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

7<sup>th</sup> Quarterly Report  
3<sup>rd</sup> Quarter Year 2002

Report prepared for EUROCHLOR

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**ACRONYMS**

<b>ABV</b>	Anaerobic Bioventing
<b>BCs</b>	Brominated Compounds
<b>CDDs</b>	Chlorinated Dibenzo- <i>p</i> -Dioxins
<b>CDFs</b>	Chlorinated Dibenzo- <i>p</i> -Furans
<b>CT</b>	Carbon Tetrachloride
<b>2,4-D</b>	2,4-Dichlorophenoxyacetate
<b>1,2-DCA</b>	1,2-Dichloroethane
<b><i>Cis</i>-DCE</b>	<i>Cis</i> -1,2-dichloroethene
<b><i>Trans</i>-DCE</b>	<i>Trans</i> -1,2-dichloroethene
<b>DCM</b>	Dichloromethane
<b>DhlA</b>	Haloalkane Dehalogenase A
<b>DiCDD</b>	Di-chlorinated Dibenzo- <i>p</i> -Dioxins
<b>DF</b>	Dibenzofuran
<b>DGGE</b>	Denaturing Gradient Gel Electrophoresis
<b>DNAPL</b>	Dense Non-Aqueous Phase Liquid
<b>E-acceptor</b>	Electron Acceptor
<b>E-donor</b>	Electron Donor
<b>ETH</b>	Ethene
<b>HCB</b>	Hexachlorobenzene
<b>HCH</b>	Hexachlorohexane
<b>HDBPs</b>	Halogenated Dimethyl Bipyrrroles
<b>MeBr</b>	Methyl Bromide
<b>MPN</b>	Most Probable Number
<b>PCBs</b>	Polychlorinated Biphenyls
<b>PCE</b>	Tetrachloroethylene
<b>PCR</b>	Polymerase Chain Reaction
<b>RTm-PCR</b>	Real Time Polymerase Chain Reaction
<b>sMMO</b>	Soluble Methane Monooxygenase
<b>1,1,2-TCA</b>	1,1,2-Trichloroethane
<b>TCE</b>	Trichlorethylene
<b>TeCDD</b>	Tetra-chlorinated dibenzo- <i>p</i> -dioxins
<b>TSS</b>	Total Suspended Solids
<b>VC</b>	Vinyl Chloride

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

**7<sup>th</sup> Quarterly Report**

**3<sup>rd</sup> Quarter– Year 2002**

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## **1. INTRODUCTION**

This report presents a review of scientific literature published during the third quarter of 2002 (covering September to November, 2002) 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 on microbial dechlorination in this quarter are several reports on anaerobic vinyl chloride degradation, a report on the anaerobic dechlorination of hexachlorobenzene and a fungus found to degrade 2,7-dichlorodibenzo-*p*-dioxin. Also a simple clever new assay for detecting halohydrin dehalogenase was developed.

Two articles describe vinyl chloride degradation under anaerobic conditions resulting in the formation of the environmentally benign product, ethene (14, 16). The first order rate

constant for anaerobic vinyl chloride degradation was reported to be  $0.015 \text{ d}^{-1}$ . Microorganisms degrading chlorinated solvents degrade compounds with  $^{12}\text{C}$  faster than those with  $^{13}\text{C}$  so there is some isotope enrichment. The isotopic ratios of carbon in vinyl chloride can be used to discern the source of vinyl chloride (e.g. whether it is an intermediate accumulating from higher chloroethanes or high chloroethenes) as well as determine if vinyl chloride is being degraded.

An anaerobic enrichment culture was found that dechlorinated hexachlorobenzene to 1,3,5-trichlorobenzene (53). Based on molecular techniques, the authors could determine that Bacterium DF-1 was most likely responsible for the dechlorination. This bacterium was previously implicated in polychlorinated biphenyl dechlorination.

An extensive screening program was conducted to find fungi capable of 2,7-dichlorodebenzo-*p*-dioxin degradation. Of the 11 strains found, the best one was identified as *Panellus stypticus*, a wood rotting fungus. 3-Chlorocatechol was identified as an intermediate in the oxidation of 2,7-dichlorodebenzo-*p*-dioxin (43).

An intelligent new method of assaying halohydrin dehalogenase was developed based on the use of *p*-nitro-2-bromo-1-phenylethanol as a substrate which has a distinct absorption-spectrum compared to its respective product *p*-nitrostyrene oxide (46). Halohydrin dehalogenase is an important enzyme in the aerobic pathway of chlorinated alcohols. Some halohydrin dehalogenases can also be used to selectively degraded enantiomers of halohydrins, the new assay can also be used to estimate the enantioselectivity.

## 2.b. Microbial Chlorination

The most important findings on microbial chlorination in this quarter is a report on the formation of organochlorines associated with decomposition and weathering of plant material (31). The evidence was based in situ x-ray spectroscopy of fresh and decomposed plant material as well as mature soil humus. As plant material is converted to humus the chlorine in the organic matter becomes progressively more organically bound. In a related study in which organochlorine content of humus was measured in remote and pristine peat bogs (20), an estimate of the global natural organochlorine content of the Earth's peat lands was made of 300 to 1100 million tonnes Cl.

Another interesting finding is that plants are the main source of chloromethanes in salt marshes (40). The evidence was based on the diurnal pattern of chloromethane emissions as well as a correlation in emissions from subplots with the plant biomass in the subplot.

This quarter an extensive review on natural sources of chloroform was published (22), indicating that soil is the main natural terrestrial source.

### 3. MICROBIAL DECHLORINATION

#### 3.a. General Reviews

In this quarter there were two review articles on microbial dechlorination. Both articles were general review articles (9, 51). One of these articles published in the *Journal of Pesticide Science* (51) was not immediately available to the author of this report. The other review discusses the main issues of bioremediation, including references to the bioremediation of chlorinated pollutants (9). Several underlying principles of bioremediation are reviewed. The review also discusses recent advances in the molecular genetics of biodegradation.

#### 3.b. Microbial Dechlorination

##### **Vinyl chloride and Other Chlorinated Ethenes**

As indicated in previous reports, 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 and dichloroethenes, are found in these studies. Below we separate chloroethene studies based on the starting compound investigated, either lower chloroethenes (vinyl chloride or dichloroethenes) or higher chloroethenes (perchloroethylene or trichloroethene).

**Vinyl Chloride (VC) and Dichloroethenes (DCE).** In this quarter only three studies which actually directly investigated the biodegradation of lower chlorinated ethenes. Two of the studies evaluated their degradation under anaerobic conditions. The first study evaluated isotopic fractionation of carbon during the formation of the intermediate VC from various chlorinated solvent precursors (16). The three isomers of dichloroethene (DCE) (*cis*-1,2-, *trans*-1,2- and 1,1-DCE) were tested in laboratory microcosms composed of anaerobic sediment and groundwater from a contaminated site. The three isomers were stoichiometrically converted to

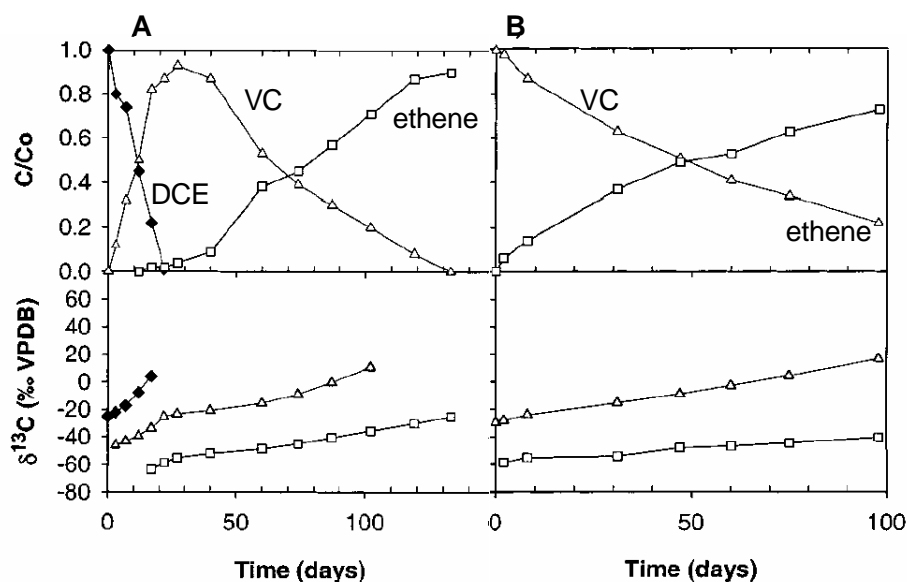
ethene with temporal accumulation of VC. The *cis*-DCE isomer was converted to VC within 20 days, and accumulated VC was completely converted to ethene after an additional 120 days (Figure 1A). In all three microcosms,  $^{13}\text{C}$  was observed to be enriched in the residual DCE. The first VC formed was enriched in  $^{12}\text{C}$ , however, as the degradation proceeded, the  $\delta^{13}\text{C}$  (relative ratio of  $^{13}\text{C}$  to some known standard) of the VC returned to the values of the DCE substrate. Later as the VC was reductively dechlorinated to ethene, VC became enriched with  $^{13}\text{C}$  and the initial ethene formed became enriched with  $^{12}\text{C}$  (Figure 1A). VC was also tested as a substrate to the microcosm and it was converted to ethene with a similar carbon isotope fractionation pattern to that observed in the assays with DCE (Figure 1B). A first-order rate constant for the anaerobic degradation of VC of  $0.015\text{ d}^{-1}$  was reported. The authors point out that differences in isotope fractionation can potentially be used as a tool to discern the source of VC. The  $\delta^{13}\text{C}$  may provide clues as to whether VC was formed from higher chlorinated ethenes, chlorinated ethanes or is present as the original pollutant.

In the second study, the conversion of *cis*-DCE and VC to ethene was evaluated in sediment-free enrichment cultures derived from a contaminated site, and acetate was shown to be an e-donor supporting reductive dehalogenation (14). Acetate alone supported the conversion of *cis*-DCE or VC to ethene; however, the rates were considerably accelerated by spiking the microcosms with hydrogen gas ( $\text{H}_2$ ). VC was converted to ethene in approximately 150 days with  $\text{H}_2$ .

The last study considered the aerobic cooxidation of 1,1-DCE by a butane-oxidizing enrichment culture (21). The kinetics of 1,1-DCE cooxidation were measured in the presence of butane as cosubstrate. The maximum velocity of 1,1-DCE oxidation was  $1.2\ \mu\text{mol}/\text{mg}$  total suspended solids (TSS) per hour and the half-saturation constant was observed to be  $1.5\ \mu\text{M}$ , indicating a high level of affinity for its oxidation. The study also reports inhibition constants of DCE for butane oxidation.

**Perchloroethylene (PCE) and Trichloroethene (TCE).** In this quarter, there were nine reports on the biodegradation of higher chlorinated ethenes. Four additional studies touched on the topic of higher chlorinated ethenes biodegradation, but were actually concerned more with aspects of bioremediation technology, than biodegradation (see section 3.e. *New Techniques for Bioremediation Technology*). Three of the studies evaluated anaerobic degradation, four of the studies evaluated aerobic degradation, and two studies evaluated sequential anaerobic-aerobic degradation.

The first anaerobic study assessed acetate as an e-donor for the reductive dechlorination of PCE in sediments from a contaminated site in Michigan (USA) (14). Typically acetate is



**Figure 1.** Reductive dechlorination of *cis*-DCE (A) and VC (B). Relative concentrations and carbon isotope ratio ( $\delta^{13}\text{C}$ ) of dichloroethene (filled markers), vinyl chloride (open triangle), and ethene (open square).

considered as a poor e-donor for reductive dehalogenation, thus the findings are remarkable. Some of the microcosms receiving acetate could completely dechlorinate PCE to ethene, albeit there was a rapid initial conversion to *cis*-DCE. The initial conversion appeared to be truly dependent on acetate, since microcosms incubated with  $\text{H}_2$ , did not begin to reduce PCE to *cis*-DCE until after  $\text{H}_2$  was first converted to acetate (by  $\text{H}_2/\text{CO}_2$  acetogens).

The second anaerobic study evaluated the degradation of PCE in dense non-aqueous phase liquids (DNAPLs) (54). While enhanced dissolution of DNAPL was previously reported due to anaerobic microbial activity, this study considered the effect of different electron donating substrates. Pentanol, calcium oleate and olive oil were compared. PCE degradation was observed and the major degradation product formed was *cis*-DCE. Significant amounts of VC and ethane were also found with some columns. Extensive methanogenesis, which reduced PCE transformation, occurred in both the pentanol-fed and oleate-amended columns, but not in the olive-oil-amended column. The best results were obtained with olive oil since methanogenesis competes for reducing equivalents needed for reductive dechlorination.

The third anaerobic study evaluated the chemical conversion of TCE by the enzyme cofactor, vitamin  $\text{B}_{12}$  (cyanocobalamin), which is well-known for its ability to catalyze the

reductive dechlorination of various chlorinated compounds (32). The study considers molecular theory to clarify why TCE is predominantly converted to *cis*-DCE by vitamin B12. The selectivity in product formation can be explained on the basis of density functional and coupled-cluster theory. The reaction is suggested to proceed via TCE radical anion, which is unstable and readily results in C-Cl cleavage. The resulting product is most stable when the two remaining chlorine atoms occupy the *cis* configuration. Once formed, the *cis*-1,2-dichloroethen-1-yl radical is about 6 kJ/mol more stable than the corresponding trans radical and 21 kJ/mol more stable than the 1,1-dichloroethen-2-yl radical.

Two studies evaluated a sequential degradation scheme in which PCE is first rapidly converted to DCE under anaerobic conditions. Subsequently, accumulated DCE is further degraded by aerobic cooxidation. The philosophy behind the strategy is that PCE degradation is only feasible under anaerobic conditions; whereas DCE degradation is more rapid under aerobic than anaerobic conditions. The first study was conducted with a laboratory sequential anaerobic-aerobic bioreactor system, which consisted of an anaerobic fixed film reactor and aerobic chemostats (24). The anaerobic reactor was inoculated with a soil enrichment culture capable of reductive dechlorination, the microorganisms were immobilized on a ceramic support material. The aerobic chemostats were inoculated with a phenol-oxidizing culture of *Alcaligenes*, known for its ability to cooxidize *cis*-DCE in the presence of phenol. The anaerobic reactor performed well, with more than 99% conversion of PCE to *cis*-DCE. The aerobic reactor performed poorly due to oxygen limitations. Through the use of H<sub>2</sub>O<sub>2</sub> to supply oxygen, the system was improved, with a maximum DCE-removal efficiency of 54%.

Sequential anaerobic-aerobic degradation was also evaluated under field conditions in the second study (4). The study evaluated an aquifer plume contaminated with PCE (1.1 μM) and benzene that first past through an anaerobic zone before entering an aerobic zone. PCE was eliminated in the anaerobic zone, where *cis*-DCE and VC were found to accumulate. However, *cis*-DCE and VC were eliminated in the aerobic zone, together with the co-contaminant, benzene. At the end of the experiment, pore water was equilibrated with sediment, to determine if contaminants would rebound. The sediment in the treated area did not release PCE or its intermediates into the pore water, demonstrating a successful remediation.

TCE degradation under aerobic conditions was evaluated under aerobic conditions in four studies. One study reports on two bacterial strains isolated from contaminated soil, *Alcaligenes odorans* N6 and *Nocardia* sp. H17 responsible for TCE metabolism (25). *A. odorans* N6 and *Nocardia* sp. H17 degraded 84% of the initial amount of TCE in a basal salts medium, containing 0.2 mM TCE as the sole source of carbon and energy, in a day. If this observation

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can be independently confirmed, it would be remarkable since biodegradation of TCE as a primary substrate has never been described before. The study also reports on a toluene-degrading mixed culture that can oxidize TCE but only in the presence of toluene (in keeping with the expectation).

In the second study, the occurrence of organisms with soluble methane monooxygenase (sMMO) was characterized in Lake Washington sediments (2). Environmental clone banks for a gene encoding a diagnostic sMMO subunit (mmoX) were generated using DNA extracted from Lake Washington sediment and subjected to restriction fragment length polymorphism (RFLP) analysis. A high frequency of Type I *Methylomonas* mmoX was encountered in the sediment samples. Direct hybridization of Lake Washington sediment DNA was carried out using a series of sMMO- and *Methylomonas*-specific probes to assess the significance of these sMMO-containing *Methylomonas*-like strains in the sediment. The results suggest that the major methanotrophic population in Lake Washington sediment consists of sMMO-containing *Methylomonas*-like (Type I) methanotrophs. The whole-cell TCE degradation kinetics of strain, LW15, isolated from this environment, were determined and found to be similar to values reported for other sMMO-containing methanotrophs. The study indicates that methanotrophs capable of degrading halogenated pollutants are present in sediment environments.

The third study evaluated cometabolism of TCE by an aerobic toluene-degrading consortium in a batch soil-slurry reactor (23). Two types of soil were compared, one freshly polluted with TCE and another in which the pollution was aged (containing only a desorption-resistant fraction of TCE). In aqueous phase experiments run for comparison, the TCE degradation was associated with toluene additions, and TCE degradation rates could be correlated with toluene dioxygenase enzyme activity over time, clearly indicating the cometabolic pattern of TCE degradation. In soil-slurry experiments containing freshly contaminated soil, a TCE degradation rate of approximately 150  $\mu\text{g TCE/kg/h}$  was observed during the first 39-h period. Thereafter, the TCE degradation rate slowed considerably to 0.84  $\mu\text{g TCE/kg/h}$ . The TCE degradation rate in the soil-slurry microcosm containing aged TCE-contaminated soil was approximately 0.32  $\mu\text{g TCE/kg/h}$ . The results clearly indicate that mass transfer into the aqueous phase limited TCE degradation rates in the contaminated soil.

The last study describes a mathematical model used to predict TCE biodegradation in a biofiltration used to purify a TCE-containing gas (8). The model incorporated important aspects such as mass transfer, biodegradation, and adsorption processes. The biokinetic and adsorption parameters for the contaminant were determined independently from a series of

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mini-biofilters and mini-adsorber column experiments. Bench-scale biofilter experiments employing granular activated carbon columns indicated the good predictive capability of the model for the removal of TCE. Sensitivity analysis demonstrated that the biofilter performance was greatly influenced by the Monod coefficients and the biofilm thickness.

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

This quarter there are no reports on the biodegradation of chloroform and only one article on the biodegradation of carbon tetrachloride. The article reports on the degradation of CT in laboratory aquifer columns operated with a pulsed microbial feeding strategy (36). CT biodegradation was achieved with *Pseudomonas stutzeri* strain KC, a denitrifying bacterium that cometabolically converts CT to harmless end products (without chloroform formation). The CT degradation rate in the columns was lower than values obtained from batch studies, and processes in addition to the growth and decay of strain KC cells (due to native flora) are necessary to describe the observed nitrate consumption. A mathematical model based on modified saturation kinetics and on a two-site sorption model was developed to describe the linked physical, chemical, and biological processes involved in CT degradation was developed.

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

This quarter there are no reports on the biodegradation of chloromethane or dichloromethane. The only publication related to this topic reports on the cooxidation of methylbromide by a native soil population of nitrifying bacteria that oxidizes ammonia (10). In cultures actively oxidizing ammonia, methylbromide (MeBr) degradation rate was 20-30 nmol/ml/h that could be sustained for approximately 12 h. Although the MeBr degradation rates were linear for the first 10-12 h of incubation, they could not be sustained regardless of  $\text{NH}_4^+$  level and declined to zero over 20 h of incubation.

#### **Dichloroethane (1,2-DCA) and other chlorinated ethanes**

Only one publication actually tested the biodegradation of 1,2-dichloroethane (1,2-DCA) this quarter. 1,2-DCA was incubated in an anaerobic microcosms with sediments from a contaminated site (16). 1,2-DCA was rapidly converted to ethene with trace formation of VC. Near stoichiometric conversion of 1,2-DCA to ethene occurred within 70 days. Residual 1,2-DCA was enriched with  $^{13}\text{C}$ . The results suggest that dichloroelimination was the predominant pathway for anaerobic 1,2-DCA degradation, as has been reported in the past. In the same

study, 1,1,2-trichloroethane (1,1,2-TCA) was also tested and was found to be slowly converted to VC, which in turn was subsequently converted to ethene.

Screening for a gene, haloalkane dehalogenase gene (*dhmA*), responsible for the aerobic degradation of 1,2-dichloroethane is discussed in another section (*3.d. New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds*).

Several other articles describe biodegradation studies evaluating other chlorinated ethanes. The kinetics of the aerobic cooxidation of 1,1-DCA and 1,1,1-TCA was measured with a butane-degrading enrichment culture (21). The maximum velocity of butane, 1,1-DCA and 1,1,1-TCA degradation; respectively, was 2.6, 0.49 and 0.19  $\mu\text{mol/mg TSS/hour}$ . The half saturation constants were 19, 19 and 12  $\mu\text{M}$ ; respectively. In another study, the rate of chloroethane oxidation by a nitrifying enrichment culture was tested, and chloroethane was found to degrade at a rate of 20-30  $\text{nmol/ml/h}$  (10).

### Chlorobenzenes

**Mono-chlorobenzene, Dichlorobenzenes and Trichlorobenzenes:** No publications regarding the microbial degradation of mono-, di- and/or tri-chlorobenzenes were found in the third quarter of the year 2002.

**Hexachlorobenzene (HCB):** One study reported on an anaerobic enrichment culture derived from aquatic sediments capable of reductively dechlorinating HCB (53). The pattern of dechlorination was  $\text{HCB} \rightarrow \text{pentachlorobenzene} \rightarrow 1,2,3,5\text{-tetrachlorobenzene} \rightarrow 1,3,5\text{-trichlorobenzene}$ . No other isomers of tetrachlorobenzenes were dechlorinated by the culture and no trichlorobenzenes was converted. The pattern of dechlorination coincides with that commonly observed in sediments. Bacterium DF-1, known to be involved in polychlorinated biphenyl (PCB) dechlorination, was detected by PCR/Denaturing Gradient Gel Electrophoresis (DGGE) analysis following dechlorination of pentaclorobenzene but was not detected when a chlorinated benzene was not dechlorinated; detection of other members in the community was unaffected by the presence or absence of dechlorination. Thus the involvement of bacterium DF-1 is implied.

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

In this quarter, two studies report on the degradation of chlorinated dibenzo-*p*-dioxins (CDDs). The first study screened 1500 basidiomycetous fungal strains for capacity to degrade 2,7-dichlorodibenzo-*p*-dioxins (2,7-DiCDD) (43). Of the 1500 strains, 11 strains were found that

significantly removed 2,7-DiCDD beyond bioadsorption. The best strain was identified as *Panellus stypticus*, which could remove close to 100% of the added 2,7-DCDD. During degradation assays, 3-chlorocatechol was identified as a degradation intermediate based on GC-MS analysis. The *Panellus stypticus* strain did not produce extracellular lignin oxidizing enzymes (lignin peroxidase, laccase nor manganese peroxidase), thus a role of intracellular enzymes was suspected. The involvement of cytochrome P450 monooxygenase was implicated by using a specific cytochrome P450 inhibitor.

In the second study, the degradation of 2,3-DiCDD in soil inoculated with the bacterium, *Pseudomonas resinovorans* strain CA10, was evaluated (52). The survival of the inoculated (bioaugmented) bacterium was studied. The survival of the inoculated cells in soil microcosms was strongly influenced by pH and organic matter. Survival decreased rapidly at pH 6 with low organic matter, a high cell density was maintained at pH 7.3 with 2.5% organic matters up to 21 days after inoculation. Single inoculation of *P. resinovorans* cells into the soil slurry system of 2,3-DiCDD-contaminated soil enhanced the degradation of 2,3-DiCDD from 25 to 37%. By repeated inoculations (every 2 days) almost all of the 2,3-DiCDD (1 µg/kg) was degraded within 14 days.

### **Hexachlorobutadiene and Octachlorostyrene**

No publications on the microbial dechlorination of hexachlorobutadiene and octachlorostyrene were found in the third quarter of 2002.

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

In this quarter six studies reported on the microbial dechlorination of PCBs. Three publications reported on aerobic degradation, one on anaerobic degradation and two publications studied PCB congener profiles in sediments for which evidence of microbial alterations were presented. The first aerobic article evaluated the degradation of PCB mixture Delor 103 (Aroclor 1242) by *Pseudomonas* sp. strain P2 (33). Biphenyl was used as the main substrate, and Delor 103 was added to the culture. Under these conditions, 70% PCB degradation was observed. Addition of an amino acid mixture improved degradation. Various chlorinated benzoic acids were identified as intermediates, of which 2,5-dichlorobenzoic acid predominated.

In the second aerobic study, various methods of immobilizing *Pseudomonas* sp. strain P2 were compared and tested with Delor 103 (33). Degradation was monitored by formation of colored intermediates, which was probably not very quantitative.

In the third aerobic study, the effect of earthworms on the degradation of PCB in soil columns bioaugmented with the PCB-degrading bacteria *Ralstonia eutrophs* and *Rhodococcus* sp. strain ACS was tested (26). Earthworms improved the dispersal of the bioaugmented bacteria as well as provide an environment that enhanced the native biphenyl degrading population. The net effect was that PCB was degraded in the top 9 cm when earthworms were applied as opposed to only the top 3 cm in treatments lacking earthworms.

The anaerobic study evaluated rates of PCB degradation in microcosms prepared from sediment obtained from the Hudson River (New York) (6). Rates of PCB dechlorination were evaluated at ten initial concentrations ranging from 0 to 11.2  $\mu\text{mol/g}$  sediment applied as Aroclor 1242. The dechlorination rates increased linearly with PCB concentration, after a clear lag phase. When rates were normalized against measured most probable number (MPN) of dechlorinating organisms they were comparable to values obtained from another microbial sediment consortium (St. Lawrence River) that was tested with Aroclor 1248. The results further support the idea that PCB dechlorination is tightly linked to the growth of dechlorinating microorganisms.

Two studies by the same author (17, 18) analyzed congener profiles in river sediments, Fox River (Wisconsin) and Ashtabula River (Ohio). In the Ashtabula River, the analysis revealed the main source of PCB pollution was Aroclor 1248; whereas, in the Fox River the main source of PCB pollution was determined to be Aroclor 1242. In both cases, congener profiles with increases in lower chlorinated congeners indicated alteration of the original pollution by anaerobic reductive dechlorination.

### Miscellaneous Chlorinated Compounds

This quarter there were five reports on the biodegradation of miscellaneous chlorinated pollutants. These included: atrazine, 2,4-dichlorophenol (2,4-DCP), 2,4-dichlorophenoxyacetate (2,4-D), dichloromethylcatechols, and hexachlorocyclohexane (HCH). The purpose of the study on atrazine was to characterize atrazine-degrading microorganisms in soil (35). Soil samples were tested for their ability to mineralize  $^{14}\text{C}$ -ring-labeled atrazine to  $^{14}\text{CO}_2$  and microorganisms were characterized with dot-blot hybridization of DNA extracted from soil. Probes used for the dot-blot were based on catabolic genes involved in atrazine degradation. Extensive mineralization of  $^{14}\text{C}$ -ring-labeled atrazine was observed but the probe for the gene responsible for ring cleavage was seldom detected. Specific inhibitors for bacteria and fungi suggested that both populations were involved in atrazine mineralization.

The study on 2,4-DCP examined the use of laccase (an extracellular oxidative enzyme) of a white rot fungus, *Trametes villosa*, to polymerize the chlorophenol in soil (1). 2,4-DCP was either transformed to methanol-soluble polymeric products (11-32%) or covalently bound to soil organic matter (53-85%). Residual unaltered 2,4-DCP could be recovered from soil by methanol extraction (0-38%) at the completion of a 14-day incubation period.

The study on 2,4-D describes various catabolic genes in the bacterium *Ralstonia eutropha* JMP134, which was isolated while grown on 2,4-D (38). The paper considers two similar gene operons (sets of genes commonly regulated) responsible for the pathway from chlorocatechols to 3-oxoadipate, which are all expressed during growth on 2,4-D.

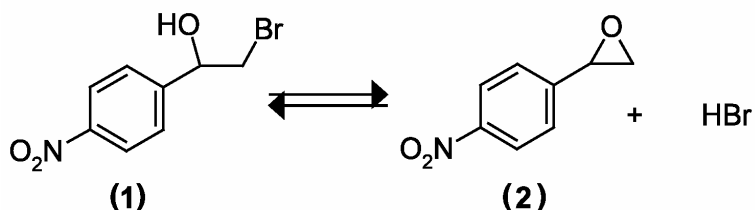
The study on the degradation of chloro-methyl-catechols elucidated different pathways of degradation for different isomers of chloromethylcatechols, occurring as metabolites during the degradation of dichlorotoluenes by *Ralstonia* sp. strain PS12 (39).

The last of the miscellaneous studies investigated the degradation of four isomers of HCH ( $\alpha$ ,  $\beta$ ,  $\delta$ ,  $\gamma$ ) by the bacterium, *Pandoraea* sp. (44). The  $\alpha$ -HCH and  $\beta$ -HCH isomers were degraded by 42% after four weeks in liquid culture; whereas,  $\gamma$ -HCH and  $\delta$ -HCH were degraded by 34 and 79%, respectively.  $\alpha$ -HCH and  $\gamma$ -HCH degradation could be improved with a culture pH of 8 (60-67% degradation). In soil slurries these compounds were degraded by 51-54% and the extent of degradation decreases with increased ratios of water to soil.

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

In this quarter, one article reported on the *in vitro* degradation of a halogenated compound by enzymes. A new method has been proposed for detecting halohydrin dehalogenase (46). This enzyme is responsible for the reversible formation of epoxides from vicinal halohydrins. Halohydrin dehalogenases are important enzymes in the degradation of chloroalcohol intermediates in the pathway of the aerobic degradation of chlorinated alkanes, such as 1,2-DCA. Also the reaction is important for the enantioselective enrichment of halohydrin isomers. The new method proposes the use of the halohydrin, *p*-nitro-2-bromo-1-phenylethanol as an enzyme assay substrate based on differences in absorption with the epoxide product, *p*-nitrostyrene oxide (see Figure 2). The time course of the absorption curve can also be used to determine the enantioselectivity of the reaction. For example one of the halohydrin dehalogenases tested, originating from *Agrobacterium radiobacter*, was shown to have enantioselective dehalogenation of *p*-nitro-2-bromo-1-phenylethanol. Since one of the isomers

was more rapidly degraded, this causes a biphasic absorption time-course graph with a plateau corresponding to the slowly degraded isomer.



**Figure 2.** New substrate for assaying activity of halohydrin dehalogenase based on difference in absorption spectrum of halohydrin, *p*-nitro-2-bromo-1-phenylethanol (46).

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

#### New Methods for Compound Detection

An abstract was presented that summarizes recent developments in the use of isotopic ratios to determine the progress of organochlorine pollutant degradation in the environment (15). Kinetic isotope effects have been observed for carbon during the anaerobic dechlorination of chlorinated ethenes as well as the aerobic cooxidation of 1,2-dichloroethane. These processes degrade <sup>13</sup>C containing pollutants more slowly than their <sup>12</sup>C counterparts, enriching the residuals with <sup>13</sup>C and producing degradation products enriched with <sup>12</sup>C. Isotope ratios can potentially be used to monitor degradation.

#### Characterization of Microbial Populations

One publication used real time polymerase chain reaction (RTm-PCR) to monitor the survival of a recombinant bacterium, *Rhodococcus* sp. strain RHA added to soil to improve PCB degradation (41). Two sets of primers were used, one which was specific for a region of the 16s RNA gene that is unique to *Rhodococcus* and close relatives. The other set was specific for the recombinant gene, which codes for a 4-chlorobenzoic acid operon (*frb*). RTm-PCR enabled monitoring cells in the range of 10<sup>2</sup> to 10<sup>7</sup> cells/ml. The results demonstrated that RTm-PCR values corresponded to colony forming units of *Rhodococcus* sp. strain RHA.

### Gene screening

One study found a haloalkane dehalogenase genes among organisms not typically associated with organochlorine compound degradation (19). Haloalkane dehalogenases are microbial enzymes that catalyze cleavage of the carbon-halogen bond by a hydrolytic mechanism. The haloalkane dehalogenase gene *dhmA* was cloned from *Mycobacterium avium* subsp. *avium* N85, a pathological bacterium in swine. The gene codes for haloalkane dehalogenase protein, DhmA, well known for its ability to catalyze the pollutant, 1,2-DCA. The study confirms that a hydrolytic dehalogenase is present in a pathogen *M. avium*. The presence of dehalogenase-like genes in the genomes of other mycobacteria, including the obligate pathogens *Mycobacterium tuberculosis* and *Mycobacterium bovis*, as well as in other bacterial species, including *Mesorhizobium loti*, *Xylella fastidiosa*, *Photobacterium profundum*, and *Caulobacter crescentus*, leads to the speculation that haloalkane dehalogenases have some other physiological function besides catalysis of hydrolytic dehalogenation of halogenated substances.

### 3.e. New Techniques for Bioremediation Technology

In this quarter several new bioremediation techniques and tools are discussed in relation to chlorinated priority pollutants. The first technique is anaerobic bioventing (ABV), which is a method to treat polychlorinated pollutants (*e.g.* PCE) in the unsaturated vadose zone. Since the vadose zone typically contains air, oxygen needs to be displaced by alternative gases (*e.g.* N<sub>2</sub>) to promote anaerobic activity. This quarter one article reports on oxygen displacement experiments conducted at the pilot scale (29). Numerical simulations were used to predict the collected experimental data. In general, reasonable agreement was found between observed and predicted oxygen concentrations. Use of impervious covers was observed to significantly reduce the volume of displacing gas used.

Another study proposes a technique to inject hydrogen into a PCE-contaminated aquifer as an e-donor in order to promote the reductive dechlorination. The method proposed is based on using hollow fiber membrane to facilitate passive diffusion of H<sub>2</sub> into the groundwater (11). The research evaluates the gas transfer behavior of hollow-fiber membranes under conditions typical of groundwater flow and assesses the effect of membrane configuration on gas transfer performance.

Two other articles evaluated and modeled the performance of a field-scale aerobic TCE bioremediation project at Edwards Air Force base (California). The project utilized toluene as

the cosubstrate to support TCE oxidation. One study analyzes the hydraulics flow patterns between extraction and injection wells that are situated in such a way to provide a "conveyer belt" flow pattern between a lower and upper aquifer separated by an aquitard (12). Bromide was used a tracer to verify several flow models. The second article (part 2) presents a model that predicts the degradation of TCE and forecasts the expansion of treated zone in the aquifer (13).

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## 4. MICROBIAL CHLORINATION

### 4.a. General Reviews

This quarter two review articles have been written on the natural production of halomethanes. The first review is an extensive review on the terrestrial sources of chloroform (CF) (22). The review first explains why CF is a compound of concern due to its impact on stratospheric and tropospheric ozone layers as well as its toxicity in groundwater. The article reviews the main origins of chloroform in the terrestrial environment, which can be anthropogenic point sources, atmospheric deposition, release by vegetation and production directly in the soil. CF production in soil is the most important terrestrial source (see Table 1). The calculated annual biogenic global chloroform emission is 700 Gg, and marine and terrestrial environments are nearly equal contributors. It is noteworthy that the estimated emissions from anthropogenic sources account for less than 10% of the estimated total emissions from all sources.

The second article reviews the mechanisms of natural production of halomethanes by plants, algae and fungi (28). The authors point out that plants only produce methyl halides; whereas fungi and algae also produce polyhalomethanes (editors note: actually there are examples of CF production by plants, *e.g. Brassica*). Polyhalomethanes are by-products of halogenation of certain organic compounds by haloperoxidases in marine algae and perhaps some fungi and they may be indirectly produced in aquatic environments by algal release of oxidized halogen species. It has been proposed that methyl halide production may provide a mechanism to regulate chloride levels in halotolerant plants. In algae, plants and some fungi, methyl halides may be a result of the insertion of ubiquitous halides into the active site of numerous methyl-transferases. The authors direct the discussion to viewing halomethanes as by-products or “*accidents*” of metabolism.

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

#### Chloromethanes

One study reports on the natural formation of chloromethane and bromomethane in salt marshes of Southern California (40). The study is extremely extensive and a series of evidences are laid out pointing to the predominant role of vegetation in the salt marshes as the main source of chloromethane and bromomethane emissions from the marsh. The main line of evidence is the correlation of the emissions with certain plant types, the diurnal pattern of the emissions and the lack of any correlation to other hypothesized biogenic sources. Also diurnal

variations in the carbon isotope compositions of CH<sub>3</sub>Cl and CH<sub>3</sub>Br and their relative ratios of emissions are consistent with simultaneously competing mechanisms of uptake and production. The authors conclude that salt marshes are globally important sources of these halomethanes despite their limited land area.

**Table 2.** Biogenic and anthropogenic sources and sinks of chloroform (22).

	annual average emission [Gg]	range [Gg]	reference
<b>source – biogenic</b>			
macroalgae <sup>a</sup>	0.84	0.009–3.1	Nightingale et al. 1995
microalgae <sup>b</sup>	23	7.9–49	Scarratt & Moore 1999
oceanic flux <sup>c</sup>	340		Khalil et al. 1999
forests <sup>d</sup>	4.9	0.4–24	Haselmann et al. 2000
soil <sup>e</sup>	200	100–400	Khalil et al. 1999
termites	100	10–100	Khalil et al. 1990b
rice fields <sup>f</sup>	23	7.7–50	Khalil et al. 1998
peatland ecosystems	4.7	0.1–150	Dimmer et al. 2001
<b>source – anthropogenic</b>			
pulp & paper manufacturing	34	25–43	Aucott et al. 1999
water treatment	22	7.9–35	Aucott et al. 1999
biomass burning	2		Lobert et al. 1999
biogas pits	0.000015	0.000006–0.00002	Khalil et al. 1990a
others	15	9–20	Aucott et al. 1999
<b>sink</b>			
chemical reactions with OH radicals	560		Keene et al. 1999
transport to stratosphere	2		Keene et al. 1999

<sup>a</sup>calculated with estimated global macroalgae biomass of  $2.8 \times 10^{14}$  g (Carpenter & Liss 2000).

<sup>b</sup>calculated with estimated global microalgae biomass of  $1.44 \times 10^{12}$  g chlorophyll *a* (calculated from data presented by Behrenfeld et al. 2001).

<sup>c</sup>oceanic flux minus emissions from micro and macroalgae.

<sup>d</sup>for Northern temperate forests.

<sup>e</sup>global land area excluding polar areas.

<sup>f</sup>calculated with estimated global rice field area of  $1.45 \times 10^{12}$  m<sup>2</sup> (Redeker et al. 2000).

### Other Chlorinated Compounds

Measurements of many different types of volatile halogenated organic compounds (VHOC) were conducted in European estuaries (7). The compounds tested were chloromethanes, bromomethanes, chloroethanes, chloroethenes, bromochloromethanes, and fluorochloromethanes. These compounds were identified and quantified, generally ranging from 0.1 ng/l to 350 ng/l. In some samples extraordinarily high values up to 4700 ng/l were observed indicating contribution from anthropogenic sources. Generally, concentrations of halogenated compounds of anthropogenic origin dominated those of prevalent natural origin. Only chloromethane and bromomethane fit a pattern expected from natural sources.

Chlorxanthomycin, a fluorescent, chlorinated, pentacyclic pyrene from a gram-positive *Bacillus* sp. was isolated from soil samples (27). The compound was found to have a molecular formula  $C_{22}H_{15}O_6Cl$  and a molecular weight of 409.8. Chlorxanthomycin appears to be located in the cytoplasm, does not diffuse out of the cells into the culture medium, and has selective antibiotic activity.

### Chlorinated Natural Organic Matter

An article published in *Science* reports on the occurrence of organic chlorine in natural organic matter based on measurements made with a new technique, *in situ* x-ray spectroscopy (31). The data indicate that natural organic matter in soils, sediments, and natural waters contain stable, less volatile organic compounds with chlorinated phenolic and aliphatic groups as the principal Cl forms. These compounds are formed at rapid rates from the transformation of inorganic Cl during humification of plant material and, thus, play a critical role in the cycling of Cl in the environment. The results of numerous data are plotted showing the proportion of inorganic chloride, aliphatic chloride and aromatic chloride in a continuum from fresh vegetation to decayed vegetation to mature soil humus (Figure 3). The results indicate that initially chlorine is predominantly present as inorganic chloride and in mature humus it is predominantly present as aromatic chloride. The aliphatic chloride occurs during decay.

An abstract is presented reporting on the measurements of organically bound chlorine in remote pristine peat bogs in the Magellanic Moorlands of Chile (20). Organochlorine content in the peat up to 0.2% dry weight was measured. This data combined with measurements from Canadian and European peat bogs was used to estimate that from 300 to 1100 million tons of organically bound chlorine is stored in the Earth's peatlands.

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

##### Chloromethanes

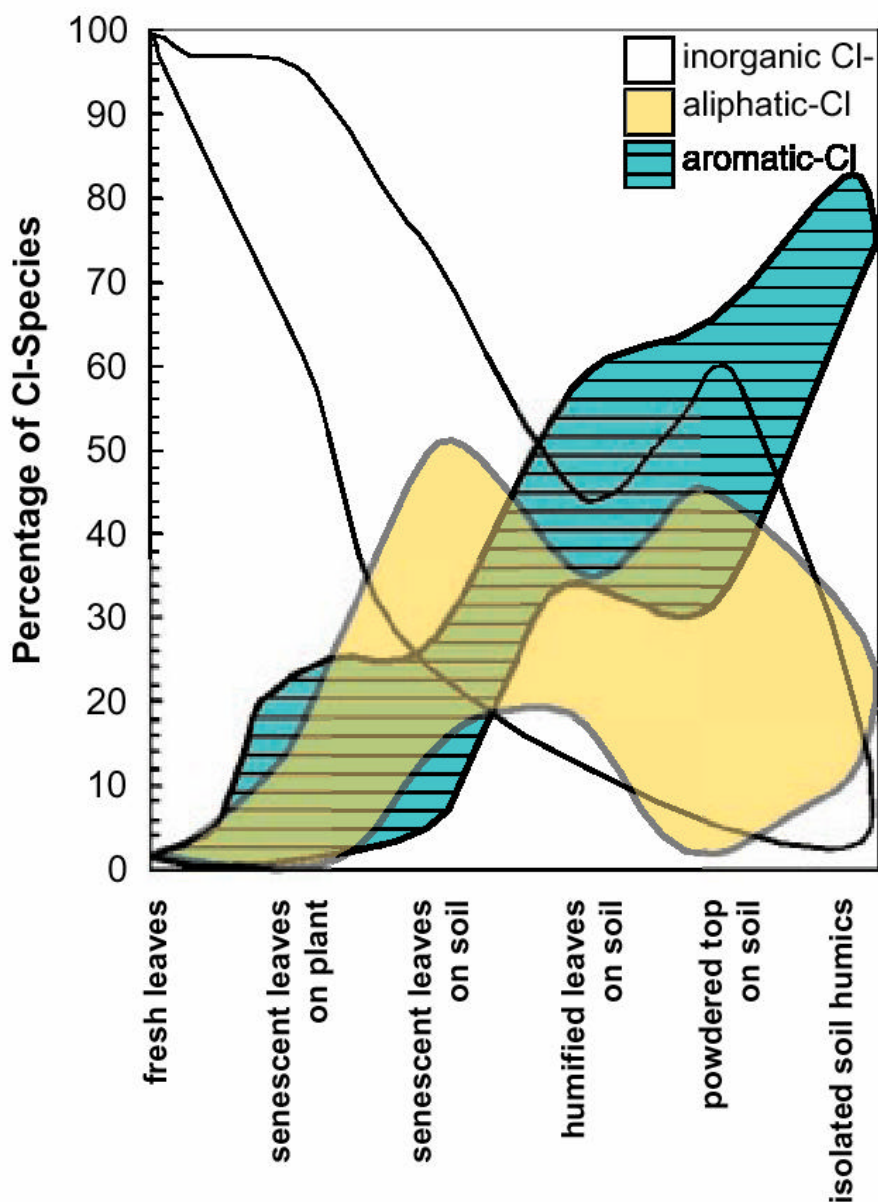
No reports concerning the formation of chloromethanes by marine and freshwater microorganisms were found during the review period.

##### Other Chlorinated Compounds

Two different studies report on the bioaccumulation of brominated organics, which are hypothesized to be naturally produced, in the blubber of marine mammals (47, 50). Four halogenated dimethyl bipyrroles (HDBPs) were quantitated in marine mammal blubber from a number of species obtained from various locations worldwide (47). HDBPs were found in samples from all locations studied. Concentrations of total HDBPs (SigmaHDBPs) ranged from 0.4 ng/g lipid weight in ringed seals (*Phoca hispida*) from the White Sea to 2,540 ng/g lipid weight in Dall's porpoise (*Phocoenoides dalli*) from the northwestern North Pacific Ocean. At their highest levels, SHDBPs made up 11% of the total quantitated organohalogen body burden of adult male Dall's porpoises. The geographical distribution of concentrations did not resemble that of the ubiquitous anthropogenic organohalogen, polychlorinated biphenyl congener CB-153. Additional results in this study also indicated that HDBPs and anthropogenic organohalogens have different sources and support the natural production hypothesis.

Several unknown, brominated compounds (BCs), which were recently detected in the blubber of dolphins and other marine mammals from Queensland (northeast Australia) (49), have been shown to be identical with natural BCs previously isolated from sponges (*Dysidea* sp.) living in the same habitat (50). In the latter study, isolates from sponges and mollusks (*Asteronotus cespitosus*) were compared with the signals detected in the mammals' tissue. Chromatographic results indicated that two compounds previously detected in blubber, BC-2 and BC-11, are identical to the sponge components, 4,6-dibromo-2-(2'-dibromo)-phenoxyanisole and 3,5-dibromo-2-(3',5'-dibromo-2'-methoxy)phenoxyanisole, respectively. Reported concentrations of the sponge halogenated natural products found in the marine mammals are frequently >1 mg/kg. These results show that marine biota can produce persistent organohalogen chemicals that accumulate to substantial concentrations in higher trophic organisms.

Volatile chlorinated compounds were isolated and identified from two tunicates, *Styela* sp. and *Phallusia* sp., occurring in the Eastern Mediterranean (phenols in *Styela* sp. and hydrocarbons in *Phallusia* sp.) (45).



**Figure 3.** Variations in inorganic Cl and organo-Cl compounds with humification of plant material in soils. The concentration of each chemical species is shown as a region, with its center and edges for any given sample corresponding to the average and the range of concentrations, respectively. The wide variations in the concentration ranges of different types of samples may be caused by the variations in the chlorination processes and related fauna and flora, and the extent of humification.

Two new minor polybrominated dibenzo-*p*-dioxins, spongiadioxin C (1) and its methyl ether (2), were isolated from an Australian marine sponge, together with the known minor metabolites methyl ethers of spongladioxins A (4) and B (6) and polybrominated diphenyl ethers (7-9) (Figure 4) (48). The structures of the new compounds were established by NMR spectroscopy and confirmed by synthesis. All isolated compounds inhibited the cell division of fertilized sea urchin eggs.

#### 4.d. Chlorinating Enzymes

In this quarter six articles report on a halogenating enzymes. Two articles deal with myeloperoxidase of human phagocytes. The first just confirms that HOCl formed from the reaction of Cl<sup>-</sup> with H<sub>2</sub>O<sub>2</sub> catalyzed by the enzymes is responsible for the antimicrobial activity of myeloperoxidase (30). The second article demonstrates that myeloperoxidases can chlorinate bacterial proteins, by analyzing for the formation of chlorotyrosine (42). The article also points out the nitrate is not used as a neutrophil since nitrated proteins could not be demonstrated when myeloperoxidase was incubated with nitrate.

Two articles report on the heterologous expression of vanadium-dependent bromoperoxidases of the macro alga *Corallina*. One study successfully expressed the recombinant vanadium-dependent bromoperoxidase gene in the yeast *Saccharomyces cerevisiae* (34). The other study successfully expressed in the bacterium *Escherichia coli* (5).

One article reports on the stability of cross-linked crystals of chloroperoxidase from *Caldariomyces fumago* (3). These catalytic crystals are more thermostable than the unmodified soluble enzyme. The enhanced stability is probably due to the structure conservation in the crystalline matrix. In addition, non-cross-linked chloroperoxidase crystals retained more activity than the soluble enzyme after incubation in an organic solvent with low water content.

The last article describes a set of PCR primers designed for locating FADH<sub>2</sub>-dependent halogenase genes in the genus *Streptomyces* (37). A halogenase gene was localized in *Streptomyces venezuelae* ISP5230, a known chloramphenicol producer. The primers were also used to localize the halogenase gene in several other *Streptomyces*.

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**ANNEX****Ahn, M. Y., J. Dec, et al. (2002). "Treatment of 2,4-Dichlorophenol polluted soil with free and immobilized laccase." *Journal of Environmental Quality* 31(5): 1509-1515.**

Enzyme treatment is currently considered for remediation of terrestrial systems polluted with organic compounds. In this study, two soils from Pennsylvania with 2.8 or 7.4% organic matter contents (Soils 1 and 2, respectively) were amended with C-14-labeled 2,4-dichlorophenol (2,4-DCP) and incubated with a laccase from *Trametes villosa* (free or immobilized on montmorillonite). 2,4-DCP was either transformed to methanol-soluble polymeric products (11-32%) or covalently bound to soil organic matter (53-85%), unaltered 2,4-DCP could be recovered from soil by methanol extraction (0-38%) at the completion of a 14-d incubation period. In Soil 1, both free and immobilized laccase removed 100% of 2,4-DCP without regard for moisture conditions. In Soil 2, immobilized laccase removed more 2,4-DCP (about 95%, regardless of moisture conditions) than free enzyme (55, 75, and 90% at 30, 55, and 100% of maximum water-holding capacity, respectively). Binding of 2,4-DCP in the humin fraction was nearly the same for free and immobilized laccase. More 2,4-DCP, however, was bound to humic and fulvic acids in the presence of immobilized laccase than in the presence of free laccase. In general, immobilized laccase performed better than free laccase. However, for practical applications, the higher activity of immobilized laccase is offset by a 23% loss in enzyme activity during immobilization, which approximates the 30% increase in free laccase needed to achieve the same level of remediation. Furthermore, immobilized laccase is more costly than free *T. villosa* laccase.

**Auman, A. J. and M. E. Lidstrom (2002). "Analysis of sMMO-containing Type I methanotrophs in Lake Washington sediment." *Environmental Microbiology* 4(9): 517-524.**

Methane-oxidizing bacteria (methanotrophs) containing soluble methane monooxygenase (sMMO) are of interest in natural environments due to the high co-metabolic activity of this enzyme with contaminants such as trichloroethylene. We have analysed sMMO-containing methanotrophs in sediment from a freshwater lake. Environmental clone banks for a gene encoding a diagnostic sMMO subunit (mmoX) were generated using DNA extracted from Lake Washington sediment and subjected to RFLP analysis. Representatives from the six RFLP groups were cloned and sequenced, and all were found to group with Type I *Methylomonas* mmoX, although a majority were divergent from known *Methylomonas* mmoX sequences. Direct hybridization of Lake Washington sediment DNA was carried out using a series of sMMO- and *Methylomonas*-specific probes to assess the significance of these sMMO-containing *Methylomonas*-like strains in the sediment. The total sMMO-containing population and the sMMO-containing *Methylomonas*-like population were estimated to be similar to previous estimates for total methanotrophs and Type I methanotrophs. These results suggest that the major methanotrophic population in Lake Washington sediment consists of sMMO-containing *Methylomonas*-like (Type I) methanotrophs. The whole-cell TCE degradation kinetics of such a strain, LW15, isolated from this environment, were determined and found to be similar to values reported for other sMMO-containing methanotrophs. The numerical significance of sMMO-containing *Methylomonas*-like methanotrophs in a mesotrophic lake environment suggests that these methanotrophs may play an important role in methanotroph-mediated transformations, including co-metabolism of halogenated solvents, in natural environments.

**Ayala, M., E. Horjales, et al. (2002). "Cross-linked crystals of chloroperoxidase." *Biochemical and Biophysical Research Communications* 295(4): 828-831.**

Chloroperoxidase from *Caldariomyces fumago* was crystallized. The crystals were modified with several cross-linkers, but only glutaraldehyde was able to produce catalytically active and insoluble crystals. Unlike other immobilized chloroperoxidase preparations, these catalytic crystals are more thermostable than the unmodified soluble enzyme. The enhanced stability is probably due to the structure conservation in the crystalline matrix. In addition, non-cross-linked chloroperoxidase crystals retained more activity than the soluble enzyme after incubation in an organic solvent with low water content. Although the cross-linked crystals were catalytically active, they showed lower specific activity than the soluble enzyme. This low activity may be due to non-specific

reactions between the cross-linker and essential residues for catalysis, Alternative cross-linking strategies are discussed. (C) 2002 Elsevier Science.

**Beeman, R. E. and C. A. Bleckmann (2002). "Sequential anaerobic-aerobic treatment of an aquifer contaminated by halogenated organics: field results." *Journal of Contaminant Hydrology* 57(3-4): 147-159.**

In situ, sequential, anaerobic to aerobic treatment of groundwater removed perchloroethene (PCE, 1.1  $\mu\text{M}$ ) and benzene (0.8  $\mu\text{M}$ ) from a contaminated aquifer. Neither aerobic nor anaerobic treatment alone successfully degraded both the chlorinated and non-chlorinated organic contaminants in the aquifer. After the sequential treatment, PCE, trichloroethene (TCE), vinyl chloride (VC), chloroethane (CA), and benzene were not detectable in groundwater. Desorption of residual aquifer contaminants was tested by halting the groundwater recirculation and analyzing the groundwater after 3 and 7 weeks. No desorption of the chlorinated contaminants or daughter products was observed in the treated portion of the aquifer. Sequential anaerobic to aerobic treatment was successful in remediating the groundwater at this test site and may have broad applications at other contaminated sites. Over the 4-year course of the project, the predominant microbial environment of the test site varied from aerobic to sulfate-reducing, to methanogenic, and back to aerobic conditions. Metabolically active microbial populations developed under all conditions, demonstrating the diversity and robustness of natural microbial flora in the aquifer. (C) 2002 Elsevier Science B.V. All rights reserved.

**Carter, J. N., K. E. Beatty, et al. (2002). "Reactivity of recombinant and mutant vanadium bromoperoxidase from the red alga *Corallina officinalis*." *Journal of Inorganic Biochemistry* 91(1): 59-69.**

Vanadium bromoperoxidase (VBPO) from the marine red alga *Corallina officinalis* has been cloned and heterologously expressed in *Escherichia coli*. The sequence for the full-length cDNA of VBPO from *C. officinalis* is reported. Steady state kinetic analyses of monochloroelmedone bromination reveals the recombinant enzyme behaves similarly to native VBPO from the alga. The kinetic parameters ( $K_m(\text{Br}^-) = 1.2 \text{ mM}$ ,  $K_m(\text{H}_2\text{O}_2) = 17.0 \text{ }\mu\text{M}$ ) at the optimal pH 6.5 for recombinant VBPO are similar to reported values for enzyme purified from the alga. The first site-directed mutagenesis experiment on VBPO is reported. Mutation of a conserved active site histidine residue to alanine (H480A) results in the loss of the ability to efficiently oxidize bromide, but retains the ability to oxidize iodide. Kinetic parameters ( $K_m(\text{I}^-) = 33 \text{ mM}$ ,  $K_m(\text{H}_2\text{O}_2) = 200 \text{ }\mu\text{M}$ ) for iodoperoxidase activity were determined for mutant H480A. The presence of conserved consensus sequences for the active sites of VBPO from marine sources shows its usefulness in obtaining recombinant forms of VBPO. Furthermore, mutagenesis of the conserved extra-histidine residue shows the importance of this residue in the oxidation of halides by hydrogen peroxide. (C). 2002 Elsevier Science Inc. All rights reserved.

**Cho, Y. C., R. C. Sokol, et al. (2002). "Kinetics of polychlorinated biphenyl dechlorination by Hudson River, New York, USA, sediment microorganisms." *Environmental Toxicology and Chemistry* 21(4): 715-719.**

The kinetics of polychlorinated biphenyl (PCB) dechlorination by Hudson River (New York, USA) sediment microorganisms were investigated using Aroclor 1242 at 10 concentrations ranging from 0 to 900 ppm (0-11.2  $\mu\text{mol Cl/g}$  sediment). The time course of PCB dechlorination and population growth were determined by congener-specific analysis and the most-probable-number technique, respectively, over a 44-week incubation period. Dechlorination rate ( $\text{nmol Cl removed/g sediment/d}$ ) was a linear function of PCB concentrations similar to the dechlorination of Aroclor 1248 by sediment microorganisms from the St. Lawrence River (New York, USA). However, the rate was much slower, with the linear slope being only 24% that of the St. Lawrence River. The threshold concentration below which no dechlorination occurs was (mean  $\pm$  standard deviation)  $1.06 \pm 0.18 \text{ }\mu\text{mol Cl/g sediment}$  ( $85 \pm 14 \text{ ppm}$ ), threefold higher than that for the dechlorination of Aroclor 1248. The maximum extent of dechlorination was greater at higher Aroclor concentrations. Dechlorinating microorganisms did not show any significant growth until late in the lag phase of dechlorination, and their maximum was greater at higher initial Aroclor 1242 concentrations. Although dechlorination rates were significantly lower with the Hudson River inoculum, when normalized to the maximum number of dechlorinating organisms, they were not significantly different from those for Aroclor 1248 by St. Lawrence River microorganisms. These results further support the idea that PCB dechlorination is tightly linked to the growth of dechlorinating microorganisms.

**Christof, O., R. Seifert, et al. (2002). "Volatile halogenated organic compounds in European estuaries." *Biogeochemistry* 59(1-2): 143-160.**

Sources, sinks, and distribution patterns of volatile halogenated organic compounds (VHOC) in estuaries were investigated during 5 cruises within the BIOGEST programme. Due to their chemical and physical properties (e.g. toxicity, persistence, mobility) these compounds are of considerable environmental concern. A wide range of compounds has been identified and quantified generally ranging from 0.1 ng l<sup>-1</sup> to 350 ng l<sup>-1</sup>. In some samples extraordinarily high values up to 4700 ng l<sup>-1</sup> were observed indicating contribution from anthropogenic sources. Generally, concentrations of halogenated compounds of anthropogenic origin dominated those of prevalent natural origin. Data of selected VHOC are presented in relation to salinity, particular organic carbon, and total suspended matter. Furthermore the observed concentrations are compared with established water quality regulations. Distribution patterns of VHOC along the estuary indicated common sources for specific halogenated compounds. Decreasing concentrations of most VHOC along the estuary confirm that degassing to the atmosphere and dilution with sea water are the dominating processes controlling the fate of these compounds in estuaries.

**Den, W. and M. Pirbazari (2002). "Modeling and design of vapor-phase biofiltration for chlorinated volatile organic compounds." *Aiche Journal* 48(9): 2084-2103.**

A mathematical model was developed for biofilter design and performance prediction with reference to the purification of contaminated gas streams. The model incorporated important aspects such as mass transfer, biodegradation, and adsorption processes. A systematic modeling protocol incorporated the development of a scale-up strategy based on dimensional analysis and similitude. Trichloroethylene (TCE) was employed as the model contaminant for biofiltration testing and model verification. The biokinetic and adsorption parameters for the contaminant were determined independently from a series of minibiofilter and miniadsorber column experiments, specifically designed to simulate the actual biofilter operational regimes in a miniature scale. Bench-scale biofilter experiments employing granular activated carbon columns indicated the good predictive capability of the model for the removal of TCE. Dynamic simulation studies were performed to assess the transient- and steady-state behavior of the model under various operating conditions. Model sensitivity was studied to evaluate the influence of adsorption equilibrium, transport and biological parameters on the biofilter dynamics. The results demonstrated that the biofilter performance was greatly influenced by the Monod coefficients and the biofilm thickness.

**Dua, M., A. Singh, et al. (2002). "Biotechnology and bioremediation: successes and limitations." *Applied Microbiology and Biotechnology* 59(2-3): 143-152.**

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

**Duddleston, K. N., D. J. Arp, et al. (2002). "Biodegradation of monohalogenated alkanes by soil NH<sub>3</sub>-oxidizing bacteria." *Applied Microbiology and Biotechnology* 59(4-5): 535-539.**

Although cooxidative biodegradation of mono-halogenated hydrocarbons has been well studied in the model NH<sub>3</sub>-oxidizing bacterium, *Nitrosomonas europaea*, virtually no information exists about cooxidation of these compounds by native populations of NH<sub>3</sub>-oxidizing bacteria. To address this subject, nitrifying activity was stimulated to 125-400 nmol NO<sub>3</sub><sup>-</sup> produced g<sup>-1</sup> soil h<sup>-1</sup> by first incubating a Ca(OH)<sub>2</sub>-amended, silt loam soil (pH 7.0 +/- 0.2) at field capacity (270 g H<sub>2</sub>O kg<sup>-1</sup> soil) with 10 Enrol NH<sub>4</sub><sup>+</sup> g<sup>-1</sup> soil for 14 days, followed by another 10 days of incubation in a shaken slurry (2:1 water:soil, v/w) with periodic pH adjustment and maintenance of 10 mM NH<sub>4</sub><sup>+</sup>. These slurries actively degraded both methyl bromide (MeBr) and ethyl chloride (EtCl) at maximum rates of 20-30 nmol ml<sup>-1</sup> h<sup>-1</sup> that could be sustained for approximately 12 h. Although the MeBr degradation rates were linear for the first 10-12 h of incubation, they could not be sustained regardless of NH<sub>4</sub><sup>+</sup> level and declined to zero over 20 h of incubation. The transformation capacity of the slurry enrichments (similar to 1 μmol MeBr ml<sup>-1</sup> soil slurry) was similar to the value measured previously in cell suspensions of *N. europaea* with similar NH<sub>3</sub>-oxidizing activity. Several MeBr-degrading characteristics of the nitrifying enrichments were found to be similar to those documented in the literature for MeBr-degrading methanotrophs and facultatively methylotrophic bacteria.

**Fang, Y., R. M. Hozalski, et al. (2002). "Passive dissolution of hydrogen gas into groundwater using hollow-fiber membranes." *Water Research* 36(14): 3533-3542.**

A new hollow-fiber membrane remediation system has recently been developed to passively supply groundwater with dissolved hydrogen (H<sub>2</sub>) to stimulate the biodegradation of chlorinated solvents. Understanding the mass transfer behavior of membranes under conditions of creeping flow is critical for the design of such systems. Therefore, the objectives of this research were to evaluate the gas transfer behavior of hollow-fiber membranes under conditions typical of groundwater flow and to assess the effect of membrane configuration on gas transfer performance. Membrane gas transfer was evaluated using laboratory-scale glass columns operated at low flow velocities (8.6-12,973 cm/d). H<sub>2</sub> was supplied to the inside of the membrane fibers while water flowed on the outside and normal to the fibers (i.e. crossflow). Membrane configuration (single fiber and fabric) and membrane spacing for the fabric modules did not affect gas transfer performance. Therefore, the results from all of the experiments were combined to obtain the following dimensionless Sherwood number (Sh) correlation expressed as a function of Reynolds number (Re) and Schmidt number (Sc):  $Sh = 0.824Re^{0.39} Sc^{0.33}$  ( $0.0004 < Re < 0.6$ ). This correlation is useful for predicting the rate of transfer of any gas from clean membranes to flowing water at low Re. This correlation provides a basis for estimating the membrane surface area requirements for groundwater remediation as illustrated by a simple example. (C) 2002 Elsevier Science Ltd. All rights reserved.

**Gandhi, R. K., G. D. Hopkins, et al. (2002). "Full-scale demonstration of in situ cometabolic biodegradation of trichloroethylene in groundwater - 1. Dynamics of a recirculating well system." *Water Resources Research* 38(4): U196-U210.**

Recirculating well systems provide an engine for the in situ treatment of subsurface contaminants. Although numerous recirculating wells have been installed in the field, for such systems, there is a paucity of comprehensive monitoring data and models constrained to data appearing in the research literature. Here we present an extensive data set combined with detailed inverse and simulation analyses for a two-well groundwater recirculation system used for in situ bioremediation at Edwards Air Force Base in southern California. The "conveyor belt" flow system, which was established for in situ treatment of trichloroethylene (TCE) in two bioactive zones, was created by pumping water upward in one well and downward in another well, each well being screened in both the upper and lower aquifers. A bromide tracer test was conducted and extensively monitored for 60 days. Combined inverse analysis was conducted on hydraulic heads from 38 monitoring wells, 32 bromide concentration histories, and a constraint on the degree of recirculation that was based on TCE concentration data. Four different formulations involving alternative weighting schemes used in a nonlinear weighted least squares simulation-regression analysis were explored. The best formulation provided parameter estimates with tight bounds on estimated covariances, suggesting that the model provides a reasonable description of the hydrogeologic system. Our investigation indicates the geometry of the recirculation zone and the degree of recirculation under two different sets of

operating conditions. Surprisingly, our analysis suggests that the effects of aquifer heterogeneity are not significant at this site under the conditions of forced recirculation. Furthermore, anomalous flow through an open monitoring well created significant vertical short-circuiting between the generally insulated aquifers. Flow through this small open conduit was equivalent to as much as 33% of the flow through the pumping wells. Using the model as a guide, we treated the aquifer system and bioactive zones as an equivalent mixed reactor to develop simple expressions relating effluent concentrations to influent concentrations. We demonstrate how these expressions are useful in predicting the removal of TCE that had undergone in situ bioremediation in the recirculatory treatment well system. The finite element model developed in this work serves as the foundation for a reactive transport simulator that we developed to analyze bioremediation which occurred during a 444 day experiment [Gandhi et al., 2002].

**Gandhi, R. K., G. D. Hopkins, et al. (2002). "Full-scale demonstration of in situ cometabolic biodegradation of trichloroethylene in groundwater - 2. Comprehensive analysis of field data using reactive transport modeling." *Water Resources Research* 38(4): art. no.-1040.**

[1] We present an analysis of an extensively monitored full-scale field demonstration of in situ treatment of trichloroethylene (TCE) contamination by aerobic cometabolic biodegradation. The demonstration was conducted at Edwards Air Force Base in southern California. There are two TCE-contaminated aquifers at the site, separated from one another by a clay aquitard. The treatment system consisted of two recirculating wells located 10 m apart. Each well was screened in both of the contaminated aquifers. Toluene, oxygen, and hydrogen peroxide were added to the water in both wells. At one well, water was pumped from the upper aquifer to the lower aquifer. In the other well, pumping was from the lower to the upper aquifer. This resulted in a "conveyor belt" flow system with recirculation between the two aquifers. The treatment system was successfully operated for a 410 day period. We explore how well a finite element reactive transport model can describe the key processes in an engineered field system. Our model simulates TCE, toluene, oxygen, hydrogen peroxide, and microbial growth/death. Simulated processes include advective-dispersive transport, biodegradation, the inhibitory effect of hydrogen peroxide on biomass growth, and oxygen degassing. Several parameter values were fixed to laboratory values or values from previous modeling studies. The remaining six parameter values were obtained by calibrating the model to 7213 TCE concentration data and 6997 dissolved oxygen concentration data collected during the demonstration using a simulation-regression procedure. In this complex flow field involving reactive transport, TCE and dissolved oxygen concentration histories are matched very well by the calibrated model. Both simulated and observed toluene concentrations display similar high-frequency oscillations due to pulsed toluene injection approximately one half hour during each 8 hour period. Simulation results indicate that over the course of the demonstration, 6.9 kg of TCE was degraded and that in the upper aquifer a region 40 m wide extending 25 m down gradient of the treatment system was cleaned up to less than 100  $\mu\text{g L}^{-1}$  from initial concentrations of approximately 700  $\mu\text{g L}^{-1}$ . A smaller region was cleaned up to less than 30  $\mu\text{g L}^{-1}$ . Simulations indicate that the cleaned up area in the upper aquifer would continue to expand for as long as treatment was continued.

**He, J. Z., Y. Sung, et al. (2002). "Acetate versus hydrogen as direct electron donors to stimulate the microbial reductive dechlorination process at chloroethene-contaminated sites." *Environmental Science & Technology* 36(18): 3945-3952.**

A study to evaluate the dechlorination end points and the most promising electron donors to stimulate the reductive dechlorination process at the chloroethene-contaminated Bachman Road site in Oscoda, MI, was conducted. Aquifer materials were collected from inside the plume and used to establish microcosms under a variety of electron donor conditions using chlorinated ethenes as electron acceptors. All microcosms that received an electron donor showed dechlorination activity, but the end points depended on the sampling location, indicating a heterogeneous distribution of the dechlorinating populations in the aquifer. Interestingly, several microcosms that received acetate as the only electron donor completely dechlorinated PCE to ethene. All acetate-amended microcosms rapidly converted PCE to cis-DCE, whereas PCE dechlorination in H<sub>2</sub>-fed microcosms only occurred after a pronounced lag time and after acetate had accumulated by H<sub>2</sub>/CO<sub>2</sub> acetogenic activity. The microcosm experiments were corroborated by defined co-culture experiments, which demonstrated that H<sub>2</sub> sustained PCE to cis-DCE dechlorination by acetotrophic populations in the presence of H<sub>2</sub>/CO<sub>2</sub> acetogens. In sediment-free nonmethanogenic enrichment cultures derived from ethene-producing microcosms, acetate alone

supported complete reductive dechlorination of chloroethenes to ethene, although the addition of H<sub>2</sub> resulted in higher cisDCE and VC dechlorination rates. Measurements of H<sub>2</sub> production and consumption suggested that syntrophic acetate-oxidizing population(s) were active in the enrichment cultures. These findings demonstrated that either acetate or H<sub>2</sub> alone can be sufficient to promote complete reductive dechlorination to ethene, provided syntrophic acetate-oxidizing population(s) and H<sub>2</sub>/CO<sub>2</sub> acetogenic population(s) are present, respectively. Approaches that result in increased fluxes of both electron donors (e.g., by addition of fermentable substrates) seem most promising to sustain complete high rate reductive dechlorination to ethene in the contaminated zone of the Bachman aquifer, although acetate or H<sub>2</sub> alone may be sufficient to drive the dechlorination process to completion.

**Hunkeler, D. and R. Aravena (2002). "Use of stable isotopes to evaluate the fate of chlorinated hydrocarbons in the subsurface." *Geochimica Et Cosmochimica Acta* 66(15A): A348-A348.**

**Hunkeler, D., R. Aravena, et al. (2002). "Carbon isotopes as a tool to evaluate the origin and fate of vinyl chloride: Laboratory experiments and modeling of isotope evolution." *Environmental Science & Technology* 36(15): 3378-3384.**

Accumulation of vinyl chloride (VC) is often a main concern at sites contaminated with chlorinated ethenes and ethanes due to its high toxicity. Since there can be several possible sources of VC and ethene at such sites, assessing the origin and fate of VC can be complicated. Aim of this study was to evaluate carbon isotope fractionation associated with various anaerobic processes that lead to the production of VC and ethene in view of using isotopes to evaluate the origin and fate of these compounds in groundwater. Microcosms were constructed using sediments and groundwater from a contaminated site and amended with potential precursors for VC and ethene production. In the microcosms with dichloroethene isomers, sequential reductive dechlorination was observed, and isotopic enrichment factors of -19.9±1.5‰ for cis-1,2-dichloroethene -30.3±1.9‰ for trans-1,2-dichloroethene, and -7.3±0.4‰ for 1,1,1-dichloroethane were obtained. In microcosms with chlorinated ethanes, 1,2-dichloroethane (1,2-DCA) and 1,1,2-trichloroethane (1,1,2-TCA) were predominantly transformed by dichloroelimination to ethene and VC, respectively, and enrichment factors of -32.1±1.1‰ for 1,2-DCA and -2.0±0.2‰ for 1,1,2-TCA were observed. Except for 1,1,2-TCA, a strong C-13 enrichment in each of the potential precursor of VC was observed, which opens the possibility to trace the origin of VC based on the isotope ratio of potential precursors. Furthermore, it was possible to model the isotope evolution of VC present as substrate or intermediate product as a function of time. The study demonstrates that carbon isotope ratios can potentially be used for qualitative and possibly quantitative evaluation of the origin and fate of VC at sites with complex contaminant mixtures.

**Imamoglu, I. and E. R. Christensen (2002). "PCB sources, transformations, and contributions in recent Fox River, Wisconsin sediments determined from receptor modeling." *Water Research* 36(14): 3449-3462.**

The PCB contamination in lower Fox River sediments was investigated in order to identify possible PCB sources, contributions, and transformations, using two receptor models. Congener specific sediment PCB data from sites immediately upstream of DePere dam to Green Bay that had been gathered for the Green Bay/Fox River Mass Balance Study, were used in this analysis. The first receptor model is a self training factor analysis (FA) model with non-negative constraints that was applied to identify the PCB sources and significant congener patterns. The second is a chemical mass balance model (CMB) in which published Aroclor sources, inferred from our FA model, were used to apportion these Aroclors to each sample. The FA model indicated two significant factors, the major one being Aroclor 1242 and the other, a profile dominated by low chlorinated congeners, indicating a possible PCB alteration profile. This profile had significant contributions to samples at or around sites with total PCB concentrations higher than 50 ppm, indicating a potential anaerobic dechlorination activity. It was also deduced from the FA model that very small contributions of more highly chlorinated Aroclors may be present in the system. The results from the CMB model confirmed that the system is dominated by Aroclor 1242. Its average contribution was 95%, with small amounts of Aroclor 1254 (2%) and 1260 (1%). Two of the samples, located in the vicinity of point sources, showed high contributions of Aroclor 1016 by the CMB model. This is interpreted as an altered Aroclor profile resembling the less chlorinated Aroclor 1016. Contributions obtained from the CMB and FA models show similar patterns. (C) 2002 Elsevier Science Ltd. All rights reserved.

**Imamoglu, I., K. Li, et al. (2002). "PCB sources and degradation in sediments of Ashtabula River, Ohio, USA, determined from receptor models." *Water Science and Technology* 46(3): 89-96.**

The PCB pollution in Ashtabula River sediments was evaluated using a factor analysis (FA) model with non-negative constraints, and a chemical mass balance (CMB) model. The FA model identified Aroclor 1248 as the major PCB source, and also a congener pattern significantly different from that of any Aroclor. The CMB model that uses linear combinations of Aroclors, failed to reproduce the sample congener profiles with good statistical fit. The findings from both models indicate that the PCBs in Ashtabula River sediments have undergone significant alterations changing their profiles from those of the original sources. These alterations may be explained by the anaerobic dechlorination of highly chlorinated congeners, according to dechlorination activities H/H.

**Jesenska, A., M. Bartos, et al. (2002). "Cloning and expression of the haloalkane dehalogenase gene dhmA from *Mycobacterium avium* N85 and preliminary characterization of DhmA." *Applied and Environmental Microbiology* 68(8): 3724-3730.**

Haloalkane dehalogenases are microbial enzymes that catalyze cleavage of the carbon-halogen bond by a hydrolytic mechanism. Until recently, these enzymes have been isolated only from bacteria living in contaminated environments. In this report we describe cloning of the dehalogenase gene dhmA from *Mycobacterium avium* subsp. *avium* N85 isolated from swine mesenteric lymph nodes. The dhmA gene has a G+C content of 68.21% and codes for a polypeptide that is 301 amino acids long and has a calculated molecular mass of 34.7 kDa. The molecular masses of DhmA determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by gel permeation chromatography are 34.0 and 35.4 kDa, respectively. Many residues essential for the dehalogenation reaction are conserved in DhmA; the putative catalytic triad consists of Asp123, His279, and Asp250, and the putative oxyanion hole consists of Glu55 and Trp124. Trp124 should be involved in substrate binding and product (halide) stabilization, while the second halide-stabilizing residue cannot be identified from a comparison of the DhmA sequence with the sequences of three dehalogenases with known tertiary structures. The haloalkane dehalogenase DhmA shows broad substrate specificity and good activity, with the priority pollutant 1,2-dichloroethane. DhmA is significantly less stable than other currently known haloalkane dehalogenases. This study confirms that a hydrolytic dehalogenase is present in the facultative pathogen *M. avium*. The presence of dehalogenase-like genes in the genomes of other mycobacteria, including the obligate pathogens *Mycobacterium tuberculosis* and *Mycobacterium bovis*, as well as in other bacterial species, including *Mesorhizobium loti*, *Xylella fastidiosa*, *Photobacterium profundum*, and *Caulobacter crescentus*, led us to speculate that haloalkane dehalogenases have some other function besides catalysis of hydrolytic dehalogenation of halogenated substances.

**Keppler, F., H. Biester, et al. (2002). "Natural enrichment of organic halogens during peat formation." *Geochimica Et Cosmochimica Acta* 66(15A): A395-A395.**

**Kim, Y., D. J. Arp, et al. (2002). "Kinetic and inhibition studies for the aerobic cometabolism of 1,1,1-trichloroethane, 1,1-dichloroethylene, and 1,1-dichloroethane by a butane-grown mixed culture." *Biotechnology and Bioengineering* 80(5): 498-508.**

Batch kinetic and inhibition studies were performed for the aerobic cometabolism of 1,1,1-trichloroethane (1,1,1-TCA), 1,1-dichloroethylene (1,1-DCE), and 1,1-dichloroethane (1,1-DCA) by a butane-grown mixed culture. These chlorinated aliphatic hydrocarbons (CAHs) are often found together as cocontaminants in groundwater. The maximum degradation rates ( $k(\max)$ ) and half-saturation coefficients ( $K_s$ ) were determined in single compound kinetic tests. The highest  $k(\max)$  was obtained for butane (2.6  $\mu\text{mol}/\text{mg TSS}/\text{h}$ ) followed by 1,1-DCE (1.3  $\mu\text{mol}/\text{mg TSS}/\text{h}$ ), 1,1-DCA (0.49  $\mu\text{mol}/\text{mg TSS}/\text{h}$ ), and 1,1,1-TCA (0.19  $\mu\text{mol}/\text{mg TSS}/\text{h}$ ), while the order of  $K_s$  from the highest to lowest was 1,1-DCA (19  $\mu\text{M}$ ), butane (19  $\mu\text{M}$ ), 1,1,1-TCA (12  $\mu\text{M}$ ) and 1,1-DCE (1.5  $\mu\text{M}$ ). The inhibition types were determined using direct linear plots, while inhibition coefficients ( $K_{ic}$  and  $K_{iu}$ ) were estimated by nonlinear least squares regression (NLSR) fits to the kinetic model of the identified inhibition type. Two different inhibition types were observed among the compounds. Competitive inhibition among CAHs was indicated from direct linear plots, and the CAHs also competitively inhibited butane utilization. 1,1-DCE was a stronger inhibitor than the other CAHs. Mixed inhibition of 1,1,1-TCA, 1,1-DCA, and 1,1-DCE transformations by butane was observed. Thus, both competitive and mixed inhibitions are important in

cometabolism of CAHs by this butane culture. For competitive inhibition between CAHs, the ratio of the K<sub>s</sub> values was a reasonable indicator of competitive inhibition observed. Butane was a strong inhibitor of CAH transformation, having a much lower inhibition coefficient than the K<sub>s</sub> value of butane, while the CAHs were weak inhibitors of butane utilization. Model simulations of reactor systems where both the growth substrate and the CAHs are present indicate that reactor performance is significantly affected by inhibition type and inhibition coefficients. Thus, determining inhibition type and measuring inhibition coefficients is important in designing CAH treatment systems. (C) 2002 Wiley Periodicals, Inc.

**Kuncova, G., J. Triska, et al. (2002). "The influence of immobilization of *Pseudomonas* sp 2 on optical detection of polychlorinated biphenyls." *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* 21(1-2): 195-201.**

The cells *Pseudomonas* sp. 2 were immobilized on porous glass surface (S), encapsulated into alginate beads (A), encapsulated into alginate beads coated with a silica layer (AC) and entrapped into silica gel (C). The silica layer thickness of 2.5 μm was prepared from prepolymerized tetramethoxysilane by dipping of the alginate beads. The production of stable yellow intermediates of biodegradation of PCBs, congeners with three chlorine atoms, was used for the detection of commercial mixture of PCBs, Delor 103. In the initial stage of biodegradation of Delor 103, the highest and the most rapid increase of concentration of yellow color was observed in the case of biodegradation with AC but the selectivity of the detection method was decreased by the simultaneous production of orange compounds. The necessary conditions for production of orange intermediates were concentration of biphenyl or PCBs with biphenyl 5 g/l together with 2-6 vol.% of methanol. Trihydroxyphenyl pyridine, phenyl pyridine, dimethylhydrazide of benzoic acid, pyridine carboxylic acid and trimethylindane were identified in the lyophilized solution of the orange compounds. The metabolic pathways of the creation of orange compounds are unknown and for that reason, utilization of these metabolites for optical detection of PCBs needs further research. (C) 2002 Elsevier Science B.V. All rights reserved.

**Laternus, F., K. F. Haselmann, et al. (2002). "Terrestrial natural sources of trichloromethane (chloroform, CHCl<sub>3</sub>) - An overview." *Biogeochemistry* 60(2): 121-139.**

The widespread use of volatile chlorinated compounds like chloroform, trichloroethene and tetrachloroethene in industrialized societies causes a large annual release of these compounds into the environment. Due to their role as a source for halogen radicals involved in various catalytic atmospheric reaction cycles, including the regulation of the stratospheric and tropospheric ozone layers, these compounds also constitute a risk for drinking water resources as they can be transported to the groundwater from contaminated field sites or even from atmospheric deposition. Therefore, identification and investigation of sources and sinks of volatile chlorinated compounds are of particular interest. Chloroform, a major contributor to natural gaseous chlorine, was found to be emitted by several anthropogenic and natural sources including the oceans and terrestrial areas. The origin of chloroform in the terrestrial environment can be anthropogenic point sources, atmospheric deposition, release by vegetation and production directly in the soil. The calculated annual biogenic global chloroform emission is 700 Gg, and marine and terrestrial environments are nearly equal contributors. The estimated emissions from anthropogenic sources account for less than 10% of the estimated total emissions from all sources. Among terrestrial sources, forests have recently been identified as contributing to the release of chloroform into the environment. With the data available, annual emissions of chloroform to the atmosphere from forest sites were calculated and compared to other natural sources. At present knowledge, forests are only a minor source in the total biogenic flux of chloroform, contributing less than 1% to the annual global atmospheric input. However, it should be noted that data are available for Northern temperate forests only. The large tropical forest areas may provide a yet unknown input of chloroform.

**Lee, S., W. M. Moe, et al. (2002). "Effect of sorption and desorption resistance on aerobic trichloroethylene biodegradation in soils." *Environmental Toxicology and Chemistry* 21(8): 1609-1617.**

Biodegradation of trichloroethylene (TCE) by toluene-degrading bacteria was measured under aerobic conditions in aqueous and soil-slurry batch microcosms. For soil-phase experiments, a freshly contaminated soil and a soil containing only the desorption-resistant fraction of TCE were tested. In both cases, presence of soil resulted in

biodegradation rates substantially lower than those determined in the absence of soil. In aqueous-phase experiments, an appreciable increase in the rate and extent of TCE biodegradation was observed in microcosms when toluene was added multiple times. The TCE degradation rates were clearly correlated with toluene dioxygenase (TOD) enzyme activity over time, thus providing an indication of the cometabolic pathway employed by the microbial population. In soil-slurry experiments containing freshly contaminated soil, a TCE degradation rate of approximately 150  $\mu\text{g TCE/kg/h}$  was observed during the first 39-h period, and then the TCE degradation rate slowed considerably to 0.59 and 0.84  $\mu\text{g TCE/kg/h}$  for microcosms receiving one and two additions of toluene, respectively. The TCE degradation rates in soil-slurry microcosms containing the desorption-resistance fraction of TCE-contaminated soil were approximately 0.27 and 0.32  $\mu\text{g TCE/kg/h}$  in microcosms receiving one and two additions of toluene, respectively. It is clear from these results that mass transfer into the aqueous phase limited bioavailability of TCE in the contaminated soil.

**Lee, T. H., M. Ike, et al. (2002). "A reactor system combining reductive dechlorination with cometabolic oxidation for complete degradation of tetrachloroethylene." *Journal of Environmental Sciences-China* 14(4): 445-450.**

A laboratory sequential anaerobic-aerobic bioreactor system, which consisted of an anaerobic fixed film reactor and two aerobic chemostats, was set up to degrade tetrachloroethylene (PCE) without accumulating highly toxic degradation intermediates. A soil enrichment culture, which could reductively dechlorinate 900  $\mu\text{M}$  (ca. 150  $\text{mg/L}$ ) of PCE stoichiometrically into cis-1,2-dichloroethylene (cis-DCE), was attached to ceramic media in the anaerobic fixed film reactor. A phenol degrading strain, *Alcaligenes* sp. R5, which can efficiently degrade cis-DCE by co-metabolic oxidation, was used as inoculum for the aerobic chemostats consisted of a transformation reactor and a growth reactor. The anaerobic fixed film bioreactor showed more than 99% of PCE transformation into cis-DCE in the range of influent PCE concentration from 5  $\mu\text{M}$  to 35  $\mu\text{M}$  at hydraulic retention time of 48h. On the other hand, efficient degradation of the resultant cis-DCE by strain R5 in the following aerobic system could not be achieved due to oxygen limitation. However, 54% of the maximum cis-DCE degradation was obtained when 10  $\mu\text{mol}$  of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was supplemented to the transformation reactor as an additional oxygen source. Further studies are needed to achieve more efficient co-metabolic degradation of cis-DCE in the aerobic reactor.

**Lee, W. S., C. S. Park, et al. (2002). "Characterization of TCE-degrading bacteria and their application to wastewater treatment." *Journal of Microbiology and Biotechnology* 12(4): 569-575.**

Two bacterial strains capable of degrading trichloroethylene (TCE), isolated from soils contaminated with various chlorinated alkenes, were identified as *Alcaligenes odorans* N6 and *Nocardia* sp. H17. In addition, four KCTC strains, including three strains of *Pseudomonas putida* and one strain of *Sphingomonas chlorophenolica*, exhibited an ability to degrade toluene. *A. odorans* N6 and *Nocardia* sp. H17 degraded 84% of the initial amount of TCE in a basal salts medium (BSM), containing 0.2  $\text{mM}$  TCE as the sole source of carbon and energy, in a day. The optimal pH for growth was within a range of 7.0-8.0. A mixed culture of the four toluene-degrading isolates degraded 95% of 0.2  $\text{mM}$  TCE with 1.5  $\text{mM}$  toluene as an inducer, whereas no TCE was degraded by the same mixture without an inducer. When a mixed culture of all 6 isolates was used, the degradation efficiency of 0.2  $\text{mM}$  TCE was 72% without an inducer, in a day, and 82% with toluene as an inducer. In a continuous treatment, 1,000  $\text{mg/l}$  of TCE in an artificial wastewater was completely removed within 18 h when an activated sludge was used along with the microbial mixture, which was 27 h faster than when only an activated sludge was used. Accordingly, it would appear that such a microbial mixture could be effectively applied to the biological treatment of wastewater containing TCE with or without an inducer.

**Luepromchai, E., A. C. Singer, et al. (2002). "Interactions of earthworms with indigenous and bioaugmented PCB-degrading bacteria." *Fems Microbiology Ecology* 41(3): 191-197.**

Partial bioremediation of polychlorinated biphenyl (PCB)-contaminated soils has been achieved using bioaugmentation with PCB-degrading bacteria and earthworms. To further study the contribution of earthworms to bioremediation, an experiment was conducted in which the changes in indigenous and bioaugmented PCB-

degrading bacteria were analyzed during treatment of contaminated soil using earthworms (*Pheretima hawayana*) alone or in combination with the PCB-degrading bacteria, *Ralstonia eutrophus* and *Rhodococcus* sp. ACS. Bacteria used for bioaugmentation were induced with carvone and salicylic acid in culture and were repeatedly applied every 3-4 days to the surface of unmixed, 20-cm long soil columns containing 100 ppm Aroclor 1242. After 9 weeks of treatment, the soil bacterial communities were analyzed using PCR primers for the *bph* genes. Results showed that approximately 50% of the PCBs were removed in the top 9 cm using a combination of earthworms and bioaugmentation, whereas bioaugmentation or earthworms applied alone were effective only for removing PCBs from the top 3 cm of the soil columns. Enhanced removal of PCBs caused by earthworms was associated with an increase in the population size of culturable, indigenous biphenyl-degrading bacteria, and an increase in the level of the *bphA* and *bphC* genes. The results suggest that earthworms facilitate PCB bioremediation by enhancing the dispersal of PCB-degrading bacteria in bioaugmented columns, as well as providing environmental conditions that favor the growth and activity of indigenous PCB-degrading bacteria. (C) 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Microbiological Societies.

**Magyarosy, A., J. Z. Ho, et al. (2002). "Chloroxanthomycin, a fluorescent, chlorinated, pentacyclic pyrene from a *Bacillus* sp." *Applied and Environmental Microbiology* 68(8): 4095-4101.**

A gram-positive *Bacillus* sp. that fluoresces yellow under long-wavelength UV light on several common culture media was isolated from soil samples. On the basis of carbon source utilization studies, fatty acid methyl ester analysis, and 16S ribosomal DNA analysis, this bacterium was most similar to *Bacillus megaterium*. Chemical extraction yielded a yellow-orange fluorescent pigment, which was characterized by X-ray crystallography, mass spectrometry, and nuclear magnetic resonance spectroscopy. The fluorescent compound, chloroxanthomycin, is a pentacyclic, chlorinated molecule with the molecular formula  $C_{22}H_{15}O_6Cl$  and a molecular weight of 409.7865. Chloroxanthomycin appears to be located in the cytoplasm, does not diffuse out of the cells into the culture medium, and has selective antibiotic activity.

**Manley, S. L. (2002). "Phytogenesis of halomethanes: A product of selection or a metabolic accident?" *Biogeochemistry* 60(2): 163-180.**

Phytoplankton (microalgae), seaweeds (macroalgae), higher plants and fungi produce halomethanes. Algae and fungi produce both methyl halides and polyhalomethanes, whereas plants are known to produce only methyl halides. Why these organisms produce halomethanes is a question frequently asked by chemists and biologists. This question implies that halomethanes have a function and have a selective value to the producing organism. Except for some fungi, the evolutionary advantage of producing halomethanes may not presently exist. Polyhalomethanes are by-products of halogenation of certain organic compounds by haloperoxidases in marine algae and perhaps some fungi, and they may be indirectly produced in aquatic environments by algal release of oxidized halogen species. A main function of this enzyme is to rid the cell of harmful oxidants such as hydrogen peroxide. Monohalomethanes (methyl halides) are products of methyltransferase activity. It has been proposed that methyl halide production may provide a mechanism to regulate chloride levels in halotolerant plants. The examination of halide cellular concentrations, halomethane production rates, and enzyme characteristics raises questions about this possible function. In algae, plants and some fungi, methyl halides may be a result of the insertion of ubiquitous halides into the active site of numerous methyltransferases. Therefore, halomethanes may be by-products or 'accidents' of metabolism.

**Milopoulos, P. G., M. T. Suidan, et al. (2002). "Numerical modeling of oxygen exclusion experiments of anaerobic bioventing." *Journal of Contaminant Hydrology* 58(3-4): 209-220.**

A numerical and experimental study of transport phenomena underlying anaerobic bioventing (ABV) is presented. Understanding oxygen exclusion patterns in vadose zone environments is important in designing an ABV process for bioremediation of soil contaminated with chlorinated solvents. In particular, the establishment of an anaerobic zone of influence by nitrogen injection in the vadose zone is investigated. Oxygen exclusion experiments are performed in a pilot scale flow cell (2 X 1.1 X 0.1 m) using different venting flows and two different outflow boundary conditions (open and partially covered). Injection gas velocities are varied from  $0.25 \times 10^{-3}$  to  $1.0 \times 10^{-3}$  cm/s and are correlated with the ABV radius of influence. Numerical simulations are used to predict the collected experimental data. In general, reasonable agreement is found between observed and predicted oxygen concentrations. Use of impervious covers can significantly reduce the volume of forcing gas used, where an

increase in oxygen exclusion efficiency is consistent with a decrease in the outflow area above the injection well.  
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**Murav'ev, R. A., P. G. But, et al. (2002). "The mechanism of bactericidal activity in phagosomes of neutrophils." *Biology Bulletin* 29(4): 356-359.**

Myeloperoxidase plays the key role in antimicrobial of phagocytes. This enzyme uses hydrogen peroxide and chloride to catalyze hypochlorous acid formation. HOCl is the most probable agent in the oxygen-dependent bactericidal activity in the phagocyte phagosome. Chlorination markers indicate HOCl generation in the quantities lethal for bacteria. Enzymatic assay for myeloperoxidase indicates proceeding of other reactions involved in bactericidal activity. Superoxide integrates many activities of this kind and is important for physiological function of myeloperoxidase. Elucidation of phagosomes biochemistry can help us to understand why certain pathogens survive in such unfavorable environment.

**Myneni, S. C. B. (2002). "Formation of stable chlorinated hydrocarbons in weathering plant material." *Science* 295(5557): 1039-1041.**

Though several chlorinated organic compounds produced by humans are carcinogenic and toxic, some are also produced by the biotic and abiotic processes in the environment. In situ x-ray spectroscopy data indicate that natural organic matter in soils, sediments, and natural waters contain stable, less volatile organic compounds with chlorinated phenolic and aliphatic groups as the principal Cl forms. These compounds are formed at rapid rates from the transformation of inorganic Cl during humification of plant material and, thus, play a critical role in the cycling of Cl and of several major and trace elements in the environment and may influence human health.

**Nonnenberg, C., W. A. van der Donk, et al. (2002). "Reductive dechlorination of trichloroethylene: A computational study." *Journal of Physical Chemistry A* 106(37): 8708-8715.**

Vitamin B-12 catalyzes the reductive dechlorination of several ubiquitous pollutants including the conversion of trichloroethylene (TCE) to similar to 95% cis-1,2-dichloroethylene (DCE) and small amounts of trans-DCE and 1,1-DCE. The origins of this unexpected selectivity were investigated using density functional and coupled-cluster theory. At all levels of theory considered, the initially formed trichloroethylene radical anion is an unstable species. Breakage of one of the three C-Cl bonds during the dissociative process gives the most stable ion complex when the two remaining chlorines occupy a cis geometry. Once formed, the cis-1,2-dichloroethen-1-yl radical is about 6 kJ/mol more stable than the corresponding trans radical and 21 kJ/mol more stable than the 1,1-dichloroethen-2-yl radical. The calculated relative energies can be rationalized by delocalization of the impaired electron over the nonbonding orbitals of the alpha-chlorine. The computed geometries of the radicals suggest significant interactions between the orbital occupied by the unpaired electron and the sigma\* orbital of the beta C-Cl bond trans to the radical. The barrier for interconversion of the two 1,2-dichlorinated vinyl radicals lies between similar to 30-40 kJ/mol depending on the level of theory. The reactivities of the three radicals with respect to hydrogen atom abstraction from methanol (C-H or O-H) as well as chlorine elimination were investigated. All three radicals show a strong preference for abstraction of the alpha-hydrogen atom of methanol (17-25 kJ/mol), with a significant positive reaction energy for chlorine elimination (6080 kJ/mol). These results are discussed further in relation to the experimentally observed product distribution.

**Novakova, H., M. Vosahlikova, et al. (2002). "PCB metabolism by *Pseudomonas* sp. P2." *International Biodeterioration & Biodegradation* 50(1): 47-54.**

The degradation of Delor 103, a mixture of polychlorinated biphenyl (PCB) congeners, by *Pseudomonas* sp. P2, an indigenous soil bacterium was studied. In mineral medium with biphenyl as sole carbon source the extent of PCB degradation monitored by GC exceeded 70%. The efficiency of Delor 103 degradation by strain P2 was compared with that of *Ralstonia eutropha* H850. The addition of saccharose or agar improved PCB degradation, whereas the addition of glycerol or pyruvate substantially reduced the degradation efficiency. The presence of an amino acid mixture enhanced PCB degradation. The following chlorobenzoic acids (CBA): 2,5 CBA, 2,4 CBA, 4 CBA, 2 CBA and 2,5-diCBA were detected as metabolites of Delor 103, with 2,5-diCBA as the major product. Although

2,5-diCBA inhibited the growth of *Pseudomonas* sp, P2 the degradation of 2,5-diCBA was unaffected for upto 14 days. (C) 2002 Elsevier Science Ltd. All rights reserved.

**Ohshiro, T., W. Hemrika, et al. (2002). "Expression of the vanadium-dependent bromoperoxidase gene from a marine macro-alga *Corallina pilulifera* in *Saccharomyces cerevisiae* and characterization of the recombinant enzyme." *Phytochemistry* 60(6): 595-601.**

The vanadium-dependent bromoperoxidase from the marine macro- alga *Corallina pilulifera* was heterologously expressed in *Saccharomyces cerevisiae*. The enzyme was purified and crystals in "tear drop" form were obtained. The catalytic properties of the recombinant enzyme were studied and compared with those of the native enzyme purified from *C. pilulifera*. Differences in thermal stability and chloroperoxidase activity were observed. The recombinant enzyme retained full activity after preincubation at 65 degreesC for 20 min, but the native enzyme was completely inactivated under the same conditions. The chlorinating activity of the native enzyme was more than ten times higher than that of the recombinant enzyme. Other properties, such as K-m values for KBr and H<sub>2</sub>O<sub>2</sub>, and optimal temperature and pH, were similar for each source of *C. pilulifera* bromoperoxidase. (C) 2002 Elsevier Science Ltd. All rights reserved.

**Ostrofsky, E. B., J. B. Robinson, et al. (2002). "Analysis of atrazine-degrading microbial communities in soils using most-probable-number enumeration, DNA hybridization, and inhibitors." *Soil Biology & Biochemistry* 34(10): 1449-1459.**

The purpose of this study was to determine whether there was an association between kinetics of atrazine mineralization, the number and type of atrazine-degrading microorganisms, and the presence of three genes representing different steps in the degradative pathway of atrazine in three soils with different histories of atrazine application. Composite soil samples were collected from two agricultural fields and one riparian zone soil. The samples were amended with atrazine, and mineralization was measured in biometers as (CO<sub>2</sub>)-C-14 evolution in samples spiked with [ring-U-C-14]-labeled atrazine. Atrazine-degrading microorganisms were enumerated by a most-probable-number (MPN) method, which was based on loss of atrazine (HPLC assay) in acetate media. Dot-blot hybridization assays were performed on total DNA extracted from the MPN samples. The DNA probes used in dot-blot assays were the genes *atzA* (atrazine chlorohydrolase from *Pseudomonas* ADP), *atrA* (cytochrome P-450 from *Rhodococcus* TE1), and *trzD* (cyanuric acid amidohydrolase from *Pseudomonas* NRRLB-12228). The herbicide amendment enhanced the subsequent rate of mineralization of atrazine in all three soil samples. The MPN numbers were in the range of 10(0) 10(2) cells g<sup>-1</sup> dry wt. soil, indicating that atrazine-degrading microorganisms could not be quantitatively enumerated by this technique. Positive hybridization signals of DNA extracted from MPN samples were frequent with the *atzA* probe; the *atrA* dot blots had fewer positive signals. The *trzD* signals were negligible or undetectable, although the parallel mineralization studies showed fast and extensive (CO<sub>2</sub>)-C-14 evolution from [ring-U-C- 14]-atrazine. The results suggested that *trzD*, the only gene known to encode s-triazine ring-cleavage, is not dominant among the atrazine-degrading populations of these soils. Streptomycin and cycloheximide were added to soil samples in biometers to determine the relative contributions of bacteria and fungi in the soils to the mineralization of atrazine. The relative suppression of mineralization in the presence of the bacterial or fungal specific inhibitor was approximately the same, indicating that both groups contributed to the mineralization of atrazine. (C) 2002 Elsevier Science Ltd. All rights reserved.

**Phanikumar, M. S., D. W. Hyndman, et al. (2002). "Simulation of microbial transport and carbon tetrachloride biodegradation in intermittently-fed aquifer columns." *Water Resources Research* 38(4): art. no.-1033.**

[1] This paper evaluates the microbial transport and degradation processes associated with carbon tetrachloride (CT) biodegradation in laboratory aquifer columns operated with a pulsed microbial feeding strategy. A seven component reactive transport model based on modified saturation kinetics and on a two-site sorption model was developed to describe the linked physical, chemical, and biological processes involved in CT degradation by *Pseudomonas stutzeri* KC, a denitrifying bacterium that cometabolically converts CT to harmless end products. After evaluating several expressions for attachment and detachment, we selected a dynamic partitioning model in which strain KC detachment decreases at low substrate concentrations. The resulting model enabled improved understanding of the complex coupled processes operative within our system and enabled us to test a model for field-scale design and transport studies. Batch studies were used to identify initial degradation and microbial

transport processes, and constrained optimization methods were used to estimate a set of reaction rates that best describe the column experiment data. The optimal set of parameters for one column provided a reasonable prediction of solute and microbial concentrations in a second column operated under different conditions, providing an initial test of the model. This modeling strategy improved our understanding of biodegradation processes and rates. The CT degradation rate in the columns was lower than values obtained from batch studies, and processes in addition to the growth and decay of strain KC cells (due to native flora) are necessary to describe the observed nitrate consumption.

**Pirae, M. and L. C. Vining (2002). "Use of degenerate primers and touchdown PCR to amplify a halogenase gene fragment from *Streptomyces venezuelae* ISP5230." *Journal of Industrial Microbiology & Biotechnology* 29(1): 1-5.**

Consensus amino acid sequences of FADH(2)-dependent bacterial halogenases were used to design PCR primers amplifying a halogenase gene fragment from the chloramphenicol producer *Streptomyces venezuelae* ISP5230. The sequence-specific degenerate primers (MPF1 and MPR2) were used with a touchdown PCR procedure in the first PCR-assisted cloning of a halogenase gene fragment. In the region of the 290-by PCR product containing the reverse primer, the deduced amino acid sequence exhibited characteristics of a beta-alpha-beta fold present in FAD-binding sites of certain monooxygenases. When used to probe Southern blots of restriction-enzyme-digested DNA, the [ $\alpha$ - P-32]dCTP-labeled PCR product hybridized specifically with DNA fragments from genomic DNA of *S. venezuelae* ISP5230. Primers MPF1 and MPR2 also allowed amplification by PCR of approximately 290-by DNA fragments from several other streptomycetes. The fragments from *Streptomyces aureofaciens* NRRL2209 and *Streptomyces coelicolor*A3(2) showed sequence identity with halogenase genes from these species. Thus, the PCR primers are of potential value for amplification and subsequent isolation of actinomycete halogenase genes.

**Plumeier, I., D. Perez-Pantoja, et al. (2002). "Importance of different *tfd* genes for degradation of chloroaromatics by *Ralstonia eutropha* JMP134." *Journal of Bacteriology* 184(15): 4054-4064.**

The *tfdC(I)D(I)E(I)F(I)*, and *tfdD(II)C(II)E(II)F(II)* gene modules of plasmid pJP4 of *Ralstonia eutropha* JMP134 encode complete sets of functional enzymes for the transformation of chlorocatechols into 3-oxoadipate, which are all expressed during growth on 2,4-dichlorophenoxyacetate (2,4-D). However, activity of *tfd(1)*-encoded enzymes was usually higher than that of *tfd(II)*-encoded enzymes, both in the wild-type strain grown on 2,4-D and in 3-chlorobenzoate-grown derivatives harboring only one *tfd* gene module. The *tfdD(II)*-encoded chloromuconate cycloisomerase exhibited special kinetic properties, with high activity against 3-chloromuconate and poor activity against 2-chloromuconate and unsubstituted muconate, thus explaining the different phenotypic behaviors of *R. eutropha* strains containing different *tfd* gene modules. The enzyme catalyzes the formation of an equilibrium between 2-chloromuconate and 5-chloro- and 2-chloromuconolactone and very inefficiently catalyzes dehalogenation to form trans-dienelactone as the major product, thus differing from all (chloro)muconate cycloisomerases described thus far.

**Pollmann, K., S. Kaschabek, et al. (2002). "Metabolism of dichloromethylcatechols as central intermediates in the degradation of dichlorotoluenes by *Ralstonia* sp strain PS12." *Journal of Bacteriology* 184(19): 5261-5274.**

*Ralstonia* sp. strain PS12 is able to use 2,4-, 2,5-, and 3,4- dichlorotoluene as growth substrates. Dichloromethylcatechols are central intermediates that are formed by TecA tetrachlorobenzene dioxygenase-mediated activation at two adjacent unsubstituted carbon atoms followed by TecB chlorobenzene dihydrodiol dehydrogenase-catalyzed rearomatization and then are channeled into a chlorocatechol ortho cleavage pathway involving a chlorocatechol 1,2-dioxygenase, chloromuconate cycloisomerase, and dienelactone hydrolase. However, completely different metabolic routes were observed for the three dichloromethylcatechols analyzed. Whereas 3,4-dichloro-6-methylcatechol is quantitatively transformed into one dienelactone (5-chloro-2-methyldienelactone) and thus is degraded via a linear pathway, 3,5-dichloro-2-methylmuconate formed from 4,6-dichloro-3-methylcatechol is subject to both 1,4- and 3,6- cycloisomerization and thus is degraded via a branched metabolic route. 3,6-Dichloro-4-methylcatechol, on the first view, is transformed predominantly into one (2-chloro-3-methyl- trans-) dienelactone. In situ  $^1\text{H}$  nuclear magnetic resonance analysis revealed the intermediate formation of 2,5-dichloro-4-methylmuconolactone, showing that both 1,4- and 3,6- cycloisomerization occur with

this muconate and indicating a degradation of the muconolactone via a reversible cycloisomerization reaction and the dienelactone-forming branch of the pathway. Diastereomeric mixtures of two dichloromethylmuconolactones were prepared chemically to proof such a hypothesis. Chloromuconate cycloisomerase transformed 3,5-dichloro-2-methylmuconolactone into a mixture of 2-chloro-5-methyl-cis- and 3-chloro-2-methyldienelactone, affording evidence for a metabolic route of 3,5-dichloro-2-methylmuconolactone via 3,5-dichloro-2-methylmuconate into 2-chloro-5-methyl-cis-dienelactone. 2,5-Dichloro-3-methylmuconolactone was transformed nearly exclusively into 2-chloro-3-methyl-trans-dienelactone.

**Rhew, R. C., B. R. Miller, et al. (2002). "Environmental and biological controls on methyl halide emissions from southern California coastal salt marshes." *Biogeochemistry* 60(2): 141-161.**

Methyl bromide (CH<sub>3</sub>Br) and methyl chloride (CH<sub>3</sub>Cl) emission rates from southern California coastal salt marshes show large spatial and temporal variabilities that are strongly linked to biological and environmental factors. Here we discuss biogeochemical lines of evidence pointing to vegetation as the primary source of CH<sub>3</sub>Br and CH<sub>3</sub>Cl emissions from salt marshes. Sediments and macroalgae do not appear to be major producers of these compounds, based on observations that the highest fluxes are not inhibited by soil inundation; their emissions are not correlated with those of certain gases produced in soils; and emissions from mudflat- and macroalgae-dominated sites are relatively small. In contrast, the seasonal and spatial variabilities of methyl halide fluxes in these salt marshes are consistent with the production of these compounds by vascular plants, although the possibility of production by microflora or fungi associated with the salt marsh vegetation is not ruled out. Flux chamber measurements of emission rates are largely correlated to the overall plant biomass enclosed in the chamber, but appear also to be highly dependent on the predominant plant species. Emission rates follow a diurnal trend similar to the trends of ambient air temperature and photosynthetically active radiation, but not surface soil temperature. Diurnal variabilities in the carbon isotope compositions of CH<sub>3</sub>Cl and CH<sub>3</sub>Br and their relative ratios of emissions are consistent with simultaneously competing mechanisms of uptake and production.

**Rodrigues, J. L. M., M. R. Aiello, et al. (2002). "Use of both 16S rRNA and engineered functional genes with real-time PCR to quantify an engineered, PCB-degrading *Rhodococcus* in soil." *Journal of Microbiological Methods* 51(2): 181-189.**

A real-time PCR (RTm-PCR) assay using fluorescently labeled oligonucleotides (TaqMan probes) was used to detect and quantify the recombinant *Rhodococcus* sp. strain RHA1 (fcb) in soil. One primer and probe set targeted a hypervariable region of the 16S rRNA gene unique to strain RHA1(fcb) and its phylogenetic relatives, and the other set targeted the recombinant chlorobenzoate (4-CBA) degradation operon (fcb) and was strain-specific. The method had a 6-log dynamic range of detection (10<sup>2</sup> - 10<sup>7</sup> cells ml<sup>-1</sup>) for both probes when DNA from pure cultures was used. Although the method was less sensitive in soil, the estimated number of cells in soil by real-time PCR corresponded to the measured number of RHA1(fcb) cells determined by colony-forming units. (C) 2002 Elsevier Science B.V. All rights reserved.

**Rosen, H., J. R. Crowley, et al. (2002). "Human neutrophils use the myeloperoxidase-hydrogen peroxide-chloride system to chlorinate but not nitrate bacterial proteins during phagocytosis." *Journal of Biological Chemistry* 277(34): 30463-30468.**

The generation of extracellular oxidants by neutrophils has been widely investigated, but knowledge about the chemical reactions that occur in the phagolysosome, the cellular compartment that kills pathogens, is more limited. One important pathway may involve the production of potent halogenating agents such as hypochlorous acid (HOCl) by the myeloperoxidase-hydrogen peroxide-halide system. However, explorations of the oxidation chemistry of phagolysosomes have been hampered by the organelle's inaccessibility. To overcome this limitation, we recovered *Escherichia coli* that had been internalized by human neutrophils. We then analyzed the bacterial proteins for 3-chlorotyrosine, a stable marker of damage by HOCl. Mass spectrometric analysis revealed that levels of 3-chlorotyrosine in *E. coli* proteins increased markedly after the bacteria were internalized by human neutrophils. This increase failed to occur in *E. coli* exposed to neutrophils deficient in NADPH oxidase or myeloperoxidase, implicating H<sub>2</sub>O<sub>2</sub> and myeloperoxidase in the halogenation reaction. The extent of protein chlorination by normal neutrophils paralleled bacterial killing. Our observations support the view that the phagolysosome of human neutrophils uses the myeloperoxidase-hydrogen peroxide-chloride system to chlorinate

bacterial proteins. In striking contrast, human neutrophils failed to nitrate bacterial proteins unless the medium was supplemented with 1 mM nitrite, and the level of nitration was low. Protein chlorination associated with bacterial killing was unaffected by the presence of nitrite in the medium. Nitration required NADPH oxidase but appeared to be independent of myeloperoxidase, suggesting that neutrophils can nitrate proteins through a pathway that requires nitrite but is independent of myeloperoxidase.

**Sato, A., T. Watanabe, et al. (2002). "Screening for basidiomycetous fungi capable of degrading 2,7-dichlorodibenzo-p-dioxin." *Fems Microbiology Letters* 213(2): 213-217.**

We devised a screening method to obtain basidiomycetous fungi capable of degrading dioxins. About 200 fungal strains were selected from more than 1500 strains by their ability to decolorize Remazol brilliant blue R dye as an indicator. To attempt to eliminate 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 levels of this chemical. In only 11 strains was there a significant difference between the two treatments. We chose *Panellus stypticus* strain 99-334 as a new, effective dioxin degrader, because it gave a close to 100% decrease in 2,7-DCDD levels (from an initial concentration of 10  $\mu$ M) after 40 days of exposure. The detection of a metabolic intermediate (1-chloro-3,4-dihydroxybenzene) by gas chromatography-mass spectrometry analysis supported the ability of this strain to degrade 2,7-DCDD. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Siddique, T., B. C. Okeke, et al. (2002). "Temperature and pH effects on biodegradation of hexachlorocyclohexane isomers in water and a soil slurry." *Journal of Agricultural and Food Chemistry* 50(18): 5070-5076.**

This study was conducted to monitor the biodegradation of alpha-, beta-, gamma-, and delta-hexachlorocyclohexane (HCH) isomers in liquid culture by a *Pandoraea* species and determine the influence of pH and temperature on the biodegradation of alpha- and gamma-HCH in liquid as well as in soil slurry cultures. The *Pandoraea* species degraded 79.4% delta-HCH and 34.3% gamma HCH in liquid culture at 4 weeks of incubation. (alpha- and beta-HCH exhibited almost identical rates (41.6 and 42.4%, respectively) of degradation. The highest degradation of alpha- and gamma-HCH (67.1 and 60.2%, respectively) was observed at an initial pH of 8.0 in liquid; 58.4 and 51.7% rates of degradation of alpha- and gamma-HCH, respectively, at an initial pH of 9.0 were found in soil slurry cultures. An incubation temperature of 30 degreesC was optimum for effective degradation of (alpha- and gamma-HCH isomers (62.5 and 57.7%, respectively) in liquid culture, and 54.3 and 51.9% rates of degradation of alpha- and gamma-HCH isomers, respectively, were found in a soil slurry. Increasing the soil/water ratio decreased the extent of degradation of both HCH isomers. Degradation of HCH isomers occurred concomitant with bacterial growth. Byproducts of growth from *Pandoraea* species significantly decreased the pH of the liquid and the soil slurry during the growth on HCH isomers. The results of this study suggest that this bacterial strain may effectively be used for remediating polluted sites and water contaminated with different HCH isomers over a range of environmental conditions.

**Slantchev, K., F. Yalcin, et al. (2002). "Composition of lipophylic extracts from two tunicates, *Styela* sp and *Phallusia* sp from the Eastern Mediterranean." *Zeitschrift Fur Naturforschung C-a Journal of Biosciences* 57(5-6): 534-540.**

Sterols, volatiles and lipids were isolated and identified from lipophylic extracts from two tunicates, *Styela* sp. and *Phallusia* sp., occurring in the Eastern Mediterranean. Seventeen sterols were identified. The sterol composition of the two organisms appeared to be similar except for the concentrations of 5alpha-stanols. Both tunicates were characterized by the presence of sterols with a (22Z)-double bond. In the volatiles significant amounts of chlorinated compounds were found (phenols in *Styela* sp. and hydrocarbons in *Phallusia* sp.). The fatty acid composition of triacylglycerols and phospholipids of the two tunicates showed significant differences.

**Spelberg, J. H. L., L. X. Tang, et al. (2002). "Exploration of the biocatalytic potential of a halohydrin dehalogenase using chromogenic substrates." *Tetrahedron-Asymmetry* 13(10): 1083-1089.**

Halohydrin dehalogenases are bacterial enzymes that catalyse the reversible formation of epoxides from vicinal halohydrins. A spectrophotometric assay for halohydrin dehalogenases based on the absorption difference between

the halohydrin para-nitro- 2-bromo-1-phenylethanol and the epoxide para-nitrostyrene oxide was developed. The enantioselectivity of ring-closure reactions catalysed by three different halohydrin dehalogenases could be estimated from the shape of progress Curves. Evaluation of ring-opening reactions catalysed by halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 established that, in addition to Cl<sup>-</sup> and Br<sup>-</sup>, nucleophiles such as N<sup>3-</sup>(-), CN<sup>-</sup> and NO<sub>2</sub><sup>-</sup> are also accepted for the ring opening of para- nitrostyrene oxide. The ring-opening reactions with these nucleophiles resulted in highly enantioselective kinetic resolutions, which expands the scope of synthetically valuable conversions catalysed by a halohydrin dehalogenase. (C) 2002 Elsevier Science Ltd. All rights reserved.

**Tittlemier, S., A. Borrell, et al. (2002). "Global distribution of halogenated dimethyl bipyrrroles in marine mammal blubber." *Archives of Environmental Contamination and Toxicology* 43(2): 244-255.**

Four halogenated dimethyl bipyrrroles (HDBPs), hypothesized to be naturally produced, were quantitated in marine mammal blubber from a number of species obtained from various locations worldwide. HDBPs were found in samples from all locations studied. Concentrations of total HDBPs (SigmaHDBPs) ranged from 0.4 ng/g lipid weight in ringed seals (*Phoca hispida*) from the White Sea to 2,540 ng/g lipid weight in Dall's porpoise (*Phocoenoides dalli*) from the northwestern North Pacific Ocean. At their highest levels, ΣHDBPs made up 11% of the total quantitated organohalogen body burden of adult male Dall's porpoises. In two beluga (*Delphinapterus leucas*) data subsets, it was found that males contained significantly higher concentrations of ΣHDBPs than females. No significant effects of age or sex on SigmaHDBPs were observed in harbor seal (*Phoca vitulina*) and bowhead whale (*Balaena mysticetus*) data subsets. The geographical distribution of concentrations did not resemble that of the ubiquitous anthropogenic organohalogen, polychlorinated biphenyl congener CB-153. Higher concentrations of HDBPs and different patterns of congeners were observed in samples from Pacific as opposed to non-Pacific Ocean influenced environments. Concentrations of HDBPs in beluga from the Arctic and St. Lawrence River were similar. Their high abundance in north Pacific Ocean biota and widespread occurrence suggest that HDBPs undergo extensive transport from a source located primarily in the Pacific Ocean. Evidence from HDBP congener patterns indicates that both ocean currents and atmospheric transport likely play a role in the movement of HDBPs. These results imply that HDBPs and anthropogenic organohalogens have different sources and support the natural production hypothesis.

**Utkina, N. K., V. A. Denisenko, et al. (2002). "Two new minor polybrominated dibenzo-p-dioxins from the marine sponge *Dysidea dendyi*." *Journal of Natural Products* 65(8): 1213-1215.**

Two new minor tribromodibenzo p dioxins, spongiadioxin C (1) and its methyl ether (2), were isolated from an Australian marine sponge *Dysidea dendyi*, together with the known minor metabolites methyl ethers of spongiadioxins A (4) and B (6) and polybrominated diphenyl ethers (7-9) The structures of the new compounds were established by 1D and 2D NMR spectroscopy and confirmed by synthesis of 2 from diphenyl ether 9 All isolated compounds inhibited the cell division of fertilized sea urchin eggs.

**Vetter, W., E. Scholz, et al. (2001). "Anthropogenic and natural organohalogen compounds in blubber of dolphins and dugongs (*Dugong dugon*) from northeastern Australia." *Archives of Environmental Contamination and Toxicology* 41(2): 221-231.**

A range of organohalogen compounds (10 polychlorinated biphenyl (PCB) congeners, DDT and metabolites, chlordane-related compounds, the potential natural organochlorine compound Q1, toxaphene, hexachlorobenzene, hexachlorocyclohexanes, dieldrin, and several yet unidentified brominated compounds) were detected in the blubber of four bottlenose dolphins (*Tursiops truncatus*), one common dolphin (*Delphinus delphis*), and seven dugongs (*Dugong dugon*), as well as in adipose tissue of a green turtle (*Chelonia mydas*) and a python (*Morelia spilota*) from northeast Queensland (Australia). The green turtle and dugongs accumulated lower organohalogen levels than the dolphins. Lower levels in dugongs were expected because this species is exclusively herbivorous. Highest PCB and DDT levels recorded in dugongs were 209 and 173 mug/kg lipids, respectively. Levels of the nonanthropogenic heptachlorinated compound Q1 (highest level in dugongs was 160 mug/kg lipids) were estimated using the ECD response factor of trans-nonachlor. Highest organohalogen levels were found in blubber of dolphins for sumDDT (575-52,500 mug/kg) and PCBs (600-25,500 mug/kg lipids). Furthermore, Q1 was a major organohalogen detected in all samples analyzed, ranging from 450-9,100 mug/kg lipids. The highest

concentration of Q1 determined in this study represents the highest concentration reported to date in an environmental sample. Levels of chlordane-related compounds were also high (280-7,700 µg/kg, mainly derived from trans-nonachlor), but concentrations of hexachlorobenzene, hexachlorocyclohexanes, dieldrin, and toxaphene were relatively low and contributed little to the overall organohalogen contamination. Furthermore, a series of three major (BC-1, BC-2, and BC-3) and six minor (BC-4 through BC-9) unknown brominated compounds were observable by extracting m/z 79 and m/z 81 from the GC/ECNI-MS full scan run. Structural proposals were made for the two major recalcitrant compounds (referred to as BC-1 and BC-2). BC-2 appears to be a tetrabromo-methoxy-diphenylether (512 u) and BC-1 has 14 u (corresponding with an additional CH<sub>2</sub> group) more relative to BC-1. In general, the organohalogen pattern observed in blubber of dolphins was different compared to similar samples from other locations in the world, which is apparent from the fact that the four major abundant signals in the GC/ECD chromatogram of *D. delphis* originated from the four unknown compounds Q1, BC-1, BC-2, and BC-3.

**Vetter, W., E. Stoll, et al. (2002). "Sponge halogenated natural products found at parts-per-million levels in marine mammals." *Environmental Toxicology and Chemistry* 21(10): 2014-2019.**

Several unknown, abundant brominated compounds (BCs) were recently detected in the blubber of dolphins and other marine mammals from Queensland (northeast Australia). The BC were interpreted as potential natural products due to the lack of anthropogenic sources for these compounds. This study investigated whether some of the BCs accumulated by diverse marine mammal species are identical with natural BCs previously isolated from sponges (*Dysidea* sp.) living in the same habitat. Isolates from sponges and mollusks (*Asteronotus cespitosus*) were compared with the signals detected in the mammals' tissue. Mass spectra and gas chromatography retention times on four different capillary columns of the isolates from sponges and mammals were identical in all respects. This proves that the chemical name of the compound previously labeled BC-2 is 4,6-dibromo-2-(2'-dibromo)phenoxyanisole and that the chemical name of BC-11 is 3,5-dibromo-2-(3',5'-dibromo-2'-methoxy)phenoxyanisole. Using a quantitative reference solution of BC-2, we established that the concentrations of the brominated metabolites found in the marine mammals are frequently >1 mg/kg. The highest concentration (3.8 mg/kg), found in a sample of pygmy sperm whale (*Kogia breviceps*), indicates that BC-2 is a bioaccumulative, natural organohalogen compound. This is supported by the concentrations of the BCs in our samples being equal to the highest concentrations of anthropogenic BCs in any environmental sample. The quantitative determination of BC-2 in blubber of marine mammals from Africa and the Antarctic suggests that BC-2 is wide-spread. These results are direct proof that marine biota can produce persistent organic chemicals that accumulate to substantial concentrations in higher trophic organisms.

**Watanabe, K. (2002). "Biodegradation of synthetic compounds and agro-chemicals by soil microorganisms in field soils." *Journal of Pesticide Science* 27(3): 287-292.**

**Widada, J., H. Nojiri, et al. (2002). "Enhanced degradation of carbazole and 2,3-dichlorodibenzo-p-dioxin in soils by *Pseudomonas resinovorans* strain CA10." *Chemosphere* 49(5): 485-491.**

We studied the degradation of carbazole (CAR) and 2,3-dichlorodibenzo-p-dioxin (2,3-DCDD) in soils inoculated with carbazole- and dioxin-degrader *Pseudomonas resinovorans* strain CA10. By using Tn5-based transposon delivery systems, this bacterium was chromosomally marked with a tandem green fluorescent protein (gfp) gene. Real-time competitive PCR and direct counting using the (gfp) marker were employed to monitor the total number of carbazole 1,9a-dioxygenase gene (carAa) and survival of CA10 cells in the soil and soil slurry microcosms. Bioaugmentation studies indicated that the survival of the marked CAM cells in soil microcosms was strongly influenced by pH and organic matter. While the number of the marked CA10 cells decreased rapidly in pH 6 with low organic matter, a high cell density was maintained in pH 7.3 with 2.5% organic matters up to 21 days after inoculation. In pH 7.3 soil, the period needed for complete degradation of CAR (100 µg kg<sup>-1</sup>) was markedly shortened from 21 to 7 days by the inoculation with the CA10 cells. Single inoculation of CAM cells into the soil slurry system of 2,3-DCDD-contaminated soil enhanced the degradation of 2,3-DCDD from 25.0% to 37.0%. In this system, the population density of CAM cells and the total number of carAa gene were maintained up to 14 days after inoculation. By repeated inoculation (every 2 days) with CA10 cells each at a density of 10<sup>9</sup> CFU g<sup>-1</sup> of soil, almost all of the 2,3-DCDD (1 µg kg<sup>-1</sup>) was degraded within 14 days. Results of these experiments

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suggest that *P. resinovorans* strain CA10 may be an important resource for bioremediation of CAR and chlorinated dibenzo-p-dioxin in contaminated soils. (C) 2002 Elsevier

**Wu, Q. Z., C. E. Milliken, et al. (2002). "Dechlorination of chlorobenzenes by a culture containing bacterium DF-1, a PCB dechlorinating microorganism." *Environmental Science & Technology* 36(15): 3290-3294.**

Polychlorinated benzenes were reductively dechlorinated by an enrichment culture containing the polychlorinated biphenyl (PCB) dechlorinating bacterium DF-1. The culture dechlorinated hexachlorobenzene (hexa-CB) --> pentachlorobenzene (penta-CB) - -> 1,2,3,5-tetrachlorobenzene (1,2,3,5-CB) --> 1,3,5- trichlorobenzene (1,3,5-CB) and did not dechlorinate other tetrachlorobenzenes or any trichlorobenzenes. This restricted series of reactions is the most predominant and frequently reported pathway for the dechlorination of hexa-CB and penta-CB by enrichment cultures inoculated with either freshwater or estuarine sediments. The culture did not dechlorinate hydroxylated and methoxylated polychlorinated benzenes or a hydroxylated PCB. Bacterium DF-1 was detected by PCR/DGGE analysis following dechlorination of penta-CB but was not detected when a chlorinated benzene (CB) was not dechlorinated; detection of other members in the community was unaffected by the presence or absence of CB dechlorination. This is the first report of a bacterium that reductively dechlorinates both PCBs and CBs and the first identification of an organism that can dechlorinate a CB with more than four chlorines.

**Yang, Y. R. and P. L. McCarty (2002). "Comparison between donor substrates for biologically enhanced tetrachloroethene DNAPL dissolution." *Environmental Science & Technology* 36(15): 3400-3404.**

Tetra chloroethene (PCE) dense nonaqueous-phase liquid (DNAPL) can act as a persistent groundwater contamination source for decades. Biologically enhanced dissolution of pure PCE DNAPL has potential for reducing DNAPL longevity as indicated previously (*Environ, Sci. Technol.* 2000, 34, 2979). Reported here are expanded studies to evaluate donor substrates that offer different remediation strategies for bioenhanced DNAPL dissolution, including pentanol (soluble substrate, fed continuously), calcium oleate (insoluble substrate, placed in column initially by alternate pumping of sodium oleate and calcium chloride), and olive oil (mixed with PCE and placed in column initially). Compared with a no-substrate column control, the DNAPL dissolution rate was enhanced about three times when directly coupled with biological transformation. The major degradation product formed was cDCE, but significant amounts of VC and ethane were also found with some columns. Extensive methanogenesis, which reduced PCE transformation, occurred in both the pentanol-fed and oleate-amended columns, but not in the olive-oil-amended column, suggesting that methanogens managed to colonize column niches where PCE DNAPL was not present. Detrimental methane production in the pentanol-fed column was nearly eliminated by presaturating the feed solution with PCE. These results suggest potential DNAPL remediation strategies to enhance dehalogenation while controlling competitive methanogenic utilization of donor substrates.