic guaiacyl lignin. Since fungal CPOs occur in soils and the fungi that produce them are common inhabitants of plant debris, CPOs may have roles in the natural production of high-molecular-weight chloroaromatics and in lignin breakdown.

Padma, T. V., R. M. Dickhut, et al. (2003). "Variations in alpha-hexachlorocyclohexane enantiomer ratios in relation to microbial activity in a temperate estuary." *Environmental Toxicology and Chemistry* 22(7): 1421-1427.

Changes in the enantiomer ratios (ERs) of chiral pollutants in the environment are often considered evidence of biological alteration despite the lack of data on causal or mechanistic relationships between microbial parameters and ER values. Enantiomer ratios that deviate from 1:1 in the environment provide evidence for the preferential microbial degradation of one enantiomer. whereas ER values equal to 1 provide no evidence for microbial degradation and may mistakenly be interpreted as evidence that biodegradation is not important. In an attempt to link biological and geochemical information related to enantioselective processes, we measured the ERs of the chiral pesticide  $\alpha$ -hexachlorocyclohexane ( $\alpha$ -HCH) and bacterial activity (normalized to abundance) in surface waters of the York River (VA, USA) bimonthly throughout one year. Despite lower overall  $\alpha$ -HCH concentrations,  $\alpha$ -HCH ER Values were unexpectedly close to 1: 1 in the freshwater region of the estuary with the highest bacterial activity. In contrast, ER values were nonracemic (ER not equal 1) and  $\alpha$ -HCH concentrations were significantly higher in the higher salinity region of the estuary, where bacterial activity was lower. Examination of these data may indicate that racemic environmental ER Values are not necessarily reflective of a lack of biodegradation or recent input into the environment. and that nonenantioselective biodegradation may be important in certain areas.

Pakdeesusuk, U., D. L. Freedman, et al. (2003). "Reductive dechlorination of polychlorinated biphenyls in sediment from the Twelve Mile Creek arm of Lake Hartwell, South Carolina, USA." *Environmental Toxicology and Chemistry* 22(6): 1214-1220.

Lake Hartwell is a U.S. Army Corps of Engineers reservoir system located on the state line between South Carolina and Georgia, USA. The lake was contaminated with an estimated 200 metric tons of polychlorinated biphenyls ([PCBs]; mainly Aroclor 1016 and 1254), and the entire Twelve Mile Creek watershed and the Seneca River arm of Lake Hartwell were placed on the National Priorities List. Monitored natural attenuation was chosen as a remedy for the contaminated sediment. The relatively warm temperature of Lake Hartwell and lack of significant cocontaminants along with the PCBs distinguish this site from others that have been studied for microbially mediated, reductive dechlorination. Microcosm studies were conducted with sediment from two locations in the Twelve Mile Creek arm and confirmed the presence of indigenous microorganisms capable of reductively dechlorinating Aroclor 1254, which contains predominantly tetra-, penta-, and hexachlorobiphenyl. The average number of total chlorines per biphenyl decreased from 4.8 to 4.9 to 2.9 to 3.0, following 250 to 260 d of incubation. The maximum observed dechlorination rates were 0.29 to 0.87 mug-atoms Cl- per gram sediment dry weight per week. The onset of dechlorination activity correlated strongly with maximum methanogenesis, which occurred without a lag in samples from the site that showed signs of in situ fermentation activity. Dechlorination occurred primarily at the meta and para positions (58-63% removal), with no apparent decrease in ortho chlorines. This most closely resembles pattern M, characterized by preferential removal of unflanked and flanked meta chlorines. The microcosm results are consistent with sediment cores analyzed from the same locations, which indicate accumulation with depth of the same ortho- and para-substituted congeners. It therefore appears that the success of monitored natural attenuation for Lake Hartwell will hinge on covering the recalcitrant PCBs with a sufficient amount of uncontaminated sediment to isolate them from the food chain.

Park, Y., Y. Liu, et al. (2003). "New Bromotyrosine Derivatives from an Association of Two Sponges, *Jaspis wondoensis* and *Poecillastra wondoensis*." *J. Nat. Prod.* (ASAP Article, in press).

Three new bromotyrosine derivatives (4-6) were isolated from an association of two sponges, *Jaspis wondoensis* and *Poecillastra wondoensis*, along with the previously described (E,E)-psammaplin A (1), (E,Z)-psammaplin A (2), psammaplin D (3), bisaprasin (7), and (3-bromo-4-hydroxyphenyl)acetonitrile (8). The structures of the new compounds were established on the basis of NMR and MS spectroscopic analysis. The compounds 1, 3, and 5-7 displayed significant cytotoxicity against human lung (A549), ovarian (SK-OV-3), skin (SK-MEL-2), CNS (XF498), and colon (HCT15) cancer cell lines. Compounds 3-7 were further evaluated for antibacterial activity against methicillin- or ofloxacin-resistant *Staphylococcus* strains. Compound 4 exhibited more potent antibacterial activity than meropenem against several strains.

Pon, G., M. R. Hyman, et al. (2003). "Acetylene inhibition of trichloroethene and vinyl chloride reductive dechlorination." *Environmental Science & Technology* 37(14): 3181-3188.

Kinetic studies reported here have shown that acetylene is a potent reversible inhibitor of reductive dehalogenation of trichloroethene (TCE) and vinyl chloride (VC) by a mixed dehalogenating anaerobic culture. The mixed culture was enriched from a contaminated site in Corvallis, OR, and exhibited methanogenic, acetogenic, and reductive dehalogenation activities. The H<sub>2</sub>-fed culture transformed TCE to ethene via cis-dichloroethene (c-DCE) and VC as intermediates. Batch kinetic studies showed acetylene reversibly inhibited reduction of both TCE and VC, and the levels of inhibition were strongly dependent on acetylene concentrations in both cases. Acetylene concentrations of 192 and 12 μM, respectively, were required to achieve 90% inhibition in rates of TCE and VC transformation at an aqueous concentration of 400 μM. Acetylene also inhibited methane production (90% inhibition at 48 μM) but did not inhibit H<sub>2</sub>-dependent acetate production. Mass balances conducted during the studies of VC inhibition showed that acetogenesis, VC transformation to ethene, and

methane production were responsible for 52%, 47%, and 1% of the H<sub>2</sub> consumption, respectively. The results indicate that halorespiration is the dominant process responsible for VC and TCE transformation and that dehalorespiring organisms are the target of acetylene inhibition. Acetylene has potential use as a reversible inhibitor to probe the biological activities of reductive dechlorination and methanogenesis. It can be added to inhibit reactions and then removed to permit reactions to proceed. Thus, it can be a powerful tool for investigating intrinsic and enhanced anaerobic remediation of chloroethenes at contaminated sites. The results also suggest that acetylene produced abiotically by reactions of chlorinated ethenes with zero-valent iron could inhibit the biological transformation of VC to ethene.

Rezanka, T. and V. M. Dembitsky (2003). "Eight new brominated oxylipins and oxylipin glycosides from red sea corals (*Dendrophyllia* and *Dendronephthya*) sp." *Eur. J. Org. Chem.* 2: 309-316.

Rezanka, T. and V. M. Dembitsky (2003). "Brominated oxylipins and oxylipin glycosides from Red Sea corals." *European Journal of Organic Chemistry*(2): 309-316.

Eight new brominated oxylipins 1-8, including two glycosides, were isolated from the Red Sea invertebrates *Dendro-phyllia* sp., *Dendronephthya* sp. (red variety), *Dendronephthya* sp. (yellow variety), and *Tubipora musica*. Their structures were elucidated mainly on the basis of NMR spectroscopic data. The relative and absolute configurations were determined by analysis of NOESY and CD data and by the modified Mosher method. The compounds gave positive results in a brine shrimp toxicity assay, a sea urchin eggs test (*Paracentrotus lividus*), and a crown gall tumor on potato disks test (*Agrobacterium tumefaciens*). ((C) Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheirri, Germany, 2003).

Rockne, K. J. and S. E. Strand (2003). "Amplification of marine methanotrophic enrichment DNA with 16S rDNA PCR primers for Type II alpha proteobacteria methanotrophs." *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* 38(9): 1877-1887.

Type II alpha proteobacteria methanotrophs are capable of a wide range of cometabolic transformations of chlorinated solvents and polycyclic aromatic hydrocarbons (PAHs), and this activity has been exploited in many terrestrial bioremediation systems. However, at present, all known obligately marine methanotrophic isolates are Type I gamma proteobacteria which do not have this activity to the extent of Type II methanotrophs. In previous work in our laboratory, determining the presence of Type II alpha proteobacteria methanotrophs in marine enrichment cultures that co-metabolized PAHs required a more sensitive assay. 16S rDNA PCR primers were designed based on oligonucleotide probes for serine pathway methanotrophs and serine pathway methylotrophs with an approximate amplification fragment size of 870 base pairs. Comparison of the primers using double primer BLAST searches in established nucleotide databases showed potential amplification with all *Methylocystis* and *Methylosinus* spp., as well as potential amplification with *Methylocella palustris*. DNA, from *Methylosinus trichosporium* OB3b, a Type II methanotroph, amplified with the primers with a fragment size of approximately 850 base pairs, whereas DNA extracted from *Methylomonas methanica*, a Type I methanotroph, did not. The primers were used to amplify DNA extracted from two marine methanotrophic enrichment cultures: a low nitrogen/low copper enrichment to select for Type II methanotrophs and a high nitrogen/high copper enrichment to select for Type I methanotrophs. Although DNA from both cultures amplified with the PCR primers, amplification was stronger in cultures that were specifically enriched for Type II methanotrophs, suggesting the presence of higher, numbers of Type II methanotrophs. These results provide further evidence for the existence of Type II marine methanotrophs, suggesting the possibility of exploiting cometabolic activity in marine systems.

Ruppe, S., A. Neumann, et al. (2003). "Anaerobic transformation of compounds of technical toxaphene. I. Regiospecific reaction of chlorobornanes with geminal chlorine atoms." *Environmental Toxicology and Chemistry* 22(11): 2614-2621.

Technical toxaphene (Melipax) and the single compounds of technical toxaphene (CTTs) 2,2,5-endo,6-exo,8,8,9,10-octachlorobornane (B8-806), 2,2,5-endo,6-exo,8,9,9,10-octachlorobornane (B8-809), 2,2,5,5,8,9,9,10,10-nonachlorobornane (B9-1025), 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonochlorobornane (B9-1679), 2-endo,3-exo,5-endo,6-exo,8,9,10,10-octachlorobornane (B8-1414), 2-endo,3-exo,5-endo,6-exo,8,8,9,10-octachlorobornane (B8-1412), and 2-exo,3-endo,5-exo,9,9,10,10-heptachlorobornane (B7-1453) were treated with suspensions of the anaerobic bacterium *Dehalospirillum multivorans*. After 7 d, more than 50% of technical toxaphene was transformed, and the relative amount of early eluting CTTs increased. After 16 d, only 2-exo,3-endo,6-exo,8,9,10-hexachlorobornane (B6-923), 2-endo,3-exo,5-endo,6-exo,8,9,10-heptachlorobornane (B7-1001), and a few minor penta- and hexachloro-CTTs were detected in the samples. The result of the transformation was comparable with observations in naturally contaminated sediments and soil. However, the performance with *D. multivorans* was more simple and reproducible, as well as faster, than use of soil, sediment, or anaerobic sewage sludge. In agreement with reports in the literature, reductive dechlorination at geminal chlorine atoms (gem-Cl) was found to be the major CTT transformation pathway. Experiments conducted with CTTs and gem-Cl at both primary and secondary carbons clarified that the initial Cl → H substitution takes place at the secondary carbon C2. Furthermore, the 2-endo-Cl position was preferably substituted with hydrogen. In the case of B8-806, the dechlorination at the secondary carbon C2 was approximately 20-fold faster than the subsequent, slow reduction at the primary carbon C8. The three different formerly unknown heptachloro-CTTs, 2-exo,3-endo,6-exo,8,9,9,10-heptachlorobornane (B7-1473), 2-exo,3-endo,6-endo,8,9,9,10-heptachlorobornane (B7-1461), and 2-exo,3-endo,6-exo,8,8,9,10-heptachlorobornane (B7-1470) were found as intermediates of the B8-806/809 transformation. Treatment of B9-1679 with *D. multivorans* indicated that gem-Cl on the bridge (C8 and C9) are dechlorinated faster than gem-Cl on the bridgehead (C10).

Ryu, J. H., Y. Lee, et al. (2003). "The role of hydrogen peroxide produced by polychlorinated biphenyls in PMR1-deficient yeast cells." *Journal of Biochemistry* 134(1): 137-142.

Polychlorinated biphenyls (PCBs) are well-known recalcitrant environmental pollutants. Although the metabolism of the PCBs has been intensively studied, very little is known about their mechanism of toxicity in living organisms or how they are degraded. We have examined the effects of PCBs on two different yeast strains to determine their mechanism of action. One yeast strain (K601, wild type) is resistant to the growth-inhibitory effect of PCBs, whereas the other strain (AA542, PMR1 mutant) is susceptible. PCBs increased the level of intracellular hydrogen peroxide in AA542 cells but not in K601 cells. In the presence of  $\alpha$ -tocopherol or ursolic acid the growth of AA542 cells was not inhibited by treatment with PCBs. These results suggest that PCBs block cell growth through production of hydrogen peroxide in the PMR1 mutant strain, AA542. We compared superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase activities in both strains. The catalase activity in K601 cells was 10 times higher than that in AA542 cells. In contrast, there was no difference in activities of SOD and GPx between the two strains. Collectively, these observations indicate that oxidative stress causes the inhibition of cell growth observed in catalase-deficient yeast cells exposed to PCBs.

Sato, A., Y. Watanabe, et al. (2003). "Screening for dioxin-degrading basidiomycetes from temperate and tropical forests." *World Journal of Microbiology & Biotechnology* 19(7): 763-766.

A total of 500 strains of basidiomycetes isolated from temperate forests in Japan and 379 strains from tropical forests in Indonesia were subjected to a laboratory screening for dioxin-degrading ability. At first, about 200 fungal strains were selected by their ability to decolorize Remazol Brilliant Blue R dye as an indicator of ligninolytic activities. Next, for excluding the factor of dioxin sorption by mycelia, we prepared two series of living cultures exposed either long-term or short-term to 2,7-dichlorodibenzo-p-dioxin (2,7-DCDD), and compared the decreases in the remaining amounts of this model compound. We chose *Bjerkandera adusta* strain

VH57 as a promising new candidate for dioxin degradation, because it gave 40% difference in 2,7-DCDD levels between the two treatments after 30 days of exposure.

Shoeb, M. and M. Jaspars (2003). "Chlorinated C12 Fatty Acid Metabolites from the Red Alga *Gracilaria verrucosa*." *J. Nat. Prod.* (ASAP article, in press).

Three compounds containing moieties rarely encountered in nature, viz., 3-nonyloxirane-2 carboxylic acid methyl ester (1), 2-chlorododec-2-en-1-ol (2), and 2-chlorododec-2,11-dien-1-ol (3), were isolated from the red alga *Gracilaria verrucosa*, and their structures were determined by spectroscopic methods.

Sierra, I., J. L. Valera, et al. (2003). "Study of the biodegradation process of polychlorinated biphenyls in liquid medium and soil by a new isolated aerobic bacterium (*Janibacter* sp.)." *Chemosphere* 53(6): 609-618.

We have isolated and characterised a novel aerobic bacterial strain, designated MS3-02, belonging to the genus *Janibacter* sp. The capability of this new strain to degrade polychlorinated biphenyls (PCBs) in a commercial mixture (Aroclor 1242) in liquid medium and in soil (sterile and non sterile soil), under laboratory scale, has been evaluated. MS3-02 was isolated from the soil around of an incinerator, located in the east of Madrid (Spain). Gas-chromatographic analysis showed that MS3-02 was able to reduce most peaks observed in the chromatogram between 70% and 100% after seven days of incubation in a culture mineral medium containing yeast extract, but without the addition of biphenyl. The presence of biphenyl in the culture medium decreased the rate of PCB degradation by this bacterium. Comparing the performance of the MS3-02 in liquid culture medium and in soil, degradation was less efficient in sterile soil and still less efficient in non sterile soil. Under the best conditions (sterile soil and 20 weeks of incubation) MS3-02 was able to reduce, between 50% and 100%, nine of the main gas-chromatographic peaks in Aroclor 1242. (C) 2003 Elsevier Ltd. All rights reserved.

Singer, A. C., D. Smith, et al. (2003). "Impact of the plant rhizosphere and augmentation on remediation of polychlorinated biphenyl contaminated soil." *Environmental Toxicology and Chemistry* 22(9): 1998-2004.

This study investigated the interactive effects of bioaugmentation, biostimulation, and the rhizosphere during remediation of Aroclor 1242-contaminated soil. Treatments were repeatedly augmented with polychlorinated biphenyl (PCB)-degrading bacteria, inducers (carvone and salicylic acid), surfactant (sorbitan trioleate), minimal salts medium in a 20-cm high soil column, or a combination of these elements. Soils containing a single *Brassica nigra* plant achieved 61 % PCB removal in the 0 to 2 and 2 to 6 cm depths after 9 weeks of bioaugmentation, whereas only 43 and 14% PCB removal, respectively, was achieved in unplanted controls. Gas diffusion coefficients of 13.0 and 5.0 X 10<sup>-7</sup> m<sup>2</sup> s<sup>-1</sup> were calculated from a methane diffusion assay for planted and unplanted soils respectively, indicating the positive effect of plant roots on gas diffusion into the soil. A second, modified column study removed 87, 73, 63, and 45% of PCB after 12 weeks in the 0 to 5, 5 to 11, 11 to 26, and 26 to 35 cm depths, respectively, in planted-bioaugmented soils, whereas 65, 54, 53, and 47% of PCB was removed from the unplanted-minimal salts treatment, respectively. Shifts in the soil microbial community structure were demonstrated by denaturing gradient gel electrophoresis of bacterial 16S ribosomal DNA. Results support that *Brassica nigra* directly contributed to accelerated PCB removal by increased oxygen diffusion, amendment infiltration, and microbial enrichment.

Stourman, N. V., J. H. Rose, et al. (2003). "Catalytic mechanism of dichloromethane dehalogenase from *Methylophilus* sp strain DM11." *Biochemistry* 42(37): 11048-11056.

The glutathione (GSH)-dependent dichloromethane dehalogenase from *Methylophilus* sp. strain DM11 catalyzes the dechlorination of CH<sub>2</sub>Cl<sub>2</sub> to formaldehyde via a highly reactive, genotoxic intermediate, S-(chloromethyl)glutathione (GS-CH<sub>2</sub>Cl). The catalytic mechanism of the enzyme toward a series of dihalomethane

and monohaloethane substrates suggests that the initial addition of GSH to the alkylhalides is fast and that the rate-limiting step in turnover is the release of either the peptide product or formaldehyde. With the exception of CH<sub>2</sub>ClF, which forms a relatively stable GS-CH<sub>2</sub>F intermediate, the turnover numbers for a series of dihalomethanes fall in a very narrow range (1-3 s<sup>-1</sup>). The pre-steady-state kinetics of the DM11-catalyzed addition of GSH to CH<sub>3</sub>CH<sub>2</sub>Br exhibits a burst of S-(ethyl)-glutathione ( $k(b) = 96 \pm 56 \text{ s}^{-1}$ ) followed by a steady state with  $k_{at} = 0.13 \pm 0.01 \text{ s}^{-1}$ . The turnover numbers for CH<sub>3</sub>CH<sub>2</sub>Cl, CH<sub>3</sub>CH<sub>2</sub>Br, and CH<sub>3</sub>CH<sub>2</sub>I are identical, indicating a common rate-limiting step. The turnover numbers of the enzyme with CH<sub>3</sub>CH<sub>2</sub>Br and CH<sub>3</sub>CH<sub>2</sub>I are dependent on viscosity and are very close to the measured off-rate of GSEt. The turnover number with CH<sub>2</sub>I<sub>2</sub> is also dependent on viscosity, suggesting that a diffusive step is rate-limiting with dihaloalkanes as well. The rate constants for solvolysis of CH<sub>3</sub>SCH<sub>2</sub>Cl, a model for GS-CH<sub>2</sub>Cl, range between 1 s<sup>-1</sup> (1:1 dioxane/water) and 64 s<sup>-1</sup> (1:10 dioxane/water). Solvolysis of the S-(halomethyl)glutathione intermediates may also occur in the active site of the enzyme preventing the release of the genotoxic species. Together, the results indicate that dissociation of the GS-CH<sub>2</sub>X or GS-CH<sub>2</sub>OH intermediates from the enzyme may be a relatively rare event.

Sung-In, Y., C. Youn-Kyoo, et al. (2003). "Effective bead preparation of coimmobilized methanogenic and methanotrophic bacteria for tetrachloroethene degradation." *Biodegradation* 14(5): 347-355.

Three types of coimmobilized methanogenic and methanotrophic bacterial beads - Ca- alginate, Ba-alginate, and Ca- alginate chitosan - were used for tetrachloroethene (PCE) degradation. For the purpose of effective preparation of coimmobilized bacterial beads, the diameter and broken-loading of beads were measured. The activity tests to find the optimal bacteria concentration in the bead were performed. It was found that Ba-alginate beads had superiority in bacterial growth and the degree of strength of beads from the diameter and broken-loading tests. Also, it was shown that it is most effective to add 200 mL of methanogens into 500 mL of 2% alginate solution and 20 mL of methanotrophs into 500 mL to 2% alginate solution. When methanogens and methanotrophs were applied with the Ba-alginate bead in the actual dechlorination of PCE, the biological PCE dechlorination rate was 92%, and there was highly effective degradation of PCE based on the coimmobilized bead. Additionally, relation to the diameter (X) and broken-loading (Y) of the Ba- alginate bead was derived following equation,  $Y = 438.02 \exp(-1.4815 X)$ .

Tachibana, S., S. Tokuoka, et al. (2003). "Adsorption of dioxins by activated carbon and their degradation by a white-rot fungus." *Mokuzai Gakkaishi* 49(3): 227-232.

collection of dioxins in water [Dibenzo-p-dioxin (DD), Dibenzofuran (DF), 2,7-Dichlorodibenzo-p-dioxin (2,7-DCDD) and 2,4,8-Trichlorodibenzofuran (2,4,8-TCDF)] by adsorption on activated carbon was investigated. The adsorbed dioxins were then to be degraded by fungi selected from nature. The degradation of the dioxin 2,4,8-TCDF adsorbed on activated carbon was attempted by bioremediation with the selected fungus, Fungus 267. It was found that the four kinds of dioxins in water were adsorbed well by the activated carbon. It was also found that about 70% of the dioxin 2,4,8-TCDF adsorbed by the activated carbon was degraded in 30 days by bioremediation with the Fungus 267. The information obtained here was useful for the bioremediation of contamination by dioxins.

Topcu, G., Z. Aydogmus, et al. (2003). "Brominated Sesquiterpenes from the Red Alga *Laurencia obtusa*." *J. Nat. Prod.* (ASAP Article, in press).

Four new sesquiterpenes, (8R\*)-8-bromo-10-epi-, -snyderol (1), (8S\*)-8-bromo-, -snyderol (2), 5-bromo-3-(3 $\beta$ -hydroxy-3 $\beta$ -methylpent-4 $\beta$ -enylidene)-2,4,4-trimethylcyclohexanone (3), and the epoxide 4, have been isolated from the chloroform-methanol extract of *Laurencia obtusa*, together with the three known compounds R-snyderol (5), R-snyderol acetate (6), and stigmaterol. The structures of the isolated

compounds were elucidated through spectroscopic analyses. Compound 1 showed antimalarial activity, with IC50 values of 2700 and 4000 ng/mL against the D6 and W2 clones of *Plasmodium falciparum*, respectively.

van Pee, K. H. (2003). "Dehalogenation of polyhalogenated dioxins." *Angewandte Chemie-International Edition* 42(32): 3718-3720.

Vieth, A., J. Muller, et al. (2003). "In-situ biodegradation of tetrachloroethene and trichloroethene in contaminated aquifers monitored by stable isotope fractionation." *Isotopes in Environmental and Health Studies* 39(2): 113-124.

Stable carbon isotope analysis of tetrachloroethene (PCE) and trichloroethene (TCE) was applied to evaluate natural attenuation processes in the upper Quaternary and lower Tertiary aquifer in the area of a former dry-cleaning plant located in Leipzig, Germany. Groundwater samples were taken during one monitoring campaign in 2001. The C-13 enrichment in contaminants along the water flow path suggested that both, PCE and TCE were degraded in the Quaternary aquifer. The enrichment of C-13 in the residual PCE fraction and an isotope fractionation factor from laboratory experiments were used to calculate the extent of biodegradation in the Quaternary aquifer. These calculations indicated that a major portion of PCE was biodegraded in the course of the plume. In the Tertiary aquifer the carbon isotope ratios of PCE and TCE indicated that the decreasing concentrations of these contaminants were probably not caused by microbial processes.

Wang, L., Q. Zhou, et al. (2003). "The biodegradation of 1,3-dichlorobenzene by an adapted strain *Bacillus cereus* PF-11 derived from town-gas industrial effluent." *Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental Engineering* 38(9): 1837-1848.

In the present study, an adapted bacterium PF-11 with high 1,3-dichlorobenzene degradation capability was isolated from town-gas industrial effluent through continuous introducing of N-methyl-N'-nitro-N-nitrosoquandine (NTG). In suitable condition, a degradation rate of 32 mg L<sup>-1</sup>d<sup>-1</sup> of 1,3-dichlorobenzene was obtained by strain PF-11 with effective chlorion release. Strain PF-11 was tentatively identified as gram-positive *Bacillus cereus*. The substrate specificity of the strain PF-11 was relatively low, and the degradation rate for different chlorobenzenes was in the order of monochlorobenzene > 1,3-dichlorobenzene > 1,2-dichlorobenzene. Initial oxidation step was molecular oxygen attacking chlorobenzene ring catalyzed by dioxygenase.

Wang, S. C., M. D. Person, et al. (2003). "Reactions of trans-3-chloroacrylic acid dehalogenase with acetylene substrates: Consequences of and evidence for a hydration reaction." *Biochemistry* 42(29): 8762-8773.

Various soil bacteria use 1,3-dichloropropene, a component of the commercially available fumigants Shell D-D and Telone 11, as a sole source of carbon and energy. One enzyme involved in the catabolism of 1,3-dichloropropene is trans-3-chloroacrylic acid dehalogenase (CaaD), which converts the trans-isomers of 3-bromo- and 3-chloroacrylate to malonate semialdehyde. Sequence analysis suggested a relationship between the heterohexameric CaaD and the bacterial isomerase, 4-oxalocrotonate tautomerase (4-OT), thereby distinguishing CaaD from a number of dehalogenases whose mechanisms proceed through an alkyl- or aryl-enzyme intermediate. In this study, the genes for the alpha- and beta-subunits of CaaD have been synthesized using a polymerase chain reaction-based strategy, cloned into separate plasmids, and the proteins expressed and purified as the functional heterohexamer. Subsequently, the product of the reaction was confirmed to be malonate semialdehyde by H-1 and C-13 NMR spectroscopy, and kinetic constants were determined using a UV spectrophotometric assay. In view of the proposed hydrolytic nature of the CaaD-catalyzed reaction, three acetylene compounds were investigated as substrates for the enzyme. One compound, 2-oxo-3-pentynoate, a potent active site-directed irreversible inhibitor of 4-OT, is a substrate for CaaD, and was processed to

acetopyruvate with kinetic constants similar to those determined for the trans-isomers of 3-bromo- and 3-chloroacrylate. The remaining two compounds, 3-bromo- and 3-chloropropiolic acid, were transformed into potent irreversible inhibitors of CaaD. The inactivation observed for 3-bromopropiolic acid is due to the covalent modification of Pro-1 of the beta-subunit. The results provide evidence for a hydratase activity and set the stage to use the 3-halopropiolic acids as ligands in inactivated CaaD complexes that can be studied by X-ray crystallography.

Watanabe, K., M. Sekine, et al. (2003). "Isolation and Structures of New Halogenated Prostanoids from the Okinawan Soft Coral *Clavularia viridis*." *J. Nat. Prod.* (ASAP Article, in press).

Fifteen new halogenated prostanoids 9-23 were isolated as minor constituents from the Okinawan soft coral *Clavularia viridis*. Compounds 9-11 were new members of iodovulone, and compounds 12-18 were 12-O-acetyliodovulones, 12-O-acetylbromovulones, and 12-O-acetylchlorovulones. Compounds 19-23 were 10,11-epoxy congeners of iodovulone, bromovulone, and chlorovulone. The structures of these compounds were determined on the basis of spectroscopic analysis and chemical conversion.

Wilson, S. C. and A. A. Meharg (2003). "Investigation of organic xenobiotic transfers, partitioning and processing in air-soil-plant systems using a microcosm apparatus. Part II: comparing the fate of chlorobenzenes in grass planted soil." *Chemosphere* 53(5): 583-591.

A microcosm system was used to investigate and compare transfers of C-14 labeled-1,2-dichlorobenzene (DCB), 1,2,4-trichlorobenzene (TCB) and hexachlorobenzene (HCB) in an air-soil-plant system using single grass tillers planted into spiked soil. This study was the second phase of a development investigation for eventual study of a range of xenobiotic pollutants. Recoveries from the system were excellent at >90%. The predominant loss pathway for C-14 labeled-1,2-DCB and 1,2,4-TCB was volatilisation with 85% and 76% volatilisation of parent compound and volatile metabolites over 5 weeks respectively. Most of the added label in the hexachlorobenzene spiked system remained in soil. Mineralisation was <1% for all compounds. C-14 plant burdens expressed as mg parent compound/g plant fresh weight were significant and suggest that plant uptake of chlorobenzenes from soil may be an important exposure pathway for grazing herbivores. Both shoot and root uptake of C-14 was detected, with foliar uptake of volatilised compounds dominating shoot uptake, and being greatest in TCB spiked systems. The microcosm is shown as potentially an ideal system with which to investigate organic xenobiotic partitioning in air-soil-plant systems to improve understanding of the equilibria and kinetics of exchanges. However, limitations imposed by the lab based conditions must be recognized and data should be compared with field based data sets as a consequence. (C) 2003 Elsevier Ltd. All rights reserved.