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

**15<sup>th</sup> Quarterly Report  
3<sup>rd</sup> Quarter Year 2004**

**Report prepared for EUROCHLOR**

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January 13, 2005

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## TABLE OF CONTENTS

<b>Acronyms</b>	4
<b>1. Introduction</b>	6
<b>2. Summary of most important developments</b>	6
2.a. Microbial dechlorination	6
2.b. Microbial chlorination	7
<b>3. Microbial dechlorination</b>	7
3.a. General reviews	7
3.b. Microbial dechlorination	8
Vinyl chloride (VC) and other chlorinated ethenes	8
Perchloroethylene (PCE) and Trichloroethene (TCE)	9
Carbon tetrachloride (CT) and chloroform (CF)	11
Chloromethane (CM) and dichloromethane (DCM)	11
1,2-Dichloroethane (1,2-DCA) and other chlorinated ethanes	12
Chlorobenzenes	13
Chlorinated dibenzo- <i>p</i> -dioxins and -furans (CDDs/CDFs)	13
Hexachlorobutadiene and octachlorostyrene	13
Polychlorinated biphenyls (PCBs)	13
Miscellaneous chlorinated compounds	14
3.c. In vitro degradation of chlorinated compounds	15
3.d. New tools & techniques to assess the biodegradation of chlorinated compounds	16
Detection of Chlorinated Compounds	16
Detection of Microbial Populations	16
Mathematical Tools	17
Cell Based Bioassays	17

## **TABLE of CONTENTS** (*Continued*)

<b>4. Microbial chlorination</b>	17
4.a. General reviews	17
4.b. Microbial chlorination in the environments	18
Chloromethanes and other chlorinated compounds	18
4.c. Chlorination by freshwater and marine organisms	18
Chloromethanes	18
Other chlorinated compounds	19
4.d. Chlorinating enzymes	20
<b>5. References Cited</b>	21
<b>6. Annex</b>	25

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**ACRONYMS**

<b>16S rRNA</b>	16S Ribosomal RNA
<b>AOX</b>	Adsorbable Organic Halides
<b>CB</b>	Chlorobenzene
<b>CBp</b>	Chlorobiphenyl
<b>CDDs</b>	Chlorinated Dibenzo- <i>p</i> -Dioxins
<b>CDFs</b>	Chlorinated Dibenzo- <i>p</i> -Furans
<b>CF</b>	Chloroform
<b>CT</b>	Carbon Tetrachloride
<b>1,2-DCA</b>	1,2-Dichloroethane
<b>DCB</b>	Dichlorobenzene
<b>DCE</b>	Dichloroethene
<b>DCM</b>	Dichloromethane
<b>2,4-DCP</b>	2,4-dichlorophenol
<b>DDT</b>	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane
<b>DNAPL</b>	Dense Non-Aqueous Phase Liquid
<b>DTT</b>	Dithiothreitol
<b>E-acceptor</b>	Electron Acceptor
<b>E-donor</b>	Electron Donor
<b>ESA</b>	Ethanesulfonic Acid
<b>ETH</b>	Ethylene, ethene
<b>HCB</b>	Hexachlorobenzene
<b>HFCs</b>	Hydrochlorofluorocarbons
<b>HCH</b>	Hexachlorocyclohexane
<b>HoDOM</b>	Hydrophobic dissolved organic matter
<b>MCL</b>	Maximum Concentration Limit
<b>OA</b>	Oxanilic Acid
<b>PCBs</b>	Polychlorinated Biphenyls
<b>PCP</b>	Pentachlorophenol
<b>PCE</b>	Tetrachloroethylene
<b>PCR</b>	Polymerase Chain Reaction
<b>RFLP</b>	Restriction Fragment Length Polymorphism

**ACRONYMS** (*Continued*)

<b>TCA</b>	Trichloroacetic Acid
<b>TCE</b>	Trichlorethylene
<b>1,2,3,4-TeCDD</b>	1,2,3,4-Tetrachlorodibenzo- <i>p</i> -dioxin
<b>1,2,3,4-TeCDF</b>	1,2,3,4-Tetrachlorodibenzo- <i>p</i> -furan
<b>VC</b>	Vinyl Chloride

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

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## **1. INTRODUCTION**

This report presents a review of scientific literature published during the third quarter of 2004 (covering August to October 2004) on the microbial halogenation and dehalogenation of the following compounds: vinyl chloride, dichloroethane, chloroform, dichloromethane, hexachlorobenzene, chlorobenzene, 1,2,4-1,2,3-1,3,5-trichlorobenzene, hexachlorobutadiene, octachlorostyrene, dioxins and chlorinated furans. In addition, reports regarding the microbial chlorination of compounds structurally related to those listed above were also reviewed.

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

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

The most important findings in this quarter for microbial dechlorination are studies characterizing the kinetics of anaerobic vinyl chloride degradation (9), characterization of the genes of reductive dehalogenases in the halorespiring genus, *Dehalococcoides* (20, 25), and a review on the evolution of enzymes to dehalogenate new chemical inputs in the environment (38). Several enrichment cultures known for anaerobic halorespiration of vinyl chloride were carefully evaluated to compare to the kinetics (9). The growth rates with VC ranged from 0.28 to 0.49 d<sup>-1</sup> and the half-velocity coefficients were approximately 2.6 μM. The dehalogenase of a VC-degrading *Dehalococcoides* was isolated and a degenerate primer was developed and used to isolate and characterize the corresponding gene, VC reductase (*vcrA*). The gene now provides an

important specific biomarker to detect anaerobic VC-degrading capacity at contaminated sites. The dehalogenase genes were carefully evaluated in two *Dehalococcoides* strains and it was discovered that the genus contains multiple non-identical reductive-dehalogenase-homologous (20). Finally, an interesting review article was published which provides mechanistic basis on how enzymes evolve to dehalogenate new synthetic chlorinated compounds introduced into the environment (38).

## 2.b. Microbial Chlorination

The most important highlights for microbial chlorination are a report on using the natural  $^{14}\text{C}$  isotope to determine if chlorinated compounds have a natural origin (30), and a review article on natural fluorinated compounds (11). Two hydrophobic chlorinated bipyrrrole compounds, 1,1'-dimethyl-3,3',4,4'-tetrabromo-5,5'-dichloro-2,2'-bipyrrrole (DBP-Br<sub>4</sub>Cl<sub>2</sub>) and heptachloro-1'-methyl-1,2'-bipyrrrole (Q1), have been detected worldwide in mammals and are suspected of having a natural source (30). Through the isolation of a large quantity of the compounds from marine animal extracts it was possible to measure the occurrence of  $^{14}\text{C}$  isotope to determine if the source of the compounds is natural (petroleum based products would not have  $^{14}\text{C}$ ).  $^{14}\text{C}$  was measured proving the compounds have a natural origin.

This quarter an interesting review article was published describing a fluorinating enzyme (fluorinase) and the natural occurrence of a variety of natural fluorinated compounds (11). Unraveling of the natural mechanisms of fluorination is scientifically novel and is included here as an important highlight.

## 3. MICROBIAL DECHLORINATION

### 3.a. General Reviews

In this quarter, 2 review articles on biological dechlorination were published. The first article reviews the evolution of enzymes to degrade new chemical inputs in the environment (38). The article specifically focuses on dichloromethane dehalogenase, *trans*-3-chloroacrylate dehalogenase of the 1,3-dichloropropene pathway, tetrachlorohydroquinone reductive dehalogenase of the pentachlorophenol pathway, and atrazine chlorohydrolase. Bacteria use dehalogenases to remove one or more halide ions and further metabolize the compounds to support growth of the cell. The dehalogenases are seen to derive from different enzyme super-

families. There is evidence that catalytically efficient dehalogenases have arisen from precursor enzymes in the time since the introduction of pesticides or solvents into commercial use. The evolution of metabolic pathways for pollutants is facilitated by the horizontal transfer of genes on plasmids and insertion of sequence elements. These processes ultimately contribute to cleansing the environment of new chemical compounds of anthropogenic origin.

The second review article covers recent advances in the biochemistry of environmental pollutant degradation (27), including several chlorinated compounds. The review starts off with a brief discussion on the halorespiration of chlorinated solvents and chlorinated aromatics. Later in the article some examples of chemotaxis involvement in chlorinated compound degradation are reviewed, including atrazine and 2,4-dichlorophenoxyacetate. The review article is finalized by a discussion of bacterial genomics applied to unraveling the presence of degradative enzymes in bacteria.

### 3.b. Microbial Dechlorination

#### Vinyl Chloride and Other Chlorinated Ethenes

A large number of studies reported on research of the higher chlorinated ethenes, perchloroethylene (PCE) and trichloroethene (TCE) because these are major groundwater contaminants. Often information regarding the degradation of lower chlorinated ethenes, vinyl chloride (VC) and dichloroethenes (DCE), are found in these studies. The studies were categorized based on the parent compound investigated, either lower chlorinated ethenes (VC or DCEs) or higher chlorinated ethenes (PCE or TCE).

**Vinyl Chloride (VC) and Dichloroethenes (DCE).** In this quarter, there were only three studies that directly investigated the biodegradation of lower chlorinated ethenes as parent compounds. One article dealt with aerobic degradation of *cis*-DCE in a methanotrophic fixed film bioreactor (1). The degradation by the methane oxidizing bacteria was cometabolic. The presence of methane was shown to be important to obtain an appreciable rate of *cis*-DCE degradation; however a large excess of methane caused competitive inhibition of *cis*-DCE degradation. Maximum *cis*-DCE removal was observed with a methane bulk concentration ranging from 0.2 to 0.7 mg l<sup>-1</sup>.

The remaining two articles about lower chlorinated ethenes concerned the anaerobic conversion of DCE and VC by the halorespiring genus of *Dehalococcoides*. The first article compared the rates of VC degrading kinetics by three enrichment cultures principally containing

bacterial strains from *Dehalococcoides* (9). These included *Dehalococcoides* species strain VS (from Victoria, Texas), the mixed culture KB-1/VC (from Ontario) and the Pinellas mixed culture (from Pinellas, Florida). None of the enrichment cultures could grow with PCE as electron acceptor, but all three enrichment cultures displayed growth with either TCE, *cis*-DCE or VC as electron acceptor and hydrogen gas as electron donor. The growth rates with VC were 0.42 to 0.49 d<sup>-1</sup> with the VS and KB-1/VC enrichments and 0.28 d<sup>-1</sup> with the Pinellas enrichment. The three strains had similar growth rates with *cis*-DCE (0.43 to 0.46 d<sup>-1</sup>) and TCE (0.33 to 0.49 d<sup>-1</sup>). Half-velocity constants of *cis*-DCE and VC degradation by the VS enrichment were 3.3 and 2.6 μM, respectively. The half-velocity coefficient for the various enrichments on TCE was 9.0 to 10.5 μM. The study is the first report of an organism obtaining energy for growth through every step in the reduction of TCE to ethene.

In the last article, a VC-reductive dehalogenase from the *Dehalococcoides* VS enrichment was purified and the gene was cloned by creating degenerative primers with the N-terminal sequence (25) as described in “3.c. *In Vitro* Degradation of Chlorinated Compounds”

**Perchloroethylene (PCE) and Trichloroethene (TCE).** In this quarter, there were 9 publications reporting on either PCE or TCE microbial degradation. One of the citations concerns the detection of TCE in trees as an indicator of groundwater contamination (37), as is discussed in “3.d. *New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds*”. A second citation describes shifts in the population of in an aerobic bioreactor used for the cooxidation of TCE with phenol (2) and is also discussed in “3.d. *New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds*”.

Of the remaining 7 articles 2 articles are concerned with aerobic degradation of TCE and 5 articles describe anaerobic degradation of TCE and/or PCE. In the first aerobic article, the enzymatic activity of toluene monooxygenase from a bacterial toluene-oxidizing enrichment culture was tested for TCE oxidation (13). Enzyme extracts showed an optimal activity (3.03 mg of TCE (mg of protein)<sup>-1</sup> d<sup>-1</sup>) at neutral pH. The addition of a cofactor (up to 0.02 mg ml<sup>-1</sup>), NADH, led to an initial reaction rate of 5.30 mg of TCE (mg of protein)<sup>-1</sup> d<sup>-1</sup>. This observation demonstrated that the availability of the cofactor played an important role in determining the overall degradation reaction rates. The second aerobic article evaluated the use of propane for the cooxidation of TCE at a field-scale in situ bioremediation site at Dover Air Force Base (Delaware, USA) (28). The propane injection resulted in degradation of a mixture of chlorinated solvents, including TC), *cis*-DCE, and 1,1,1-trichloroethane (TCA). In only 20 d, the propane injection resulted in decreases of TCE and *cis*-DCE of > 98%, and a decrease in TCA in soil gas by approximately 70%. The decreases in chlorinated solvent concentrations were accompanied by large increases in propane-utilizing bacteria. Microbial TCE degradation activity, as

measured in microcosms, also increased with the propane injection. The highest rates of degradation were observed in microcosms with propane and nutrients, indicating the potential for higher field rates of degradation with nutrient additions.

Of the 5 anaerobic articles, one article describes the formate dehydrogenase genes of a PCE-halorespiring organism, *Sulfurospirillum multivorans* (33), an organism also known to contain a PCE-dehalogenase. Another article describes the chemical dehalogenation of TCE by sediments that were chemically reduced with sodium dithionite (35). In different aquifer sediments, 10-22% of amorphous and crystalline Fe-III-oxides were dissolved/reduced, which produced primarily adsorbed Fe-II, and some siderite. The sediment TCE reduction rate (3.3 h half-life) was chemically controlled with some additional diffusion control during reduction in sediment columns (8.0 h half-life). It was necessary to maintain neutral to high pH to maintain reduction efficiency and prevent iron mobilization, as reduction generated  $H^+$ . Sequential extractions on reduced sediment showed that adsorbed ferrous iron controlled TCE reactivity.

The third anaerobic article described the natural attenuation of PCE in plume converging on a river (8). The plume observed in the shallow streambed deposits was significantly different from what would have been predicted based on the characteristics of the upgradient plume. The most important effect of the near-river zone on the plume was the extensive anaerobic biodegradation that occurred in the top 2.5 m of the streambed, even though essentially no biodegradation of the PCE plume was observed in the upgradient aquifer. Approximately 54% of the area of the plume in the streambed consisted solely of PCE transformation products, primarily *cis*-DCE and VC.

The fourth anaerobic article describes enrichment cultures isolated from PCE contaminated aquifers that largely convert PCE to *trans*-DCE (16). These cultures reductively dechlorinated PCE and TCE to *trans*-DCE and *cis*-DCE simultaneously and in a ratio of 3:1 that was stable through serial transfers with a variety of electron donors and occurred in both methanogenic and non-methanogenic enrichments. This constitutes a noteworthy finding since most enrichment cultures found so far generally favor reduction to *cis*-DCE.

The last anaerobic article compared the reductive dehalogenases of two *Dehalococcoides* strains. One strain, CBDB1 is known for its ability to reductively dechlorinate polychlorinated benzenes and polychlorinated dioxins; whereas, the other strain, FL2, is known for its TCE-dechlorinating activity. Degenerate primers were used to amplify large fragments of reductive-dehalogenase-homologous (RDH) genes from genomic DNA of two *Dehalococcoides* strains. The amplicons (1,350 to 1,495 bp) corresponded to nearly complete open reading frames of known reductive dehalogenase genes and short fragments (approximately 90 bp) of genes encoding putative membrane-anchoring proteins. Cloning and restriction analysis revealed the presence of at least 14 different RDH genes in each strain. All amplified RDH genes showed

sequence similarity with known reductive dehalogenase genes over the whole length of the sequence and shared all characteristics described for reductive dehalogenases. Deduced amino acid sequences of seven RDH genes from strain CBDB1 were 98.5 to 100% identical to seven different RDH genes from strain FL2, suggesting that both strains have an overlapping substrate range. All RDH genes identified in strains CBDB1 and FL2 were related to the RDH genes present in the genomes of *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp. strain BAV1; however, sequence identity did not exceed 94.4 and 93.1%, respectively. This study demonstrates that the presence of multiple nonidentical RDH genes is characteristic of *Dehalococcoides* strains.

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

Phytoremediation studies showed that poplar plantations can effectively break down carbon tetrachloride (CT) into harmless components (39). Poplar trees removed more than 99% of dosed CT with minimal transpiration and diffusion of CT into the air. CT balances in plant tissue and in the root zone soils, both in vegetated and non-vegetated soils, indicated that the dominant fate of CT is degradation in plant tissues. Accumulation of CT and its metabolites in the tissues of poplars exposed to CT was negligible.

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

*Pseudomonas aeruginosa* strain NB1 was shown to utilize chloromethane (CM) as its sole source of carbon and energy under nitrate-reducing and aerobic conditions (15). Stoichiometric release of chloride and minimal accumulation of soluble metabolic products (measured as chemical oxygen demand) following CM consumption, under anoxic and aerobic conditions, indicated complete biodegradation of CM. Nitrate was stoichiometrically converted to dinitrogen gas. Acetylene did not inhibit CM use under aerobic conditions, implying that a monooxygenase was not involved in initiating aerobic CM metabolism. Under anoxic conditions, the maximum specific CM utilization rate ( $k$ ) for NB1 was  $5.01 \mu\text{mol of CM mg of TSS}^{-1} \text{ day}^{-1}$ , the maximum specific growth rate ( $\mu_{\text{max}}$ ) was  $0.0506 \text{ day}^{-1}$ , and the Monod half-saturation coefficient ( $K_s$ ) was  $0.067 \mu\text{M}$ . Under aerobic conditions, the values for  $k$ ,  $\mu_{\text{max}}$ , and  $K_s$  were  $10.7 \mu\text{M CM (mg TSS)}^{-1} \text{ day}^{-1}$ ,  $0.145 \text{ day}^{-1}$ , and  $0.93 \mu\text{M}$ , respectively, indicating that NB1 used CM faster under aerobic conditions.

A second publication reports on the biomimetic degradation of a chlorofluoromethane compound, CFC 113 or chlorofluorocarbon 113 (3). CFC113 was rapidly and completely degraded non-enzymatically under anaerobic conditions in systems containing cyanocobalamin with titanium (III) citrate as the reductant. Hydrochlorofluorocarbon 123a accounted for up to 25% of the degraded CFC113. Chlorotrifluoroethene was also detected. Increasing concentrations of cyanocobalamin increased CFC113 removal rates asymptotically and also decreased the fraction of HCFC123a remaining.

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

In this quarter there were 3 reports on chloroethane degradation. The first article describes the pollution problem associated with the use of 1,2-DCA as a leaded gasoline additive (14). Past investigations and remediation efforts at sites contaminated by leaded gasoline have rarely addressed the potential for 1,2-DCA contamination. For this reason, there is a substantial likelihood that undetected 1,2-DCA plumes above the maximum concentration limit (MCL) may exist at many sites where leaded gasoline leaked or spilled.

The second article describes the use of isotopic fractionation detection techniques to distinguish between two different pathways of aerobic 1,2-DCA degradation (19). An abstract published in the same period contains the same information (18). One known degradation pathway, utilized by *Xanthobacter autotrophicus* GJ10 and *Ancylobacter aquaticus* AD20, involves a hydrolytic dehalogenase enzyme. The initial hydrolytic dehalogenation step involves the cleavage of a C-Cl bond, producing 2-chloroethanol. The second proposed pathway, utilized by *Pseudomonas* sp. strain DCA1, is thought to involve a monooxygenase enzyme, resulting in an initial C-H bond cleavage. Current literature reports that stable carbon isotope fractionation of 1,2-DCA during aerobic biodegradation is large and reproducible (-27 to -33 parts per thousand). In this study, a significant variation in the magnitude of stable carbon isotope fractionation during aerobic biodegradation was observed. Biodegradation in experiments involving microcosms, enrichment cultures, and pure microbial cultures produced a consistent bimodal distribution of enrichment factors ( $\epsilon$ ) with one mean epsilon centered on -3.9 parts per thousand and the other on -29.2 parts per thousand. The bimodal distribution is therefore consistent with the microbial degradation of 1,2-DCA by two separate enzymatic pathways. This interpretation is further supported in this study by experiments with pure strains of *X. autotrophicus* GJ10, *A. aquaticus* AD20, and *Pseudomonas* sp. strain DCA1.

The third article describes the removal of 1,1,1-trichloroethane (1,1,1-TCA) via aerobic cooxidation with propane (28). The results from field-scale bioventing experiments at Dover Air

Force Base (Delaware, USA) indicated 70% reduction of 1,1,1-TCA 20 days after a propane injection.

### **Chlorobenzenes (CB)**

No reports were found directly about the microbial degradation of chlorobenzene compounds. The reductive dehalogenase genes of *Dehalococcoides* strain CBDB1 were described (20). This strain is known to catalyze the reductive dechlorination of polychlorinated benzenes as a result of halorespiration. The reductive dehalogenase genes were compared with trichloroethylene degrading strains of *Dehalococcoides*.

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

In this quarter, no studies directly reported on the degradation of chlorinated dibenzo-*p*-dioxins (CDDs) by anaerobic cultures. As mentioned above, the reductive dehalogenase genes of *Dehalococcoides* strain CBDB1 were described (20) and this strain is known to catalyze the reductive dechlorination of polychlorinated dioxins.

### **Hexachlorobutadiene and Octachlorostyrene**

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

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

In this quarter, 4 publications reported on the microbial degradation of polychlorinated biphenyls (PCBs). Davis ((10)) developed a model to predict the mass balance of PCBs in the San Francisco Bay (USA). The model predicts that in the case of no additional external inputs it would take 20 years to reduce the PCB mass by 50%. Specific half-lives are provided for different congeners that range from 4 years for PCB congener 18 to 30 years for PCB congener 194. The second article evaluates the degradation of PCB containing wastewaters after UV-assisted chemical oxidation and activated carbon adsorption of oxidized products (22). Wastewaters evaluated included extracts from PCB contaminated soils or PCB contaminated groundwater from PCB contaminated sites. The last two articles provide a mini-review of

different strategies towards PCB bioremediation (26) and on the status of genetically engineering bacteria to fully mineralize PCBs (34), respectively.

### Miscellaneous Chlorinated Compounds

The search query used is specifically designed to review literature on the target compounds listed in the *Introduction* section. Interesting publications concerned with compounds outside of the target list that are found in the search process are briefly discussed below. This quarter our search retrieved 8 reports on the biodegradation of miscellaneous chlorinated pollutants, including, chlorophenols, chloroanilines, hexachlorocyclohexanes, chlorinated pesticides (atrazine, dicamba,  $\gamma$ -hexachlorocyclohexane, triasulfuron, chloroacetinilide herbicides).

The first of these studies evaluated the extractability and mineralization of 2,4-dichlorophenol (2,4-DCP) in planted and unplanted soil (6). Uniformly-labeled 2,4-DCP was eliminated by 30 and 24% after 57 days. The residual 2,4-DCP was mineralized by 10 and 27% to  $^{14}\text{CO}_2$  in planted and unplanted soils, respectively; when the soils were inoculated with the chlorophenol degrading bacterium, *Burkholderia*, and incubated for 478 h. The extractability of the label by acetonitrile-water was 48% in the unplanted soil and 17% in the planted soil on day 57. The results taken as a whole suggest significant formation of bound residue in soil, with enhanced sequestration in planted soils.

The second paper examined the extractability of chloroanilines from soil with biosurfactants (4). Biosurfactants enhanced the elution of chlorinated anilines from historically polluted soil, and did not interfere with the treatment of the eluent by aerobic bacteria. In contrast, synthetic surfactants did decrease the population of aerobic bacteria during aerobic treatment of the soil wash eluents.

The third report evaluated the biomimetic dechlorination of  $\alpha$ -,  $\beta$ -,  $\delta$ -, and  $\gamma$ -hexachlorocyclohexane (HCH) by vitamin B12 with dithiothreitol (DTT) or titanium(III)-citrate as reducing agents (31). Only  $\alpha$ - and  $\gamma$ -HCH were degraded by vitamin B12/DTT system. All isomers were completely degraded and rapidly degraded by the vitamin B12/Ti(III) system.

The fourth article evaluated the mineralization and formation of non-extractable residues (bound residue) in soil microcosms from radiolabeled chlorinated pesticides, atrazine, dicamba and lindane ( $\gamma$ -HCH) (24). Atrazine and lindane mineralization and bound-residue formation was limited by ring cleavage, and most of the residuals were extractable. On the other hand, dicamba (2-methoxy-3,6-dichloro-benzoic acid) was subjected to approximately 25% mineralization in 91 days and significant formation of bound residue, approximately 72%.

The fifth article examined the effect on compost on the fate of the chlorinated sulfonylurea herbicide, triasulfuron, in soil (32). Results have shown that the adsorption of triasulfuron to soil increases in the presence of compost, and that the humic acids and hydrophobic dissolved organic matter (HoDOM) fractions are mainly responsible for this increase. The rate of hydrolysis of triasulfuron in solution was significantly higher at acidic pH and the presence of organic matter fractions extracted from compost also slightly increased the rate of hydrolysis. The rate of degradation in amended and non-amended soils is explained by two-stage degradation kinetics. During the initial phase, although triasulfuron degradation was rapid with a half-life of approximately 30 d, the presence of compost and HoDOM was found to slightly reduce the rate of degradation with respect to that in non-amended soil.

The sixth report is an article concerning a survey of drinking wells in the State of Wisconsin (USA) for the presence of chloroacetinilide herbicides and metabolites of chloroacetinilide herbicides (29). Analysis of data resulted in an estimated proportion of 38% of wells that contained detectable levels of an herbicide or herbicide metabolite. The most commonly detected compound was alachlor ethanesulfonic acid (alachlor ESA) with a proportion estimate of 28%. Other detected compounds in order of prevalence were metolachlor ESA, metolachlor oxanilic acid (metolachlor OA), alachlor OA, acetochlor ESA, and parent alachlor. Estimates of the mean concentration ranged from  $0.15 \mu\text{g l}^{-1}$  for acetochlor ESA to  $1.8 \mu\text{g l}^{-1}$  for alachlor OA.

The seventh report is an article quantifying the cell yield of a halorespiring bacterium utilizing 2-chlorophenol (2-CP) and 2,6-dichlorophenol (2,6-DCP) as an electron acceptor (17). Dechlorination was not significantly inhibited by end product phenol up to 8 mM. Specific growth rates averaged  $0.033 \text{ h}^{-1}$  at 50% substrate limitation, that was in agreement with the maximum specific growth rate of  $0.068 \text{ h}^{-1}$ .

The final article evaluated the use of a fungus, *Penicillium camemberti* to dechlorinate adsorbable organic halides (AOX) in pulping effluents (36). An upflow glass wool packed column reactor established with this fungus could be operated for nearly two years in the laboratory. At best, around 70% AOX could be removed from chlorinated pulping wastes in 7.3 h of contact with no aeration and with a minimal amount of carbon supplement ( $0.2 \text{ g l}^{-1}$ ) in the form of acetate.

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

A vinyl chloride (VC) reductive dehalogenase was purified from a highly enriched halorespiring culture, *Dehalococcoides* sp. strain VS (25). The enzyme reduced VC and all dichloroethene (DCE) isomers, but not PCE or TCE. Rates of VC and DCE conversion were high (990 nmol

(mg protein)<sup>-1</sup> min<sup>-1</sup>). Using degenerative primers the dehalogenase gene VC reductase (*vcrA*) was isolated. Based on the predicted amino acid sequence, VC reductase is a novel member of the family of corrinoid/iron-sulfur cluster containing reductive dehalogenases. The *vcrA* gene was found to be cotranscribed with *vcrB*, a gene that encodes a small hydrophobic protein presumably acting as membrane anchor for VC reductase, and *vcrC*, a gene that encodes a regulatory protein. The *vcrAB* genes were subsequently found to be present and expressed in other cultures containing VC-respiring *Dehalococcoides* organisms and could be detected in water samples from a field site contaminated with chlorinated ethenes. The results are significant since *vcrA* can now be used as a marker for VC biodegradation ability at contaminated sites.

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

#### **Detection of Chlorinated Compounds**

In this quarter one article describes the detection of TCE in trees as an indicator of groundwater contamination (37). TCE was detected in the cores of trees growing above TCE-contaminated ground at three sites. Detection was feasible even when the depth to water was 7.9 m or when the contaminated aquifer was confined beneath 3 m of clay. Additional groundwater contaminants detected in the tree cores were cis-1,2-dichloroethene(DCA) at two sites and PCE at one site. Tree coring can be a rapid and effective means of locating shallow subsurface chlorinated ethenes and possibly identifying zones of active TCE dechlorination. Tree cores collected over time were useful in identifying the onset of ground water contamination

#### **Detection of Microbial Populations**

Community analysis by terminal restriction fragment length polymorphism (T-RFLP) was used to describe shifts in the population of an aerobic sequencing batch bioreactor used for the cooxidation of TCE with phenol (2). Additionally the rate of at which biomass samples biotransformed TCE was also monitored. One bioreactor was operated with only phenol and another was operated with phenol and TCE. After initiation of the TCE loading in the second reactor, the TCE transformation rates initially decreased, but they stabilized with an average second-order rate coefficient of 0.044 L mg<sup>-1</sup> d<sup>-1</sup> for 2 years. In contrast, the phenol-fed reactor showed higher and unstable TCE transformation rates, with an average rate coefficient of 0.093

L mg<sup>-1</sup> d<sup>-1</sup>. The phenol-plus-TCE-fed reactor had marked changes in community structure during the first 100 days and remained relatively stable afterwards, corresponding to the period of stable function. In contrast, the community structure of the phenol-fed reactor changed periodically, and the changes coincided with the periodicity observed in the TCE transformation rates. Long-term TCE stress appeared to select for a different and stable community structure, with lower but stable TCE degradation rates. In contrast, the community under no stress exhibited a dynamic structure and dynamic function.

### **Mathematical Tools**

A metabolic pathway prediction system (PPS) was developed and its predictive accuracy was tested (21). The PPS, that is available free of cost at <http://umbbd.ahc.umn.edu/predict/>, predicts transformations based on metabolic rules that were designed chiefly by examining reactions catalogued in the University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD). The system correctly predicted known metabolism for 111 of the 113 selected compounds containing C and H, O, N, S, P and/or halides, and also correctly predicted 410 of the 569 known pathway branches for these compounds.

### **Cell Based Bioassays**

A review of recombinant cell bioassay systems for the detection and relative quantitation of halogenated dioxins and related chemicals was recently published (12). The review focuses on methods based on the ability of these chemicals to activate the aromatic hydrocarbon receptor (AhR) and the AhR signal transduction pathway. Based on existing evidence, the authors conclude that these cell bioassay systems provide a relatively rapid, accurate, and cost effective screening approach for the detection of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) and related HAHs in a variety of environmental, biological, food and feed samples.

## **4. MICROBIAL CHLORINATION**

### **4.a. General Reviews**

Review papers concerned with microbial chlorination were not found during the review period. An interesting work was published that reviews literature from the last four years on the enzymes and intermediates involved in fluorometabolite biosynthesis in the bacterium *Streptomyces*

*cattleya* (11). A particular emphasis is placed on the purification and characterization of the fluorinase, the C–F bond forming enzyme that initiates the biosynthesis of fluorometabolites. Interestingly, the structure and gene sequence of the fluorinase does not bear an obvious relationship to any known enzymatic activity, indicating a rather unique enzyme of limited distribution. The article presents a survey of known fluorinated metabolites in living organisms.

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

##### **Chloromethanes and Other Chlorinated Compounds**

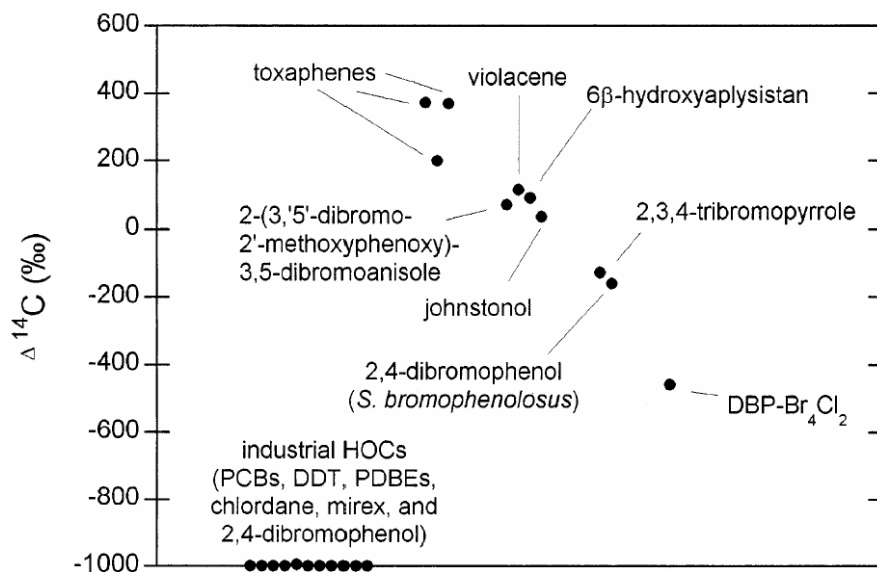
No reports were found during the review period concerning the microbial formation of chloromethane.

Radiocarbon evidence was reported supporting that the compound 1,1'-dimethyl-3,3',4,4'-tetrabromo-5,5'-dichloro-2,2'-bipyrrole (DBP-Br<sub>4</sub>Cl<sub>2</sub>) is naturally produced by a marine organism (30). Numerous studies have detected the latter compound as well as the compound heptachloro-1'-methyl-1,2'-bipyrrole (Q1) in Antarctic air, seabird eggs, the blubber of marine mammals and human milk. To date, it has been difficult to determine whether these compounds are natural products or derived from industrial synthesis, although their predominance in marine as opposed to freshwater environments, mixed halogenation (which is rare for industrial compounds), and relative structural similarity to known metabolites suggest that they are marine natural products. The results of this study confirm the presence of detectable <sup>14</sup>C in the DBP-Br<sub>4</sub>Cl<sub>2</sub>, which strongly points to at least a natural or biogenic source. Most industrial compounds are manufactured from petrochemicals (<sup>14</sup>C-free). The <sup>14</sup>C levels detected in DBP-Br<sub>4</sub>Cl<sub>2</sub> were more depleted than expected for a recently synthesized natural product (Figure 1). The authors discuss various hypotheses that could explain the latter finding.

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

##### **Chloromethanes**

This quarter reports were not found on the natural formation of chloromethanes and related methylhalides by marine microorganisms.



**FIGURE 5.** Radiocarbon content of natural and industrially synthesized HOCs. The  $^{14}\text{C}$  content of the synthetic HOCs shown in the bottom left corner, which include Aroclors 1242, 1254, 1260, and 1268, Clophen A60, Bromkal 70-5DE, DDT, Phenoclor DP-6, and chlordane, have been previously reported in ref 11. In addition, 2,4-dibromophenol (purchased from Sigma and analyzed as part of this study) resides with the latter group of compounds in this figure. The data for the three toxaphenes and 2-(3',5'-dibromo-2'-methoxyphenoxy)-3,5-dibromoanisole were previously presented in ref 13.

**Figure 1.** Radiocarbon content of natural and industrially synthesized halogenated organic compounds (30).

### Other Chlorinated Compounds

Eleven novel chlorinated lipids, taveuniamides, were isolated from two mixed Fijian collections of the marine cyanobacteria *Lyngbya majuscula* and *Schizothrix* (40). Some of these metabolites were potent brine shrimp toxins with  $\text{LD}_{50}$  values ranging between 1.7-1.9  $\mu\text{g ml}^{-1}$ . Three new iodinated tryptophan derivatives, plakohypaphorines D-F, were isolated from the Caribbean sponge *Plakortis simplex* (5). One of these compounds is unique in that it possesses both chlorine and iodine atoms on the indole nucleus. Halogenated diterpenoids were obtained from three collections of the red alga *Laurencia nipponica* (23). Bromophenol derivatives from the

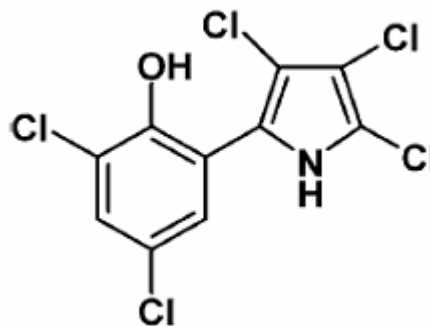
red alga *Rhodomela confervoides* were isolated and their chemical structure was elucidated (43). Dibenzyl bromophenols with diverse dimerization patterns were obtained from the extracts of the brown alga *Leathesia nana* (42)

#### 4.d. Chlorinating Enzymes

This quarter two halogenating enzymes were described involved in the synthesis of naturally chlorinated antibiotics. The first is a novel halogenase gene involved in the biosynthesis of pentachloropseudilin (Figure 2) (41). The novel halogenase gene was isolated from the actinomycete, *Actinoplanes* sp. ATCC 33002. The halogenase has high identity (55%) to the flavin-dependent monodechloroamino-pyrrolnitrin-3 halogenase from pyrrolnitrin biosynthesis and to the halogenases PltM and PltA (35% and 28%, respectively) involved in pyoluteorin biosynthesis.

The gene could be heterologously expressed in *Pseudomonas aureofaciens* ACN as soluble protein. Chlorinating activity of HalB was shown with two synthetic substrates with structural similarity to pentachloropseudilin. HalB is the first halogenase from an actinomycete and only the third halogenase for which halogenating activity could be demonstrated in vitro.

The gene of tryptophan 7-halogenase was isolated from the *Pseudomonas aureofaciens* ACN strain producing pyrrolnitrin, a chloro-containing antibiotic, and sequenced (7). A high homology degree (over 95%) was established for the genes and the corresponding halogenases from *R. aureofaciens* ACN and *P. fluorescens* BL915. The tryptophan 7-halogenase gene was amplified by PCR, and the corresponding enzyme was expressed in *Escherichia coli* cells.



**Figure 2.** The chemical structure of pentachloropseudilin.

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## 6. ANNEX

**Arcangeli, J. P. and E. Arvin (2004). "Methanotrophic biodegradation of cis-1,2-dichloroethylene in a continuously fed fixed-film bioreactor." *Water Science and Technology* 49(11-12): 231-236.**

Co-metabolic biodegradation of cis-dichloroethylene (cis-DCE) was investigated in a bench-scale fixed-film bioreactor inoculated with a mixed culture of methane oxidising bacteria. The aim of this work was to identify factors that affect the cis-DCE biodegradation. It was observed that the presence of methane was necessary to enhance the biodegradation of cis-DCE, but an excess of methane inhibited the cis-DCE removal. cis-DCE did not inhibit the methane biodegradation at concentrations up to 300 µg/L. Maximum cis-DCE removal was observed with a methane bulk concentration ranging from 0.2 to 0.7 mg/L. It was found that the activity of the biofilm was located in the upper 100 µm of the biofilm. On the basis of this study it is concluded that careful control of the oxygen and methane concentrations as well as of the biofilm thickness is necessary in order to optimise the biodegradation of cis-DCE in fixed film bioreactors.

**Ayala-del-Rio, H. L., S. J. Callister, et al. (2004). "Correspondence between community structure and function during succession in phenol- and phenol-plus-trichloroethene-fed sequencing batch reactors." *Applied and Environmental Microbiology* 70(8): 4950-4960.**

The effects of more than 2 years of trichloroethene (TCE) application on community succession and function were studied in two aerobic sequencing batch reactors. One reactor was fed phenol, and the second reactor was fed both phenol and TCE in sequence twice per day. After initiation of TCE loading in the second reactor, the TCE transformation rates initially decreased, but they stabilized with an average second-order rate coefficient of 0.044 liter mg<sup>(-1)</sup> day<sup>(-1)</sup> for 2 years. In contrast, the phenol-fed reactor showed higher and unstable TCE transformation rates, with an average rate coefficient of 0.093 liter mg<sup>(-1)</sup> day<sup>(-1)</sup>. Community analysis by terminal restriction fragment length polymorphism (T-RFLP) analysis of the 16S rRNA genes showed that the phenol-plus-TCE-fed reactor had marked changes in community structure during the first 100 days and remained relatively stable afterwards, corresponding to the period of stable function. In contrast, the community structure of the phenol-fed reactor changed periodically, and the changes coincided with the periodicity observed in the TCE transformation rates. Correspondence analysis of each reactor community showed that different community structures corresponded with function (TCE degradation rate). Furthermore, the phenol hydroxylase genotypes, as determined by restriction fragment length polymorphism analysis, corresponded to community structure patterns identified by T-RFLP analysis and to periods when the TCE transformation rates were high. Long-term TCE stress appeared to select for a different and stable community structure, with lower but stable TCE degradation rates. In contrast, the community under no stress exhibited a dynamic structure and dynamic function.

**Bagley, D. M., I. G. Sutherland, et al. (2004). "Non-enzymatic degradation of chlorofluorocarbon 113 using cyanocobalamin under anaerobic conditions." *Journal of Environmental Engineering and Science* 3(4): 295-299.**

Chlorofluorocarbon 113 (CFC113) was rapidly and completely degraded non-enzymatically in systems containing cyanocobalamin with titanium (III) citrate as the reductant. Hydrochlorofluorocarbon 123a (HCFC123a) accounted for up to 25% of the degraded CFC113. Chlorotrifluoroethene was also detected. Increasing concentrations of cyanocobalamin increased CFC113 removal rates asymptotically and also decreased the fraction of HCFC123a remaining.

**Berselli, S., G. Milone, et al. (2004). "Effects of cyclodextrins, humic substances, and rhamnolipids on the washing of a historically contaminated soil and on the aerobic bioremediation of the resulting effluents." *Biotechnology and Bioengineering* 88(1): 111-120.**

Nontoxic and biodegradable pollutant-mobilizing agents, instead of chemical surfactants, were tested in the washing of an actual-site chloroaromatic-contaminated soil. A soil historically contaminated by chlorinated anilines and benzenes, thiophenes and several polycyclic aromatic hydrocarbons was subjected to washing by suspending it (15% w/v) in water or in water with 1.0% (w/v) p-cyclodextrin (beta-CD), hydroxypropyl-beta-cyclodextrin (HP-

beta-CD), rhamnolipid (RL), dissolved humic substances (HS), or Triton X-100 (TX) in shaken batch reactors for 24 hr. The resulting wastewaters were amended with nutrients and treated aerobically in shaken reactors for 65 days. The biogenic agents markedly enhanced (by 237%, beta-CD; 265%, HP-beta-CD; 400%, RL; 566%, HS) the capability of water of eluting organic pollutants from the soil. TX enhanced the overall pollutant removal by about 660%; however, a lower depletion of the initial soil ecotoxicity, along with a more extensive impact on the soil organic matter, was observed. Furthermore, TX adversely affected the bioremediation of the resulting effluent by apparently inducing a premature decrease of specialized bacterial biomass. By contrast, the biogenic agents, and in particular HS and RL, sustained the biodegradation and dechlorination of pollutants by apparently enhancing the availability of specialized bacteria in the reactors. Thus, the biogenic agents proposed here seem to be promising nontoxic and nonaggressive soil washing agents for the integrated physicochemical (washing) and biological (aerobic posttreatment) restoration of poorly bioremediable (chloro) organics-contaminated soils. (C) 2004 Wiley Periodicals, Inc.

**Borrelli, F., C. Campagnuolo, et al. (2004). "Iodinated indole alkaloids from *Plakortis simplex* - New plakohypaphorines and an evaluation of their antihistamine activity." *European J Organic Chemistry* 15: 3227-3232.**

Three new iodinated tryptophan derivatives, plakohypaphorines D-F (4-6), have been isolated from the Caribbean sponge *Plakortis simplex*. Their structures have been determined by applying spectroscopic methods and microwave-assisted selective dehalogenation. Compound 5 is the first naturally occurring triiodinated indole, while compound 6 is a unique metabolite because it possesses both chlorine and iodine atoms on the indole nucleus. We have evaluated the antihistamine activity of the whole series of plakohypaphorines A-F, but only the diiodinated analogues proved to be active: they display a specific antagonism of the noncompetitive type. (C) Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2004.

**Boucard, T. K., R. D. Bardgett, et al. (2005). "Influence of plants on the chemical extractability and biodegradability of 2,4-dichlorophenol in soil." *Environmental Pollution* 133(1): 53-62.**

This study investigated the fate and behaviour of [UL-C-14] 2,4-dichlorophenol (DCP) in planted (*Lolium perenne* L.) and unplanted soils over 57 days. Extractability of [UL-C-14] 2,4-DCP associated activity was measured using calcium chloride (CaCl<sub>2</sub>), acetonitrile-water and dichloromethane (DCM) extractions. Biodegradability of [UL-C-14] 2,4-DCP associated activity was assessed through measurement of (CO<sub>2</sub>)-C-14 production by a degrader inoculum (*Burkholderia* sp.). Although extractability and mineralisation of [UL-C-14] 2,4-DCP associated activity decreased significantly in both planted and unplanted soils, plants appeared to enhance the sequestration process. After 57 days, in unplanted soil, 27% of the remaining [UL-C-14] 2,4-DCP associated activity was mineralised by *Burkholderia* sp., and 13%, 48%, and 38% of C-14-activity were extracted by CaCl<sub>2</sub>, acetonitrile-water and DCM, respectively. However, after 57 days, in planted soils, only 10% of the [UL-C-14] 2,4-DCP associated activity was available for mineralisation, whilst extractability was reduced to 2% by CaCl<sub>2</sub>, 17% by acetonitrile-water and 11% by DCM. This may be due to the effect of plants on soil moisture conditions, which leads to modification of the soil structure and trapping of the compound. However, the influence of plants on soil biological and chemical properties may also play a role in the ageing process. (C) 2004 Elsevier Ltd. All rights reserved.

**Burd, V. N. and K. H. van Pee (2004). "Tryptophan 7-halogenase from *Pseudomonas aureofaciens* ACN strain: Gene cloning and sequencing and the enzyme expression." *Russian Journal of Bioorganic Chemistry* 30(4): 353-357.**

The gene of tryptophan 7-halogenase was isolated from the *Pseudomonas aureofaciens* ACN strain producing pyrrolnitrin, a chlorocontaining antibiotic, and sequenced. A high homology degree (over 95%) was established for the genes and the corresponding halogenases from *R. aureofaciens* ACN and *P. fluorescens* BL915. The tryptophan 7-halogenase gene was amplified by PCR, and the corresponding enzyme was expressed in *Escherichia coli* cells using the pBSII SK+ vector.

**Conant, B., J. A. Cherry, et al. (2004). "A PCE groundwater plume discharging to a river: influence of the streambed and near-river zone on contaminant distributions." *Journal of Contaminant Hydrology* 73(1-4): 249-279.**

An investigation of a tetrachloroethene (PCE) groundwater plume originating at a dry cleaning facility on a sand aquifer and discharging to a river showed that the near-river zone strongly modified the distribution, concentration, and composition of the plume prior to discharging into the surface water. The plume, streambed concentration, and hydrogeology were extensively characterized using the Waterloo profiler, mini-profiler, conventional and driveable multilevel samplers (MLS), Ground Penetrating Radar (GPR) surveys, streambed temperature mapping (to identify discharge zones), drivepoint piezometers, and soil coring and testing. The plume observed in the shallow streambed deposits was significantly different from what would have been predicted based on the characteristics of the upgradient plume. Spatial and temporal variations in the plume entering the near-river zone contributed to the complex contaminant distribution observed in the streambed where concentrations varied by factors of 100 to 5000 over lateral distances of less than 1 to 3.5 m. Low hydraulic conductivity semi-confining deposits and geological heterogeneities at depth below the streambed controlled the pattern of groundwater discharge through the streambed and influenced where the plume discharged into the river (even causing the plume to spread out over the full width of the streambed at some locations). The most important effect of the near-river zone on the plume was the extensive anaerobic biodegradation that occurred in the top 2.5 m of the streambed, even though essentially no biodegradation of the PCE plume was observed in the upgradient aquifer. Approximately 54% of the area of the plume in the streambed consisted solely of PCE transformation products, primarily cis-1,2-dichloroethene (cDCE) and vinyl chloride (VC). High concentrations in the interstitial water of the streambed did not correspond to high groundwater discharge zones, but instead occurred in low discharge zones and are likely sorbed or retarded remnants of past high-concentration plume discharges. The high-concentration areas (up to 5529 µg/l of total volatile organics) in the streambed are of ecological concern and represent potential adverse exposure locations for benthic and hyporheic zone aquatic life, but the effect of these exposures on the overall health of the river has yet to be determined. Even if the upgradient source of PCE is remediated and additional PCE is prevented from reaching the streambed, the high-concentration deposits in the streambed will likely take decades to hundreds of years to flush completely clean under natural conditions because these areas have low vertical groundwater flow velocities and high retardation factors. Despite high concentrations of contaminants in the streambed, PCE was detected in the surface water only rarely due to rapid dilution in the river and no cDCE or VC was detected. Neither the sampling of surface water nor the sampling of the groundwater from the aquifer immediately adjacent to the river gave an accurate indication of the high concentrations of PCE biodegradation products present in the streambed. Sampling of the interstitial water of the shallow streambed deposits is necessary to accurately characterize the nature of plumes discharging to rivers. (C) 2004 Elsevier B.V. All rights reserved.

**Cupples, A. M., A. M. Spormann, et al. (2004). "Comparative evaluation of chloroethene dechlorination to ethene by Dehalococcoides-like microorganisms." *Environmental Science & Technology* 38(18): 4768-4774.**

Reductive dehalogenation of tetra chloroethene (PCE), trichloroethene (TCE), cis-1,2-dichloroethene (NE), and vinyl chloride (VC) was examined in four cultures containing Dehalococcoides-like microorganisms. Dechlorination and growth kinetics were compared using a Monod growth-rate model for multiple electron acceptor usage with competition. Included were the Victoria mixed culture containing Dehalococcoides species strain VS (from Victoria, TX), the mixed culture KB-1/VC (from southern Ontario), the Pinellas mixed culture (from Pinellas, FL), and *D. ethenogenes* strain 195. All cultures, with the exception of *D. ethenogenes* strain 195, grew with VC as catabolic electron acceptor. A dilution method was developed that allows a valid comparison to be made of dehalogenating kinetics between different mixed cultures. Using this procedure, maximum growth rates on VC were found to be similar for strain VS and KB-1/VC (0.42-0.49 +/- 0.02 d(-1)) but slower for the Pinellas culture (0.28 +/- 0.01 d(-1)). The 16S rRNA gene sequences were determined to ensure that no cross contamination between cultures had occurred. Following enrichment of the VC dechlorinating microorganisms on VC, the cultures were amended with DCE, TCE, or PCE. The three mixed cultures failed to dechlorinate PCE or did so very slowly. However, the dilution technique indicated that all experienced growth on TCE and DCE as well as on VC. Maximum growth rates on DCE alone were quite similar (0.43-0.46 d(-1)), while the Pinellas culture grew faster on TCE alone (0.49 d(-1)) than did the other two mixed cultures (0.33-0.35 d(-1)). Half-velocity and inhibition constants for growth on TCE were also determined for the three mixed cultures; both constants were found to be essentially equal and the same for the different cultures, varying between only 8.6 and 10.5 µM. The ability of the strain VS, KB-1/VC, and Pinellas cultures to utilize TCE rapidly with conversion to ethene is quite different from that of any other reported microorganism. It was separately confirmed with more traditional cell-counting techniques that strain VS coupled

TCE, as well as DCE and VC, utilization with growth. This is the first report of an organism obtaining energy for growth through every step in the reduction of TCE to ethene. Also, as suggested by the dilution technique, the dehalogenating organisms in the KB-1/VC and Pinellas cultures appear to obtain growth from TCE utilization as well. Such ability to grow while dehalogenating TCE to ethene will be an important advantage for their use in bioaugmentation.

**Davis, J. A. (2004). "The long-term fate of polychlorinated biphenyls in San Francisco Bay (USA)." *Environmental Toxicology and Chemistry* 23(10): 2396-2409.**

A simple one-box mass budget model is presented as a first step toward a quantitative understanding of the long-term fate of polychlorinated biphenyls (PCBs) in San Francisco Bay (USA). Sensitivity analysis indicated that the most influential input parameters were degradation half-life in sediment,  $K_{ow}$ , outflow, average PCB concentration in sediment, and depth of the active sediment layer. Moderately influential parameters included organic carbon content of suspended solids, sediment burial mass transfer coefficient, and Henry's law constant. If external loading could be eliminated entirely, the mass of PCBs in the bay is predicted to drop to half of the present value in 20 years. The model predicts that sustained loading of 10 kg year<sup>-1</sup> would prevent the total PCB mass in the bay from ever dropping below 10% of the present mass. With a sustained loading of 20 kg year<sup>-1</sup>, the model predicts that the total PCB mass would never fall below about 25% of the present mass. The half-lives in the bay for the individual PCB congeners evaluated in this report ranged from four years for PCB 18 to 30 years for PCB 194.

**Deng, H., D. O'Hagan, et al. (2004). "Fluorometabolite biosynthesis and the fluorinase from *Streptomyces cattleya*." *Natural Product Reports* 21(6): 773-784.**

Covering: the literature from 2000–2004. This review outlines the recent developments in uncovering the enzymes and intermediates involved in fluorometabolite biosynthesis in the bacterium *Streptomyces cattleya*. A particular emphasis is placed on the purification and characterisation of the fluorinase, the C–F bond forming enzyme which initiates the biosynthesis. Nature has hardly developed a biochemistry around fluorine, yet fluorinated organics are important commercial entities, therefore a biotransformation from inorganic to organic fluorine is novel and of contemporary interest.

**Denison, M. S., B. Zhao, et al. (2004). "Recombinant cell bioassay systems for the detection and relative quantitation of halogenated dioxins and related chemicals." *Talanta* 63(5): 1123-1133.**

Proper epidemiological, risk assessment and exposure analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related halogenated aromatic hydrocarbons (HAHs) requires accurate measurements of these chemicals both in the species of interest and in various exposure matrices (i.e. biological, environmental, food and feed). High-resolution instrumental analysis techniques are established for these chemicals, however, these procedures are very costly and time-consuming and as such, they are impractical for large scale sampling studies (i.e. for epidemiological studies and assessment of areas with widespread contamination). Accordingly, numerous bioanalytical methods have been developed for the detection of these chemicals in extracts from a variety of matrices, the majority of which take advantage of the ability of these chemicals to activate the aromatic hydrocarbon receptor (AhR) and the AhR signal transduction pathway. Here we review the currently available in vitro AhR-based cell bioassay systems with a focus on recent recombinant reporter gene cell lines that have been developed for detection and relative quantitation of TCDD and related HAHs. Comparison of the relative sensitivities of the various cell bioassays and examples of their use in screening and analysis of environmental, biological, and food and feed samples are presented. Currently available experimental results and validation studies demonstrate the utility of these cell bioassay systems to provide a relatively rapid, accurate, and cost effective screening approach for the detection of TCDD and related HAHs in a variety of environmental, biological, food and feed samples. The availability of these cell bioassay systems will not only facilitate the large scale sampling studies needed for accurate assessment of contamination and exposure to these environmental chemicals, but they provide avenues for the identification of novel classes of TCDD-like chemicals. (C) 2004 Elsevier B.V. All rights reserved.

**El-Zahab, B., L. Meza, et al. (2004). "Enzymatic degradation of trichloroethylene using enzyme extracts isolated from a bacterial consortium." *Applied Biochemistry and Biotechnology* 117(3): 165-174.**

Degradation of trichloroethylene (TCE) using crude enzyme extracts from a bacterial consortium was examined for wastewater treatment. The effects of pH, chemical induction, and cofactor were investigated. Enzyme extracts showed an optimal activity (3.03 +/- 0.03 mg of TCE/[mg of protein.d]) at neutral pH (6.5-7.5). In an attempt to increase the production of effective enzymes for TCE degradation, chemical induction using both toluene and TCE in the growth of the bacterium consortium was conducted. Although the induction increased the overall production of protein by about fourfold, the activity of the extracts was only slightly improved (up to 3.40 mg of TCE/[mg of protein.d]), indicating that the induction did not specifically enhance the production of TCE-degrading enzymes. Interestingly, the addition of a cofactor (up to 0.02 mg/mL), NADH, led to an initial reaction rate of 5.30 +/- 0.05 mg of TCE/(mg of protein.d). This observation demonstrated that the availability of the cofactor played an important role in determining the overall degradation reaction rates. The observations with NADH were in agreement with the assumption that toluene monooxygenases (which are NADH dependent) are the key enzymes for the degradation reactions.

**Falta, R. W. (2004). "The potential for ground water contamination by the gasoline lead scavengers ethylene dibromide and 1,2-dichloroethane." *Ground Water Monitoring and Remediation* 24(3): 76-87.**

Ethylene dibromide (EDB) is a synthetic organic chemical that was produced in large amounts for use as a leaded gasoline additive and pesticide. The chlorinated solvent 1,2-dichloroethane (1,2-DCA) is widely used in the chemical industry, and was also added to leaded gasoline. EDB and 1,2-DCA are classified as probable human carcinogens by the U.S. Environmental Protection Agency (U.S. EPA), and EDB's use as a pesticide was suspended in 1984. The current U.S. EPA maximum contaminant level (MCL) for EDB in drinking water is 0.05 mug/L, and the MCL for 1,2-DCA is 5 mug/L. EDB has proven to be both mobile and persistent in ground water, and contamination of ground water by EDB was documented in several states beginning in the early 1980s. The majority of this contamination is attributed to agricultural uses of EDB; however, similar to 90% of the EDB produced was used as a leaded gasoline additive and it was present in virtually all leaded gasoline sold in the United States. 1,2-DCA is commonly found as a ground water contaminant, and it is both mobile and persistent. Past investigations and remediation efforts at sites contaminated by leaded gasoline have rarely addressed the potential for EDB or 1,2-DCA contamination. For this reason, there is a substantial likelihood that undetected EDB and 1,2-DCA plumes above the MCL may exist at many sites where leaded gasoline leaked or spilled.

**Freedman, D. L., M. Swamy, et al. (2004). "Biodegradation of chloromethane by *Pseudomonas aeruginosa* strain NB1 under nitrate-reducing and aerobic conditions." *Applied and Environmental Microbiology* 70(8): 4629-4634.**

*Pseudomonas aeruginosa* strain NB1 uses chloromethane (CM) as its sole source of carbon and energy under nitrate-reducing and aerobic conditions. The observed yield of NB1 was 0.20 (+/-0.06) (mean standard deviation) and 0.28 (+/-0.01) mg of total suspended solids (TSS) mg of CM-1 under anoxic and aerobic conditions, respectively. The stoichiometry of nitrate consumption was 0.75 (+/-0.10) electron equivalents (eeq) of NO per eeq of CM, which is consistent with the yield when it is expressed on an eeq basis. Nitrate was stoichiometrically converted to dinitrogen (0.51 +/- 0.05 mol of N<sub>2</sub> per mol of NO<sub>3</sub><sup>-</sup>). The stoichiometry of oxygen use with CM (0.85 +/- 0.21 eeq of O<sub>2</sub> per eeq of CM) was also consistent with the aerobic yield. Stoichiometric release of chloride and minimal accumulation of soluble metabolic products (measured as chemical oxygen demand) following CM consumption, under anoxic and aerobic conditions, indicated complete biodegradation of CM. Acetylene did not inhibit CM use under aerobic conditions, implying that a monooxygenase was not involved in initiating aerobic CM metabolism. Under anoxic conditions, the maximum specific CM utilization rate (k) for NB1 was 5.01 (+/-0.06) mumol of CM mg of TSS-1 day<sup>-1</sup>, the maximum specific growth rate (mu(max)) was 0.0506 day<sup>-1</sup>, and the Monod half-saturation coefficient (K<sub>s</sub>) was 0.067 (+/-0.004) muM. Under aerobic conditions, the values for k, mu(max), and K<sub>s</sub> were 10.7 (+/-0.11) mumol of CM mg of TSS-1 day<sup>-1</sup>, 0.145 day<sup>-1</sup>, and 0.93 (+/-0.042) muM, respectively, indicating that NB1 used CM faster under aerobic conditions. Strain NB1 also grew on methanol, ethanol, and acetate under denitrifying and aerobic conditions, but not on methane, formate, or dichloromethane.

**Griffin, B. M., J. M. Tiedje, et al. (2004). "Anaerobic microbial reductive dechlorination of tetrachloroethene to predominately trans-1,2-dichloroethene." *Environmental Science & Technology* 38(16): 4300-4303.**

While most sites and all characterized PCE and TCE dechlorinating anaerobic bacteria produce cis-DCE as the major DCE isomer, significant amounts of trans-DCE are found in the environment. We have obtained microcosms from some sites and enrichment cultures that produce more trans-DCE than cis-DCE. These cultures reductively dechlorinated PCE and TCE to trans-DCE and cis-DCE simultaneously and in a ratio of 3(+/-0.5):1 that was stable through serial transfers with a variety of electron donors and occurred in both methanogenic and nonmethanogenic enrichments. Two sediment-free, nonmethanogenic enrichment cultures produced trans-DCE at rates of up to 2.5  $\mu\text{mol L}^{-1} \text{ day}^{-1}$ . Dehalococcoides populations were detected in both trans-DCE producing cultures by their 16S rRNA gene sequences, and trans-DCE was produced in the presence of ampicillin. Because trans-DCE can be the major product from PCE and TCE microbial dechlorination, high fractions of trans-DCE at chloroethene-contaminated sites are not necessarily from source contamination.

**He, Q. and R. A. Sanford (2004). "The generation of high biomass from chlororespiring bacteria using a continuous fed-batch bioreactor." *Applied Microbiology and Biotechnology* 65(4): 377-382.**

A continuous fed-batch reactor system was developed to rapidly obtain dense chlororespiring cultures of *Anaeromyxobacter dehalogenans* strain 2CP-C. A syringe pump continuously delivered concentrated 2,6-dichlorophenol (50-150 mM) to an anaerobic reactor vessel at a rate that sustained linear growth but prevented the substrate toxicity of chlorophenol. Dechlorination was not significantly inhibited by end product phenol up to 8 mM. A cell density of 76.8 mg protein  $\text{L}^{-1}$  was obtained in 24 h. Specific growth rates averaged 0.033  $\text{h}^{-1}$  at 50% substrate limitation, which was in agreement with the maximum specific growth rate of 0.068  $\text{h}^{-1}$ . This reactor system provides an efficient, cost-effective, and convenient method to rapidly obtain dense dechlorinating biomass and is promising to accelerate investigations of enzymes involved in chlororespiration.

**Hirschorn, S. K., M. J. Dinglasan, et al. (2004). "Pathway dependent isotopic fractionation during aerobic biodegradation of 1,2-dichloroethane." *Environmental Science & Technology* 38(18): 4775-4781.**

1,2-Dichloroethane (1,2-DCA) is a widespread groundwater contaminant known to be biodegradable under aerobic conditions via enzymatic oxidation or hydrolytic dehalogenation reactions. Current literature reports that stable carbon isotope fractionation of 1,2-DCA during aerobic biodegradation is large and reproducible (-27 to -33 parts per thousand). In this study, a significant variation in the magnitude of stable carbon isotope fractionation during aerobic biodegradation was observed. Biodegradation in experiments involving microcosms, enrichment cultures, and pure microbial cultures produced a consistent bimodal distribution of enrichment factors ( $\epsilon$ ) with one mean  $\epsilon$  centered on -3.9 +/- 0.6 parts per thousand and the other on -29.2 +/- 1.9 parts per thousand. Reevaluation of  $\epsilon$  in terms of kinetic isotope effects  $(^{12}\text{C}/^{13}\text{C})_k$  gave values of  $(^{12}\text{C}/^{13}\text{C})_k = 1.01$  and 1.06, which are typical of oxidation and hydrolytic dehalogenation ( $\text{S}(\text{N})_2$ ) reactions, respectively. The bimodal distribution is therefore consistent with the microbial degradation of 1,2-DCA by two separate enzymatic pathways. This interpretation is further supported in this study by experiments with pure strains of *Xanthobacter autotrophicus* GJ10, *Ancylobacter aquaticus* AD20, and *Pseudomonas* sp. Strain DCA1 for which the enzymatic degradation pathways are well-known. A small fractionation of -3.0 parts per thousand was measured for 1,2-DCA degradation by *Pseudomonas* sp. Strain DCA1 (monooxygenase enzyme), while degradation by the hydrolytic dehalogenase enzyme by the other two pure strains was characterized by fractionation of -32.3 parts per thousand.

**Holscher, T., R. Krajmalnik-Brown, et al. (2004). "Multiple nonidentical reductive-dehalogenase-homologous genes are common in Dehalococcoides." *Applied and Environmental Microbiology* 70(9): 5290-5297.**

Degenerate primers were used to amplify large fragments of reductive-dehalogenase-homologous (RDH) genes from genomic DNA of two *Dehalococcoides* populations, the chlorobenzene- and dioxin-dechlorinating strain CBDB1 and the trichloroethene-dechlorinating strain FL2. The amplicons (1,350 to 1,495 bp) corresponded to nearly complete open reading frames of known reductive dehalogenase genes and short fragments (approximately 90 bp) of genes encoding putative membrane-anchoring proteins. Cloning and restriction analysis revealed the presence of at least 14 different RDH genes in each strain. All amplified RDH genes showed sequence similarity with known reductive dehalogenase genes over the whole length of the sequence and shared all characteristics described for reductive dehalogenases. Deduced amino acid sequences of seven RDH genes from strain CBDB1 were 98.5 to 100% identical to seven different RDH genes from strain FL2, suggesting that both strains have an

overlapping substrate range. All RDH genes identified in strains CBDB1 and FL2 were related to the RDH genes present in the genomes of *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp. strain BAV1; however, sequence identity did not exceed 94.4 and 93.1%, respectively. The presence of RDH genes in strains CBDB1, FL2, and BAV1 that have no orthologs in strain 195 suggests that these strains possess dechlorination activities not present in strain 195. Comparative sequence analysis identified consensus sequences for cobalamin binding in deduced amino acid sequences of seven RDH genes. In conclusion, this study demonstrates that the presence of multiple nonidentical RDH genes is characteristic of *Dehalococcoides* strains.

**Hou, B. K., L. B. M. Ellis, et al. (2004). "Encoding microbial metabolic logic: predicting biodegradation." *Journal of Industrial Microbiology & Biotechnology* 31(6): 261-272.**

Prediction of microbial metabolism is important for annotating genome sequences and for understanding the fate of chemicals in the environment. A metabolic pathway prediction system (PPS) has been developed that is freely available on the world wide web (<http://umbbd.ahc.umn.edu/predict/>), recognizes the organic functional groups found in a compound, and predicts transformations based on metabolic rules. These rules are designed largely by examining reactions catalogued in the University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD) and are generalized based on metabolic logic. The predictive accuracy of the PPS was tested: (1) using a 113-member set of compounds found in the database, (2) against a set of compounds whose metabolism was predicted by human experts, and (3) for consistency with experimental microbial growth studies. First, the system correctly predicted known metabolism for III of the 113 compounds containing C and H, O, N, S, P and/or halides that initiate existing pathways in the database, and also correctly predicted 410 of the 569 known pathway branches for these compounds. Second, computer predictions were compared to predictions by human experts for biodegradation of six compounds whose metabolism was not described in the literature. Third, the system predicted reactions liberating ammonia from three organonitrogen compounds, consistent with laboratory experiments showing that each compound served as the sole nitrogen source supporting microbial growth. The rule-based nature of the PPS makes it transparent, expandable, and adaptable.

**Kastanek, F., P. Kastanek, et al. (2004). "Decontamination of wastewater contaminated by polychlorinated biphenyls (PCBs)." *Water Science and Technology* 50(2): 131-138.**

Wastewater contaminated by PCBs obtained from three different sources was treated at both laboratory and pilot plant scale conditions by ultraviolet oxidation of organics at the presence of hydrogen peroxide after partial adsorption of impurities and PCBs on activated carbon and/or activated bentonite. The procedure was conducted both with and without a Fe(II) catalyst and considerable reduction of PCB concentration was achieved in both cases. In pilot plant scale experiments, activated carbon polishing step followed UV oxidation. The following three types of contaminated waste water were examined: a) aqueous extracts originated in the course of clean-up of contaminated soil by extraction with aqueous solvents. Concentrations of PCBs in extracts were between 1 µg/L to 3,000 µg/L; b) wastewater condensates originated in the process of thermal desorption of PCB from soils. Concentrations of PCBs in condensates were between 300 µg/L and 5,000 µg/L. c) underground water contaminated by PCBs extracted from the sites of old contamination. The content of PCBs was up to 50,000 ng/L. Biodegradation of PCBs with a mixture of indigenous soil bacteria (selected strains of *Pseudomonas* and *Acetivibrio*) was also tested. It was carried out in a reactor with volume of 1.5 m<sup>3</sup> by application of the bacteria in a slurry of bentonite with adsorbed PCBs.

**Mordaunt, C. J., B. Geva, et al. (2005). "Formation of non-extractable pesticide residues: observations on compound differences, measurement and regulatory issues." *Environmental Pollution* 133(1): 25-34.**

Six major use pesticides (Atrazine, Dicamba, Isoproturon, Lindane, Paraquat and Trifluralin) with differing physico-chemical properties were evaluated for the significance of 'bound' or non extractable residue formation. Investigations were carried out in purpose-built microcosms where mineralization, volatilisation, 'soil water' extractable and organic solvent extractable residues could be quantified. Extractable residues were defined as those accessible by sequential extraction where the solvent used became increasingly non-polar. Dichloromethane was the 'harshes't solvent used at the end of the sequential extraction procedure. C-14- labelled volatilised and (CO<sub>2</sub>)-C-14 fractions were trapped on exit from the microcosm. The pesticides were categorised into 3 classes based on their behaviour. (i) Type A (Atrazine, Lindane and Trifluralin) in which ring degradation was limited as was the formation of non-extractable residues; the remainder of the C-14-activity was found in the extractable fraction. (ii)

Type B (Dicamba and Isoproturon) in which approximately 25% of the C-14-activity was mineralised and a large portion was found in the non-extractable fraction after 91 days. Finally, Type C (Paraquat) in which almost all of the C-14-activity was quickly incorporated into the non-extractable fraction. The implications of the data are discussed, with respect to the variability and significance of regulatory aspects of non-extractable residues. (C) 2004 Elsevier Ltd. All rights reserved.

**Muller, J. A., B. M. Rosner, et al. (2004). "Molecular identification of the catabolic vinyl chloride reductase from Dehalococcoides sp strain VS and its environmental distribution." *Applied and Environmental Microbiology* 70(8): 4880-4888.**

Reductive dehalogenation of vinyl chloride (VC) to ethene is the key step in complete anaerobic degradation of chlorinated ethenes. VC-reductive dehalogenase was partially purified from a highly enriched culture of the VC-respiring *Dehalococcoides* sp. strain VS. The enzyme reduced VC and all dichloroethene (DCE) isomers, but not tetrachloroethene (PCE) or trichloroethene (TCE), at high rates. By using reversed genetics, the corresponding gene (*vcrA*) was isolated and characterized. Based on the predicted amino acid sequence, VC reductase is a novel member of the family of corrinoid/iron-sulfur cluster containing reductive dehalogenases. The *vcrA* gene was found to be cotranscribed with *vcrB*, encoding a small hydrophobic protein presumably acting as membrane anchor for VC reductase, and *vcrC*, encoding a protein with similarity to transcriptional regulators of the NosR/NirI family. The *vcrAB* genes were subsequently found to be present and expressed in other cultures containing VC-respiring *Dehalococcoides* organisms and could be detected in water samples from a field site contaminated with chlorinated ethenes. Therefore, the *vcrA* gene identified here may be a useful molecular target for evaluating, predicting, and monitoring in situ reductive VC dehalogenation.

**Ohtsubo, Y., T. Kudo, et al. (2004). "Strategies for bioremediation of polychlorinated biphenyls." *Applied Microbiology and Biotechnology* 65(3): 250-258.**

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

**Parales, R. E. and J. D. Haddock (2004). "Biocatalytic degradation of pollutants." *Current Opinion in Biotechnology* 15(4): 374-379.**

Microbial reactions play key roles in biocatalysis and biodegradation. The recent genome sequencing of environmentally relevant bacteria has revealed previously unsuspected metabolic potential that could be exploited for useful purposes. For example, oxygenases and other biodegradative enzymes are benign catalysts that can be used for the production of industrially useful compounds. In conjunction with their biodegradative capacities, bacterial chemotaxis towards pollutants might contribute to the ability of bacteria to compete with other organisms in the environment and to be efficient agents for bioremediation. In addition to the bacterial biomineralization of organic pollutants, certain bacteria are also capable of immobilizing toxic heavy metals in contaminated aquifers, further illustrating the potential of microorganisms for the removal of pollutants.

**Pfiffner, S. M., A. V. Palumbo, et al. (2004). "Microbial population and degradation activity changes monitored during a chlorinated solvent biovent demonstration." *Ground Water Monitoring and Remediation* 24(3): 102-110.**

Microbial populations and degradation activity increased significantly during a chlorinated solvent bioventing bioremediation effort using propane at Dover Air Force Base in Delaware. The propane injection resulted in degradation of a mixture of chlorinated solvents, including trichloroethylene (TCE), cis-dichloroethylene (c-DCE), and 1,1,1-trichloroethane (TCA). In only 20 d, the propane injection resulted in decreases of TCE and c-DCE of > 98%, and a decrease in TCA in soil gas by similar to 70%. The degradation of the TCA may not have occurred with a methane, butane, toluene, or phenol injection. These decreases in chlorinated solvent concentrations were accompanied by large increases in propane-utilizing bacteria that ranged from below detection levels prior to the

injection to similar to 1% of the ending total aerobic heterotrophic population by the end of the propane injection. Thus, a proportional increase occurred as heterotrophic counts increased a hundredfold. Microbial TCE degradation activity, as measured in microcosms, also increased with the propane injection. The highest rates of degradation were observed in microcosms with propane and nutrients, indicating the potential for higher field rates of degradation with nutrient additions.

**Postle, J. K., B. D. Rheineck, et al. (2004). "Chloroacetanilide Herbicide Metabolites in Wisconsin Groundwater: 2001 Survey Results." *Environmental Science and Technology* 38(20): 5339 - 5343.**

Asurvey of agricultural chemicals in Wisconsin groundwater was conducted between October 2000 and April 2001 to obtain a current picture of agricultural chemicals in groundwater used for private drinking water. A stratified, random sampling procedure was used to select 336 sampling locations. Water from private drinking water wells randomly selected from within the 336 sampling locations was analyzed for 18 compounds including herbicides, herbicide metabolites, and nitrate. This report focuses on the frequency and concentration of chloroacetanilide herbicides and their metabolites. Analysis of data resulted in an estimated proportion of 38 ( 5.0% of wells that contained detectable levels of a herbicide or herbicide metabolite. The most commonly detected compound was alachlor ESA with a proportion estimate of 28 ( 4.6%. Other detected compounds in order of prevalence were metolachlor ESA, metolachlor OA, alachlor OA, acetochlor ESA, and parent alachlor. Estimates of the mean concentration for the detects ranged from 0.15 ( 0.082  $\mu\text{g/L}$  for acetochlor ESA to 1.8 ( 0.60  $\mu\text{g/L}$  for alachlor OA. Water quality standards have not been developed for these chloroacetanilide herbicide metabolites. The results of this survey emphasize the need for toxicological assessments of herbicide metabolite compounds and establishment of water quality standards at the state and federal levels.

**Reddy, C. M., L. Xu, et al. (2004). "Radiocarbon evidence for a naturally produced, bioaccumulating halogenated organic compound." *Environmental Science and Technology* 38(7): 1992-1997.**

Halogenated organic compounds (HOCs) such as 1,1'-dimethyl-3,3',4,4'-tetrabromo-5,5'-dichloro-2,2'-bipyrrole (DBP-Br4Cl2) and heptachloro-1'-methyl-1,2'-bipyrrole (Q1) have been detected worldwide, sometimes at high levels in Antarctic air, seabird eggs, the blubber of marine mammals, and, most notably, even human milk. To date, it has been difficult to determine whether these compounds are natural products or derived from industrial synthesis. Molecular-level C-14 analysis of these compounds is particularly appealing because most industrial compounds are manufactured from petrochemicals (C-14-free) and natural compounds should have "modern" or "contemporary" C-14 levels. To investigate the source of DBP-Br4Cl2, we isolated 600  $\mu\text{g}$  of this compound (150  $\mu\text{g}$  of carbon) from marine animal extracts by employing gel permeation chromatography, Florisil column chromatography, and two-dimensional preparative Capillary gas chromatography. The purified DBP-Br4Cl2 was split into two samples (75  $\mu\text{g}$  of carbon each) and analyzed by accelerator mass spectrometry for C-14 content. The  $\Delta(14)\text{C}$  values were -449parts per thousand and -467parts per thousand, corresponding to conventional C-14 ages of 4740 and 5000 years before present (BP), respectively. The presence of detectable C-14 in the DBP-Br4Cl2 strongly points to at least a natural or biogenic source. However these  $\Delta(14)\text{C}$  values for DBP-Br4Cl2 are more depleted than expected for a recently synthesized natural product. Several explanations are discussed, but additional samples from discrete locations need to be analyzed before a clear understanding of the source (or sources) of this compound (and other unknown HOCs) is fully determined.

**Rodríguez-Garrido, B., M. Camps Arbestain, et al. (2004). "Reductive Dechlorination of alpha -, beta -, delta -, and gamma-Hexachlorocyclohexane Isomers by Hydroxocobalamin in the Presence of Either Dithiothreitol or Titanium(III) Citrate as Reducing Agents." *Environmental Science and Technology* 38: 5046 - 5052.**

The effect of the reducing potential on the reductive dehalogenation of the different HCH (hexachlorocyclohexane) isomers has not yet been studied. In the present study, the potential for dehalogenation of R-,  $\beta$ -,  $\gamma$ -, and  $\alpha$ -HCH isomers by the dithiothreitol (DTT) and titanium(III) citrate (reducing potential at pH 7,-0.33 and -0.48 V, respectively), with and without the addition of hydroxocobalamin was investigated. In the presence of DTT without catalyst, there was no disappearance of any of the HCH isomers studied after 1 h of treatment. However, disappearance of the  $\alpha$ - and R-HCH isomers was observed during the same time period when titanium(III) citrate was used as the reductant in the absence of catalyst (62.9 and 16.6% disappearance, respectively). Addition of the hydroxocobalamin to the DTT system favored mainly the disappearance of  $\alpha$ - and R-HCH (92.9 and 30.8% disappearance after 1 h, respectively); disappearance of  $\gamma$ -HCH and  $\beta$ -HCH was small

(11.9%) or negligible, respectively. Addition of the hydroxocobalamin to the titanium(III) citrate system favored the degradation of all HCH isomers under study: Á- and R-HCH completely disappeared to undetectable levels (<0.1%) after 1 and 2 min, respectively; degradation of %-HCH and ,-HCH was slower than that of the other two isomers, although they had almost completely disappeared (99.9 and 99.6% disappearance, respectively) after 10 and 60 min, respectively. The order of disappearance, Á-HCH > R-HCH > %-HCH > ,-HCH, coincided with a decreasing order of the axially positioned Cl atoms of these isomers (considering their thermodynamically most stable configuration). This study is the first description of the rapid degradation of %- and ,-HCH under abiotic conditions, and the results demonstrate the effect of the reducing potential on the reductive dehalogenation of HCH isomers.

**Said-Pullicino, D., G. Gigliotti, et al. (2004). "Environmental fate of triasulfuron in soils amended with municipal waste compost." *Journal of Environmental Quality* 33(5): 1743-1751.**

The amendment of soil with compost may significantly influence the mobility and persistence of pesticides and thus affect their environmental fate. Factors like adsorption, kinetics, and rate of degradation of pesticides could be altered in amended soils. The aim of this study was to determine the effects of the addition of compost made from source-separated municipal waste and green waste, on the fate of triasulfuron [(2-(2-chloroethoxy)-N-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl]amino]carbonyl]benzenesulfonamide], a sulfonylurea herbicide used in postemergence treatment of cereals. Two native soils with low organic matter content were used. A series of analyses was performed to evaluate the adsorption and degradation of the herbicide in soil and in solution after the addition of compost and compost-extracted organic fractions, namely humic acids (HA), fulvic acids (FA), and hydrophobic dissolved organic matter (HoDOM). Results have shown that the adsorption of triasulfuron to soil increases in the presence of compost, and that the HA and HoDOM fractions are mainly responsible for this increase. Hydrophobic dissolved organic matter applied to the soils underwent sorption reactions with the soils, and in the sorbed state, served to increase the adsorption capacity of the soil for triasulfuron. The rate of hydrolysis of triasulfuron in solution was significantly higher at acidic pH and the presence of organic matter fractions extracted from compost also slightly increased the rate of hydrolysis. The rate of degradation in amended and nonamended soils is explained by a two-stage degradation kinetics. During the initial phase, although triasulfuron degradation was rapid with a half-life of approximately 30 d, the presence of compost and HoDOM was found to slightly reduce the rate of degradation with respect to that in nonamended soil.

**Schmitz, R. P. H. and G. Diekert (2004). "The fdh operon of *Sulfurospirillum multivorans*." *Fems Microbiology Letters* 237(2): 235-242.**

The complete single copy fdh operon (similar to 5.7 kb) encoding the formate dehydrogenase subunits of the gram negative, reductively dehalogenating anaerobe *Sulfurospirillum multivorans* was sequenced and analyzed. The gene fdhA encoding the catalytically active periplasmic subunit is part of an operon (fdhEABCD) containing additional structural genes. The genes fdhEABCD were cotranscribed as indicated by RT-PCR and primer extension experiments. Two mRNAs for fdhEABCD and fdhABCD were either transcribed independently from two transcription start sites upstream of fdhE and fdhA or might result from posttranscriptional processing of the full-length fdhEABCD mRNA. The operon shows a high degree of similarity to the fdh operons of *Campylobacter jejuni* and *Wolinella succinogenes* in terms of architecture and putative cofactor binding motifs of the gene products. (C) 2004 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.

**Sylvestre, M. (2004). "Genetically modified organisms to remediate polychlorinated biphenyls. Where do we stand?" *International Biodeterioration & Biodegradation* 54(2-3): 153-162.**

The biphenyl catabolic pathway transforms selected polychlorinated biphenyls (PCBs) to chlorobenzoates. The inability of bacteria to degrade the persistent PCBs is due to failure of enzymes of this pathway to catalyze their transformation. Biphenyl 2,3-dioxygenase (BPDO), 2,3-dihydroxybiphenyl 1,2-dioxygenase (HPDO) and 2-hydroxy-6-oxo-6-phenyl-2,4-hexadienoate (HOPDA) hydrolase (HOPDAH) are critical to determine the range of PCBs degraded. Investigations are under way to examine features of these enzymes that influence their interactions with chlorinated substrates and to propose ways to bypass the blockages that they cause. BPDO terminal oxygenase component is a hexamer comprised of an alpha and a beta subunit. *Burkholderia* sp. LB400 and *Pseudomonas pseudoalcaligenes* KF707 BPDOs are very similar but exhibit quite distinct substrate specificities. *Comamonas testosteroni* B-356 and *Rhodococcus globerulus* P6 BPDOs are more distantly related to LB400 BPDO and show a different range of PCB substrate used. Comparative analyses of the catalytic properties of chimeras of these

enzymes unveiled several regions of the C-terminal domain of the alpha subunit that strongly influence BPDO's substrate specificity. DNA shuffling between genes encoding these homologous BPDOs has been used to broaden the enzyme's substrate specificity. HPDOs are unable to oxygenate 3,4-dihydroxylated metabolites of chlorobiphenyls. HOPDAH are limited in their capacity to hydrolyze HOPDAs bearing chloro substituents on the dienoate moiety. Enzymes homologous to HPDO and HOPDAH but exhibiting different patterns of substrate specificity have been identified. They provide tools to examine the enzymes structural features responsible for substrate specificity and to plan strategies to extend the range of chlorinated substrates that they can transform. (C) 2004 Elsevier Ltd. All rights reserved.

**Szecsody, J. E., J. S. Fruchter, et al. (2004). "In situ chemical reduction of aquifer sediments: Enhancement of reactive iron phases and TCE dechlorination." *Environmental Science & Technology* 38(17): 4656-4663.**

In situ chemical reduction of aquifer sediments is currently being used for chromate and TCE remediation by forming a permeable reactive barrier. The chemical and physical processes that occur during abiotic reduction of natural sediments during flow by sodium dithionite were investigated. In different aquifer sediments, 10-22% of amorphous and crystalline Fe-III-oxides were dissolved/reduced, which produced primarily adsorbed Fe-II, and some siderite. Sediment oxidation showed predominantly one FeII phase, with a second phase being oxidized more slowly. The sediment reduction rate (3.3 h batch half-life) was chemically controlled (58 kJ mol<sup>-1</sup>), with some additional diffusion control during reduction in sediment columns (8.0 h half-life). It was necessary to maintain neutral to high pH to maintain reduction efficiency and prevent iron mobilization, as reduction generated H<sup>+</sup>. Sequential extractions on reduced sediment showed that adsorbed ferrous iron controlled TCE reactivity. The mass and rate of field-scale reduction of aquifer sediments were generally predicted with laboratory data using a single reduction reaction.

**Taseli, B. K., C. F. Gokcay, et al. (2004). "Upflow column reactor design for dechlorination of chlorinated pulping wastes by *Penicillium camemberti*." *Journal of Environmental Management* 72(3): 175-179.**

A *Penicillium camemberti* strain isolated in our laboratory has been studied for its ability to degrade chlorinated pulping wastes, presumably containing a variety of chlorinated polyphenols. In batch tests, the highest removals (76% AOX, 61% color and 65% TOC) were obtained with 0.2 g/l feed acetate concentration. The tendency of the fungus to dechlorinate bleachery effluents better under non-shaking conditions and to attach onto surfaces suggested the use of immobilized cells rather than freely suspended ones in further exploitation of the process. An upflow glass wool packed column reactor established with this fungus could be operated for nearly two years in the laboratory. At best around 70% AOX could be removed from chlorinated pulping wastes in 7.3 h of contact with no aeration and with a minimal amount of carbon supplement (0.2 g/l). Finally, an asymptotic mathematical formula for determining Michaelis-Menten kinetic rates has been derived. The kinetic rates  $K_m$  (the Michaelis constant or saturation constant for the substrate) and  $V_m$  (the product of maximum rate for the enzymatic reaction and biomass concentration) were then calculated as 126.386 mg/l and 2.83017 mg/l h, respectively. (C) 2004 Elsevier Ltd. All rights reserved.

**Ulrich, R., J. Nuske, et al. (2004). "Novel haloperoxidase from the agaric basidiomycete *Agrocybe aegerita* oxidizes aryl alcohols and aldehydes." *Applied and Environmental Microbiology* 70(8): 4575-4581.**

*Agrocybe aegerita*, a bark mulch- and wood-colonizing basidiomycete, was found to produce a peroxidase (AaP) that oxidizes aryl alcohols, such as veratryl and benzyl alcohols, into the corresponding aldehydes and then into benzoic acids. The enzyme also catalyzed the oxidation of typical peroxidase substrates, such as 2,6-dimethoxyphenol (DMP) or 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS). *A. aegerita* peroxidase production depended on the concentration of organic nitrogen in the medium, and highest enzyme levels were detected in the presence of soybean meal. Two fractions of the enzyme, AaP I and AaP II, which had identical molecular masses (46 kDa) and isoelectric points of 4.6 to 5.4 and 4.9 to 5.6, respectively (corresponding to six different isoforms), were identified after several steps of purification, including anion- and cation-exchange chromatography. The optimum pH for the oxidation of aryl alcohols was found to be around 7, and the enzyme required relatively high concentrations of H<sub>2</sub>O<sub>2</sub> (2 mM) for optimum activity. The apparent  $K_m$  values for ABTS, DMP, benzyl alcohol, veratryl alcohol, and H<sub>2</sub>O<sub>2</sub> were 37, 298, 1,001, 2,367 and 1,313 μM, respectively. The N-terminal amino acid sequences of the main AaP II spots blotted after two-dimensional gel electrophoresis were almost identical and exhibited almost no homology to the sequences of other peroxidases from basidiomycetes, but

they shared the first three amino acids, as well as two additional amino acids, with the heme chloroperoxidase (CPO) from the ascomycete *Caldariomyces fumago*. This finding is consistent with the fact that AaP halogenates monochlorodimedone, the specific substrate of CPO. The existence of haloperoxidases in basidiomycetous fungi may be of general significance for the natural formation of chlorinated organic compounds in forest soils.

**Vroblesky, D. A., B. D. Clinton, et al. (2004). "Ground water chlorinated ethenes in tree trunks: Case studies, influence of recharge, and potential degradation mechanism." *Ground Water Monitoring and Remediation* 24(3): 124-138.**

Trichloroethene (TCE) was detected in cores of trees growing above TCE-contaminated ground at three sites: the Carswell Golf Course in Texas, Air Force Plant PJKS in Colorado, and Naval Weapons Station Charleston in South Carolina. This was true even when the depth to water was 7.9 m or when the contaminated aquifer was confined beneath similar to 3 m of clay. Additional ground water contaminants detected in the tree cores were cis-1,2-dichloroethene at two sites and tetrachloroethene at one site. Thus, tree coring can be a rapid and effective means of locating shallow subsurface chlorinated ethenes and possibly identifying zones of active TCE dechlorination. Tree cores collected over time were useful in identifying the onset of ground water contamination. Several factors affecting chlorinated ethene concentrations in tree cores were identified in this investigation. The factors include ground water chlorinated ethene concentrations and depth to ground water contamination. In addition, differing TCE concentrations around the trunk of some trees appear to be related to the roots deriving water from differing areas. Opportunistic uptake of infiltrating rainfall can dilute prerain TCE concentrations in the trunk. TCE concentrations in core headspace may differ among some tree species. In some trees, infestation of bacteria in decaying heartwood may provide a TCE dechlorination mechanism within the trunk.

**Williamson, R. T., I. P. Singh, et al. (2004). "Taveuniamides: new chlorinated toxins from a mixed assemblage of marine cyanobacteria." *Tetrahedron* 60(33): 7025-7033.**

Brine shrimp toxicity guided fractionation of the extracts from two mixed Fijian collections of the cyanobacteria *Lyngbya majuscula* and *Schizothrix* sp. led to the isolation of eleven novel chlorinated lipids. All of these metabolites show an intriguing constellation of unsaturation (olefinic and acetylenic bonds) and chlorination at the two termini of a 15-carbon chain. The central carbon atom of the chain (C-8) is substituted in each case with an N-acetate function. Taveuniamides A-E have an adjacent carbomethoxy group at C-9 to form a protected beta-amino acid while taveuniamides F-K have a methylene group at this position. A standard assortment of 2D NMR techniques in concert with mass spectrometry and other analytical techniques were used to define the structures of these novel metabolites. Taveuniamides F, G and K were the most potent brine shrimp toxins with LD(50)s between 1.7-1.9 µg/mL. (C) 2004 Elsevier Ltd. All rights reserved.

**Wynands, I. and K. H. van Pee (2004). "A novel halogenase gene from the pentachloropseudilin producer *Actinoplanes* sp ATCC 33002 and detection of in vitro halogenase activity." *Fems Microbiology Letters* 237(2): 363-367.**

A novel halogenase gene (*halB*) was isolated from a cosmid library of the pentachloropseudilin producer *Actinoplanes* sp. ATCC 33002. The halogenase has high identity (55%) to the flavin-dependent monodechloroaminopyrrolnitrin-3 halogenase from pyrrolnitrin biosynthesis and to the halogenases *PltM* and *PltA* (35% and 28%, respectively) involved in pyoluteorin biosynthesis. The enzyme has no sequence similarity to the flavin-dependent tryptophan halogenases. The gene could be heterologously expressed in *Pseudomonas aureofaciens* ACN as soluble protein. Chlorinating activity of *HalB* was shown with two synthetic substrates with structural similarity to pentachloropseudilin. *HalB* is the first halogenase from an actinomycete and only the third halogenase for which halogenating activity could be demonstrated in vitro. (C) 2004 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.