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

2<sup>nd</sup> Quarterly Report

2<sup>nd</sup> Quarter Year 2001

Report prepared for EUROCHLOR

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Environmental Engineering

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August 2001

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**ACRONYMS**

<b>CB</b>	Monochlorobenzene
<b>CF</b>	Chloroform
<b>CM</b>	Chloromethane
<b>CPO</b>	Chloroperoxidase
<b>CT</b>	Carbon tetrachloride
<b>1,2-DCA</b>	1,2-Dichloroethane
<b>DCB</b>	Dichlorobenzenes
<b>1,1-DCE</b>	1,1-Dichloroethene
<b>1,2-DCE</b>	1,2-Dichloroethene
<b><i>Cis</i>-DCE</b>	<i>Cis</i> -1,2-dichloroethene
<b><i>Trans</i>-DCE</b>	<i>Trans</i> -1,2-dichloroethene
<b>1,2-DCP</b>	1,2-Dichloropropane
<b>DCM</b>	Dichloromethane
<b>E-acceptor</b>	Electron acceptor
<b>E-donor</b>	Electron donor
<b>ETH</b>	Ethene
<b>HCB</b>	Hexachlorobenzene
<b>HCH</b>	Hexachlorohexane
<b>NAPL</b>	Nonaqueous phase liquid
<b>PCBs</b>	Polychlorinated biphenyls
<b>PCDDs</b>	Polychlorinated dibenzo- <i>p</i> -dioxins
<b>PCDFs</b>	Polychlorinated dibenzo- <i>p</i> -furans
<b>PCE</b>	Tetrachloroethylene
<b>TCB</b>	Trichlorobenzene
<b>TCE</b>	Trichlorethylene
<b>VC</b>	Vinyl chloride

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

**2<sup>nd</sup> Quarter – Year 2001**

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## **1. INTRODUCTION**

This report presents a review of scientific literature published during the second quarter of 2001 on the microbial halogenation and dehalogenation of the following compounds: vinyl chloride, dichloroethane, chloroform, dichloromethane, hexachlorobenzene, chlorobenzene, 1,2,4-1,2,3-1,3,5-trichlorobenzene, hexachlorobutadiene, octachlorostyrene, dioxins and chlorinated furans. In addition, reports regarding the microbial chlorination of compounds structurally related to those listed above were also reviewed.

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

### **2.a. Microbial dechlorination**

The most important findings on microbial dechlorination in the second quarter are the identification of an extracellular porphorin in cell exudates of a methanogen capable of dechlorinating chloroform and carbon tetrachloride (26). Additionally a unique reductive dehalogenase capable of dechlorinating a broad range of chloroaliphatics including PCE and TCE was isolated for the first time from a fermentative bacterium, *Clostridium* (32). Degradation (as well as biohalogenation) of halogenated methanes was shown to correspond to the highest carbon isotopic fractionation known in biological systems (22, 31).

## 2.b. Microbial chlorination

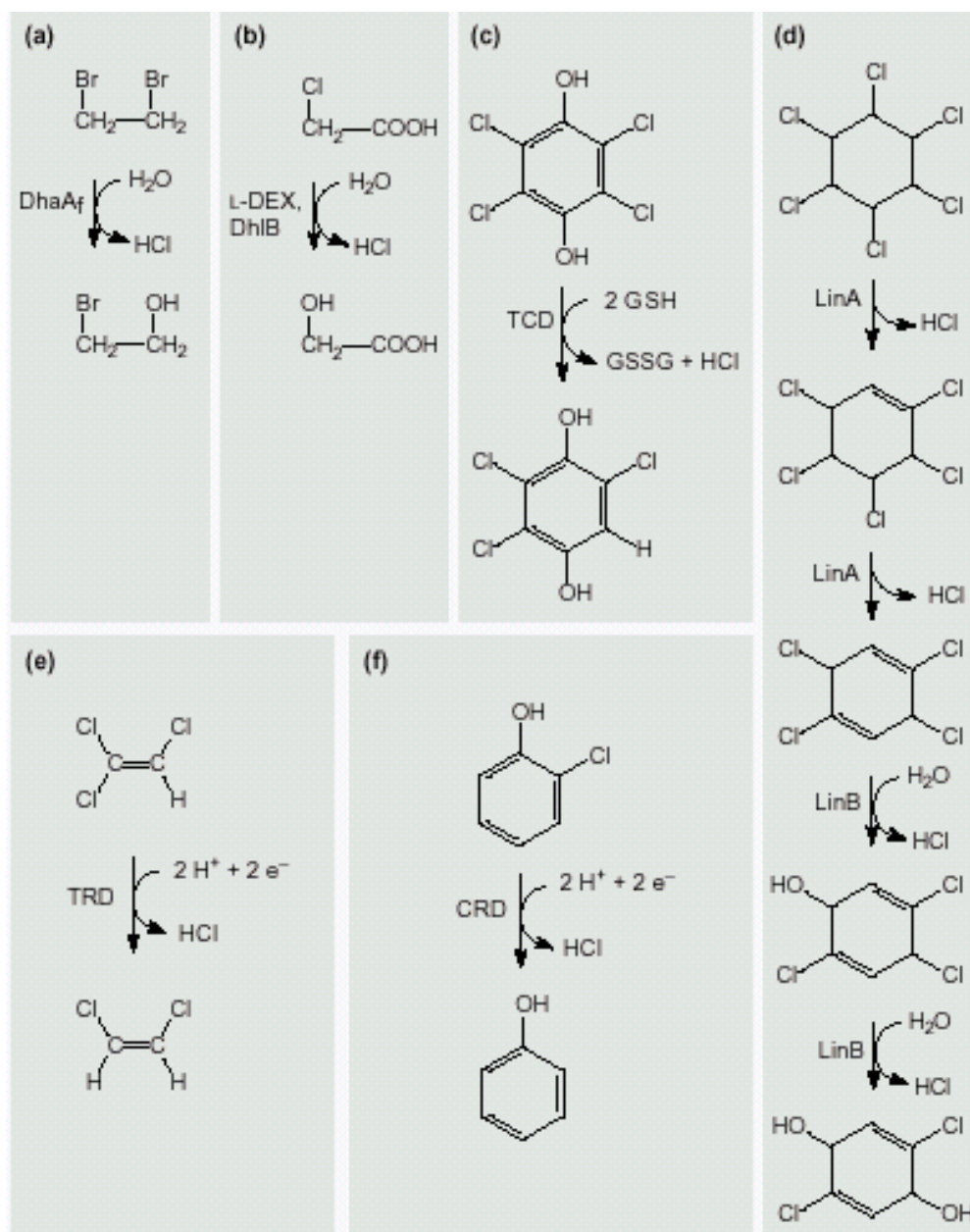
An estimate of the global production of chloromethane and chloroform from peatlands was made based on measurements from Irish peatlands (12).

## 3. MICROBIAL DECHLORINATION

### 3.a. General Reviews

Novel dehalogenases or dehalogenating pathways recently described in bacteria have been reviewed by Janssen *et al.* (20). The recently explored dehalogenation reactions covered in the review are illustrated in Figure 1. The review describes hydrolytic haloalkane dehalogenases that have been recovered from aerobic bacteria utilizing 1,3-dichloropropylene, 1,2-bromoethane, lindane ( $\gamma$ -hexachlorocyclohexane) and chloroacetate. The review goes on to discuss three enzymes involved in removing chlorines from polychlorinated aromatics prior to ring cleavage by aerobic bacteria: 1) a dioxygenase of *Burkholderia* oxidizing 1,2,4,5-tetrachlorobenzene; 2) tetrachlorohydroquinone reductive dehalogenase of *Sphingomonas chlorophenolica* involving a reductive displacement of chloro-group by glutathione-dependent reaction; and 3) 4-chlorobenzoyl coenzyme A dehalogenase, which functions by hydrolytic cleavage. There is evidence that the latter two mechanisms have evolved from other non-dehalogenating enzymes. The review article also addresses new reductive dehalogenases of halorespiring anaerobes (organisms that use chlorinated compounds as terminal electron acceptors to support respiration). These include tetrachloroethene (PCE) and trichloroethene (TCE) reductive dehalogenase of *Dehalospirillum multivorans* and *Dehalococcoides ethenogens*, respectively, as well as and *ortho*-chlorophenol reductive dehalogenase of *Desulfitobacterium dehalogens*. Dehalogenases are not constitutive and thus the review discusses recent insights on how the dehalogenase genes are regulated in various aerobic bacteria. The review closes by discussing 4 gaps in the present understanding of microbial dehalogenation: 1) thermodynamics predicts that many more halogenated compounds should be growth substrates than is observed experimentally, implying poorly understood biochemical limitations; 2) little attention has been given to auxiliary enzymes involved in the metabolism of halogenated compounds (*e.g.* involved in detoxification); 3) unraveled mechanisms of dehalogenation (*e.g.* haloalcohol dehalogenase, HCH dehydrochlorinase); 4) origin and distribution of dehalogenase genes.

Also in the second quarter of 2001, a review of the biodegradation of chlorinated aliphatics by aerobic and anaerobic bacteria has been written in the Czech language (40).



**Figure 1.** Recently explored dehalogenation reactions reviewed by Janssen *et al.* [Janssen, 2001 #156]. *i*) haloalkane dehalogenase for 1,2-dibromoethane (DhaA<sub>f</sub>); *ii*) group II dehalogenase for chloroacetate (L-DEX, DhIB); *iii*) tetrachlorohydroquinone dehalogenase (TCD); *iv*) initial steps in the dehalogenation of lindane (LinA, LinB); *v*) trichloroethene reductive dehalogenase (TRD); *f*) *ortho*-chlorophenol reductive dehalogenase (CRD)

### 3.b. Microbial dechlorination

#### Vinyl chloride and other chlorinated ethenes

As was the case in the previous quarter, most literature concerning vinyl chloride is related to this compound as an intermediate in the biodegradation of major groundwater pollutants, tetrachloroethylene (PCE) and trichloroethylene (TCE). Therefore vinyl chloride (VC) will be considered in the context of chlorinated ethenes.

The reductive dehalogenation of PCE to *cis*-1,2-dichloroethene (*cis*-DCE) in sediment slurries from a 15 y historically contaminated industrial site is described (47). The conversion required an electron donating substrate that was supplied as volatile fatty acids. Curiously only even numbered fatty acids or alcohols (acetate, butyrate, caproate, ethanol and butanol) supported dehalogenation; while odd numbered acids (formate, propionate and valerate) and methanol did not. The intermediate *cis*-DCE was dechlorinated completely to ethene (ETH) only if PCE was present and even numbered alcohols were utilized as e-donor. The requirement for PCE indicates that *cis*-DCE and VC are only cometabolized if PCE acts as terminal electron acceptor (e-acceptor).

The use of peat as a slow-release electron-donating substrate to dehalogenate PCE in biobarriers (treatment of contaminated plumes as they pass through an engineered filled trench) was evaluated (23). A laboratory-scale column experiment inoculated with acclimatized anaerobic sludge was conducted to evaluate the feasibility of this proposed system. This experiment was performed using a series of continuous-flow glass columns including a soil column, a peat column, followed by two consecutive soil columns. Effluent samples were analyzed for PCE and its degradation byproducts. Up to 98% of the PCE in simulated contaminated groundwater was removed in this passive system. PCE dechlorination resulted in VC and ethene as major products, but TCE, *cis*-DCE and ethane were also observed. Results indicate that the continuously released organics from peat column enhanced PCE biotransformation.

The first report describing growth of a sulphate-reducing bacterium in a defined two-member continuous culture by syntrophically coupling the electron and hydrogen transfer to a dehalorespiring bacterium (13). The co-culture consisting of the dehalorespiring *Desulfitobacterium frappieri* TCE1 and the sulphate-reducing *Desulfovibrio* sp. strain SULF1 was obtained via anaerobic enrichment from soil contaminated with PCE (13). In this co-culture, PCE dechlorination to *cis*-DCE was due to the activity of the dehalorespiring bacterium only. A syntrophic association between the two bacteria in the coculture via interspecies hydrogen transfer was proposed to explain the observed growth of the sulfate-reducer in cultures lacking

sulfate or other chemical e-acceptor. The authors suggest that *Desulfitobacterium frappieri* is the terminal "living" terminal electron acceptor of *Desulfovibrio*.

*Desulfitobacterium* is an important genera containing many halorespiring strains, capable of PCE and TCE dechlorination through halorespiration. In contrast to these previous observations, a *Desulfitobacterium frappieri* strain was isolated from human feces that was not capable of dechlorinating chloroethenes or chlorophenols (45).

The use of a sequential anaerobic - aerobic bioventing system was evaluated for PCE degradation (30). Bioventing is a bioremediation technology based on introducing gases into the unsaturated zone. The use of bioventing for PCE bioremediation is completely novel and has never been tested before. Hydrogen was delivered in the gas phase as a reducing agent for the anaerobic step at levels of 1%, and oxygen at 4.2% was used as an e-acceptor in the aerobic step. The anaerobic step yielded *trans*-1,2-dichloroethene (*trans*-DCE) and VC as the reductive dechlorination byproducts of PCE. The aerobic step resulted in the rapid oxidation of the VC and *trans*-DCE to carbon dioxide. The half-lives of PCE in the anaerobic step and VC in the aerobic step were less than 10 minutes, indicating that the bioventing system promoted rapid biodegradation.

A specific methanogenic inhibitor, 2-bromoethanesulfonate (BES), was applied to an anaerobic TCE-dechlorinating enrichment culture in order to determine the involvement of methanogens in TCE dechlorination (8). Long-term exposure to BES altered the bacterial community structure of the anaerobic enrichment culture but did not hinder the dechlorination of TCE or other chlorinated ethenes as previously reported. Nonetheless, different transient intermediates and end products were observed. The BES amended culture converted TCE to ethene; while the methanogenic active culture converted TCE to ethane.

Kinetics of phenol- and TCE cooxidation by 13 aerobic phenol-degrading bacteria were analyzed (16). The affinity constant ( $K_s$ ) values for TCE showed a great diversity, ranging from 11  $\mu\text{M}$  to over 800  $\mu\text{M}$ . The maximum activity ( $V_{\text{max}}$ ) is divided by  $K_s$  to evaluate the physiological relevance. The  $V_{\text{max}}/K_s$  values for phenol were three orders of magnitude higher than the values for TCE in all bacteria analyzed, suggesting that these bacteria preferentially degrade phenol rather than TCE. The authors conclude that low- $K_s$  bacteria should be selectively grown for effective bioremediation of TCE-contaminated groundwater. High- $K_s$  bacteria do not provide complete degradation of TCE at the relatively low concentrations often found in contaminated groundwater. The high  $K_s$  bacteria generally have a high  $V_{\text{max}}$  for phenol and thus they potentially will out-compete the more effective low- $K_s$  bacteria.

A field study evaluating TCE bioremediation by stimulating TCE cooxidation through methane injection was conducted in Japan at a site with inhabited buildings(14). Methane,

oxygen, nitrate, and phosphate were introduced into groundwater contaminated with 220 ug/L of TCE. After a week of biostimulation, methane concentrations gradually decreased below the detection limit, resulting in a simultaneous increase in methane oxidizing bacterial numbers. During methane injection, the biotransformation yield was 3-13 mg TCE/g CH<sub>4</sub>. No TCE removal was observed after methane injections were stopped. The authors conclude that bioremediation using methane injection is useful for the removal of TCE contamination in groundwater, particularly when safety considerations are critical (e.g., clean-up of contamination in the near vicinity of houses). Other substrates used to stimulate the cooxidation of TCE, mainly phenol and toluene, are well known for their toxicity to human health.

The cytotoxicity associated with TCE oxidation in the toluene-oxidizing bacterium *Burkholderia cepacia* strain G4 were investigated by Yeager *et al.* (50). One of the major problems with TCE cooxidation is the temporary accumulation of short-lived toxic intermediates that are damaging to the bacteria and enzymes responsible for aerobic TCE degradation. *B. cepacia* was found to suffer damage during TCE cometabolism, with injuries that impair general cellular processes, such as respiratory metabolism and cell culturability. These injuries to the cell were more important than inactivation of the responsible enzyme, toluene 2-monooxygenase (Tol). Cells that had degraded approximately 0.5 μmol TCE /mg cells lost as much as 95% of their general respiratory activity (measured by acetate-dependent O<sub>2</sub> uptake activity), but only 35% of their Tol activity. Cell culturability also decreased upon TCE oxidation; however, the extent of loss varied greatly (up to 3 orders of magnitude) with the method of assessment. Furthermore, among *B. cepacia* cells isolated from cultures that had degraded approximately 0.5 μmol TCE/mg cells, up to 90% lacked Tol and were no longer capable of TCE degradation. These results indicate that a toxicity threshold for TCE oxidation exists in *B. cepacia* G4 beyond which cell recovery is severely limited.

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

Chloroform and carbon tetrachloride degradation by the methanogen *Methanosarcina thermophila* was shown to be influenced by ferrous iron (Fe<sup>2+</sup>) and pH levels, both of which are increased by oxidation of elemental iron (Fe(0)) (4). The impact of Fe(0) and Fe<sup>2+</sup> on the anaerobic biodegradation of CT and CF is important for the application of zero-valent iron (Fe(0)) in biobarriers (engineered trenches that intercept and treat contaminated plumes). A methanogen was used as a model since these microorganisms are important in the anaerobic biodegradation of chloromethanes. Previous research by the same authors, demonstrated that Fe(0) increased the methanogenic CT degradation rate by providing H<sub>2</sub> for cell growth and reductive dechlorination. The rate of CT and CF degradation was found to increase with

increasing pH (51% and >90% CT removal after 6 h at pH 5.5 and 8.5, respectively). Ferrous iron ( $\text{Fe}^{2+}$ ) promoted rapid transformation of CF, but it did not significantly influence the elimination of CT. However, increased CF levels (60% more CF) were detected as CT degradation byproduct in  $\text{Fe}^{2+}$ -unsupplemented cultures. These results indicate that a combined Fe(0)/bioremediation system may result in enhanced halocarbon degradation. The  $\text{Fe}^{2+}$  formed from Fe(0) was found to prevent CF accumulation from CT degradation.

A peculiarity of anaerobic CT and CF degradation is the involvement of unidentified reduced cofactors in the reductive dechlorination process as opposed to direct enzymatic degradation. In many previous studies, autoclaved cells of methanogens and other anaerobes were shown to have CT and CF degrading activity, presumably due to the presence of reduced cofactors. A study by Koons *et al.* (26) provides interesting insights on the reduced cofactors excreted to the extracellular medium by a methanogen that are responsible for CF and CT dechlorination. Cell exudates from the methanogen *Methanosarcina thermophila* were shown to contain porphorin-type molecules capable of dechlorinating CT and CF. Characterization of the active excreted agents in the cell exudates suggested that they were possibly corrinoid, heme, and zinc-containing molecules.

### **Dichloromethane (DCM)**

See headings “3.c. In vitro degradation of chlorinated compounds ” and ”3.d. New tools to assess the biodegradation of chlorinated compounds” for discussion of works and Jendrzewski *et al.* (21) and by Kmuniek *et al.* (25).

### **Chloromethane (CM)**

See discussion of work by Studer *et al.* (41) in heading “3.c. In vitro degradation of chlorinated compounds”; by Kalin *et al.* (22) and Miller *et al.* (31) in heading “3.d. New tools to assess the biodegradation of chlorinated compounds”; and by Huang *et al.* (19) in heading “4.d. Chlorination by marine and fresh water organisms”

### **Dichloroethane (1,2-DCA)**

See discussion of work by Lewandowicz *et al.* (28) in heading “3.c. In vitro degradation of chlorinated compounds”; and discussion of work by Jendrzewski *et al.* (21) and Kmuniek *et al.* (25) in heading ”3.d. New tools to assess the biodegradation of chlorinated compounds”.

**Dichloropropane (1,2-DCP)**

Natural attenuation of 1,2-DCP in contaminated groundwater was investigated by Tesoreiro *et al.* (43). Field data and results from simulation studies of the pollutant fate and transport suggested that microbial degradation of 1,2-DCP occurred in iron-reducing zones of the unconfined shallow aquifer. Microcosm experiments with aquifer materials from the iron-reducing zone confirmed the microbial degradation of 1,2-DCP by hydrogenolysis with production of 1-chloropropane and 2-chloropropane in a constant ratio of about 8:1. The incomplete dechlorination of 1,2-DCP at slow rates and the positive correlation between the production of monochlorinated propanes and methane suggest that the observed dechlorination reactions in the microcosm experiments were most likely due to anaerobic cometabolism. Dechlorination of this fumigant compound was not observed in microcosm studies using aquifer material from the aerobic zone as inoculum.

**Chlorobenzenes**

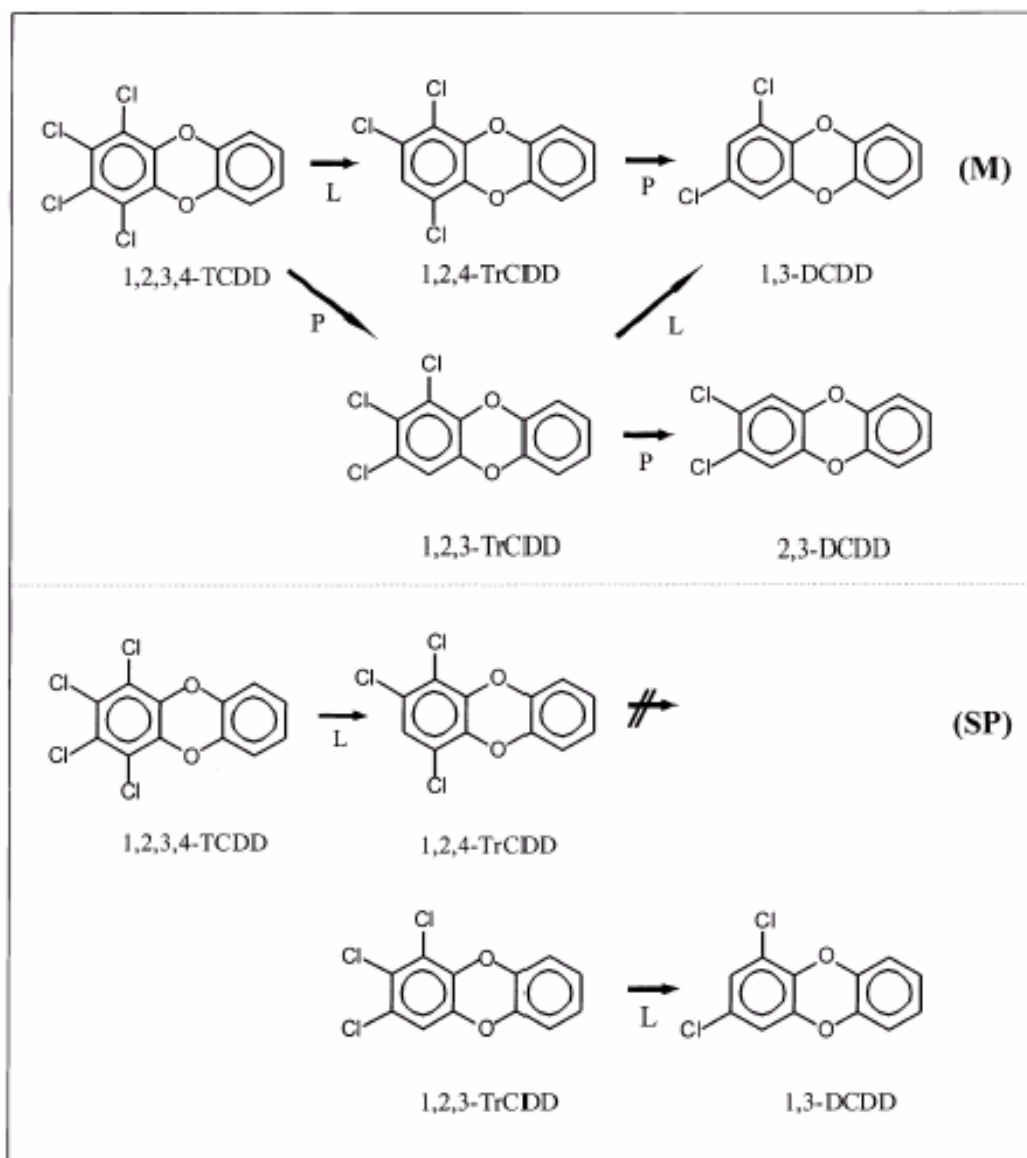
**Mono-chlorobenzene (CB) and Dichlorobenzenes (DCB):** Production of adapted biomass for the aerobic biodegradation of a CB and DCB mixture in industrial treatment systems was optimized in a batch bioreactor with pulsed supplementation of the chlorobenzenes as only source of carbon and energy (39). The optimum CB and DCB concentration, pulse duration, and CB and DCB loading rate required to maximize biomass productivity were determined. Bacterial strains identified from the mixed adapted consortia degrading CBs included *Stenotrophomonas maltophilia*, *Comamonas acidovorans*, *Pseudomonas putida* and *Escherichia hermannii*.

**Trichlorobenzenes (TCB) and Hexachlorobenzene (HCB):** Publications on the microbial degradation of TCBs and HCB were not found in the 2<sup>nd</sup> quarter of 2001.

**Polychlorinated Dibenzo-*p*-dioxins and -furans (PCDDs/PCDFs)**

Anaerobic enrichment cultures inoculated with PCDD/F-contaminated sediments were able to reductively dechlorinate spiked 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) with formation of tri- and dichlorinated products in the presence of electron-donating cosubstrate (a mixture of and formate, fumarate, acetate benzoate, pyruvate and yeast extract) (6). Analysis of subcultures spiked with 1,2,3- and 1,2,4-trichlorodibenzo-*p*-dioxin, respectively, revealed two different dechlorination pathways within the sediment cores. One pathway was characterized by the simultaneous dechlorination of *peri*-chlorine atoms (flanked only one side by chlorine atoms) and lateral-chlorine atoms (flanked by chlorine atoms on both sides). The other pathway was restricted to dechlorination lateral-chlorine atoms. These results show the occurrence of different

dechlorination pathways within a single sediment core, suggesting that different microbial populations may be involved in these processes.



**Figure 2.** Comparison of two 1,2,3,4-TeCDD-dechlorination processes (M, SP) produced in slurries of Spittelwasser sediment (Bunge *et al.* (6)). Removal of chlorine atoms in *peri*- (P) and lateral (L) positions.

Anaerobic enrichment cultures obtained from dioxin-contaminated river sediments were shown to cause reductive dechlorination of two dioxins, 1,2,3,4,7,8-HpCDD or 1,2,3,4,6,7,9-HpCDD, in assays amended with organic acids, in the absence or presence of sulfate (20 mM) at different salinity levels (15). These conditions were chosen to mimic freshwater (low salt/low sulfate), estuarine (high salt/low sulfate) and marine environments (high salt/high sulfate). The yield of dechlorination products increased with salinity, but never exceeded 20% of the initial dioxins present. The yield and nature of the detected chlorinated products was strongly affected by the imposed conditions (sulfate and salinity levels), and by the presence of humic organic matter. Under “marine” and “estuarine” conditions, hexa- and diCDD represented the dominant congener groups in incubations with 1,2,3,4,6,7,9-HpCDD, while tri- and diCDD dominate in 1,2,3,4,6,7,8-HpCDD-amended cultures. In contrast, under freshwater conditions hepta- and tetraCDD were dominant, and the final products of the dechlorination were monoCDD. The production of 2,3,7,8-TCDD increased with decreasing salinity and in the presence of dissolved organic matter.

See section 3.c., "In Vitro Degradation of Chlorinated Compounds", for a study on aerobic degradation of lower halogenated dioxins by a dioxygenase of an aerobic bacterium (36).

### **Hexachlorobutadiene and Octachlorostyrene**

In the second quarter of 2001, no publications regarding the microbial degradation of two compounds in the priority list, namely, hexachlorobutadiene, octachlorostyrene, were found.

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

Reviewing the microbial degradation of PCBs is out of the scope of this report, therefore publications found on this topic will only be introduced briefly. Growth of several aerobic isolates on dichlorobiphenyls chlorinated on both rings as a sole carbon and energy source was demonstrated by Kim *et al.* (24). One of the isolates was tentatively identified as *Alcaligenes* sp. The ability to grow on PCB isomers with two *ortho* chlorines, such as 2,2'-dichlorobiphenyl, is quite unique and previously unreported. In a different study, the kinetics of anaerobic PCB (Aroclor 1248) dechlorination by cultures obtained from PCB-contaminated sediments was investigated (35). The results indicated that the reductive dechlorination rate is tightly linked to the growth of dechlorinating microorganisms. The maximum first-order rate of PCB dechlorination was 0.24/d (a half-life of 2.9 d) with a half-saturation constant of 1.18  $\mu\text{mol/g}$  sediment. A comparison of the anaerobic reductive dechlorination of PCBs by microorganisms collected from various contaminated sediments was conducted by Chen *et al.* (7). The microbial consortia from different sources differed in the spectrum of PCB congeners they could degrade. In a related study, Cho *et al.* (9) reported on the microbial PCB dechlorination in dredged

sediments and on its enhancement by increased moisture levels. Finally, microbial reductive dechlorination was shown to increase the hormonal activity of PCBs towards in vitro uterine contraction frequency (5). Reductive biotransformation of PCBs (Aroclor 1242 and 1254) produced mixtures with an increased proportion of *ortho*-substituted congeners with one or two chlorine substitutions, and resulted in a considerably increased stimulation of contraction of pregnant rat uterus in vitro compared to the parent Aroclor mixtures.

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

Purification, cloning, and sequencing of an enzyme from a fermentative anaerobic bacterium, *Clostridium bifermentans* DPH-1 mediating the reductive dechlorination of PCE, was accomplished by Okeke *et al.* (32). The enzyme degraded a broad spectrum of chloroaliphatics (*i.e.*, PCE, TCE, *cis*-DCE, *trans*-DCE, 1,1-DCE, 1,2-dichloropropane, and 1,1,2-trichloroethane) and, catalyzed the reductive dechlorination of PCE to *cis*-DCE via TCE, at a  $V_{max}$  and  $K_m$  of 73 nmol/mg protein and 12  $\mu$ M, respectively. Enzymatic activity was independent of metal ions, oxygen sensitive and possibly involved a corrinoid cofactor. *C. bifermentans* dehalogenase appears to be unique amongst the reported anaerobic PCE dehalogenases, and molecular data on the encoding gene (designated *pceC*) indicate that a new group of microbial reductive dehalogenases may exist.

*Desulfitobacterium* strain PCE1 is able to use PCE and chloroaromatics as terminal e-acceptors for growth. In a recent study, two distinct enzyme systems were shown to be responsible for PCE and chlorophenol reductive dehalogenation in *Desulfitobacterium* PCE1 (44). The characteristics of the chlorophenol dehalogenase were similar to those of the chlorophenol reductive dehalogenase of *D. dehalogenans*. Furthermore, characterization of partially purified PCE dehalogenase indicated that this enzyme is a novel type of reductive dehalogenase.

A strictly aerobic methylotrophic bacterium, *Methylobacterium chloromethanicum* strain CM4 can grow using chloromethane (CM) as the sole carbon source. Recent work by Studer *et al.* (41) confirms that the initial dehalogenation step in *M. chloromethanicum* CM4 pathway for CM utilization requires two proteins, CmuA and CmuB. These two proteins were found to be functionally similar to components of the methylotrophic methanogen (*Methanosarcina barkeri*) system for the utilization of methylamines and methanol as growth substrates. In combination, CmuA and CmuB proteins catalyze the in vitro transfer of the methyl group of chloromethane to tetrahydrofolate, thus affording a direct link between chloromethane dehalogenation and core C1

metabolism of *Methylobacterium*. In this study, CmuA was shown to represent a novel and so far unique two-domain methyltransferase/corrinoid-binding protein involved in methyl transfer.

Ring-hydroxylating dioxygenases are key enzymes of the aerobic bacterial catabolism of aromatic compounds. Although unsubstituted vicinal aromatic carbons are the typical target sites for dioxygenation, an increasing body of literature reports exceptions to this accepted rule. For example, the bph-encoded biphenyl dioxygenase (BphA) of *Burkholderia* sp. strain LB400 is known to attack chlorinated *ortho*-carbons of some biphenyls (17, 37, 38) leading to elimination of the *ortho*-chlorine. Seeger *et al.* (36) reported recently on the attack by the BphA on a number of symmetrical *ortho*-substituted biphenyls or quasi *ortho*-substituted biphenyl analogues (*e.g.* dioxin). Their results show that conversions of differently substituted biphenyls (*i.e.*, 2,2'-Difluoro-, 2,2'-dibromo-, 2,2'-dinitro-, and 2,2'-dihydroxybiphenyl) and biphenyl analogues (*i.e.*, dibenzodioxins and dibenzofurans) into catechols by the investigated BphA take place via dioxygenation. These conversions include hydroxylations of halo-, nitro-, or phenoxy-substituted carbons. Dibenzofuran and dibenzodioxin were attacked at the "quasi *ortho*" carbon and its neighbor. While angular attack by the biphenyl dioxygenase was the main route of dibenzodioxin oxidation, lateral dioxygenation leading to dihydrodiols was the major reaction with dibenzofurans. These results indicate that the BphA enzyme is capable of hydroxylating *ortho* or angular carbons carrying a variety of substituents, which exert electron-withdrawing inductive effects.

Chlorine kinetic isotope effects were found on the hydrolytic dehalogenation of 1,2-dichloroethane (1,2-DCA) and 1-chlorobutane catalyzed by haloalkane dehalogenase from *Xanthobacter autotrophicus* (0.45 and 0.66%, respectively) (28). 1-chlorobutane is a very slow substrate of haloalkane dehalogenase as compared to 1,2-DCA. Based on analysis of the chlorine fractionation pattern, combined with known kinetic data, the authors conclude that the dehalogenation step is reversible and the overall irreversibility of the haloalkane dehalogenase-catalyzed reaction is caused by the following step, hydrolysis of the enzyme-bound intermediate.

### 3.d. New tools to assess the biodegradation of chlorinated compounds

#### Isotopic Fractionation

Continuous flow stable isotope methods using gas chromatography and compound-specific isotope ratio mass spectrometry for study of  $d^{13}C$  fractionation during halomethane production and degradation were developed by Kalin *et al.* (22). Method optimization allowed measurement of  $d^{13}C$  for several halomethanes, *i.e.*,  $CH_3Cl$ ,  $CH_3Br$  and  $CH_3I$ . Carbon isotope effects during biological production and degradation of chlorinated organics can be as much as 100 per

thousand (22, 31). The authors conclude that stable isotope analysis is a powerful tool for monitoring the biotransformation of halogenated contaminants resulting from natural attenuation or bioremediation efforts, as well as the global sources and sinks of halogenated compounds.

Large stable carbon isotopic fractionation (up to 70 per thousand) occurs during oxidation of methyl halides ( $\text{CH}_3\text{Cl}$ ,  $\text{CH}_3\text{Br}$ , and  $\text{CH}_3\text{I}$ ) by methylotrophic bacteria (31). This value is comparable to those in two methanogenic pathways,  $\text{CO}_2$  reduction with  $\text{H}_2$  and metabolism of methylated C1 compounds, which are the largest biological fractionations of stable carbon isotopes previously observed in nature. Methylotrophs and methanogens have also recently been shown to have significant genetic similarities. Only part of the observed fractionation of carbon isotopes during oxidation of the methyl halides could be accounted for by the activity of the corrinoid methyltransferase enzyme, suggesting fractionation by enzymes further along the degradation pathway. The authors cautioned that further research is required to prove whether a significant carbon isotope fractionation is associated with the microbial degradation of the various methyl halides at their respective atmospheric mixing ratios, as opposed to the higher concentrations used in their experiments.

A set of 12 samples of 5 different chlorinated hydrocarbons (TCE, PCE, DCM, 1,1,1-TCA, CF) provided by several manufacturers were characterized for both carbon and chlorine isotopic composition, using a new sensitive method (21). The results obtained suggested a very large range of variation of Cl ( $\delta^{37}\text{Cl}$  from  $-2.69$  to  $+4.00$  per thousand, standard mean ocean chloride) and C isotopic compositions ( $\delta^{13}\text{C}$  from  $-51.66$  to  $-24.05$  per thousand) for the industrial solvents. Unlike the data published previously, with one exception, all results obtained had positive delta  $\delta^{37}\text{Cl}$  values which might differentiate natural Cl from that derived from degradation of halogenated solvent. Moreover,  $\delta^{37}\text{Cl}/\delta^{13}\text{C}$  pairs seemed to be related to solvent/manufacturer. These results suggest that Cl isotopic compositions are probably highly fractionated during high temperature organic synthesis. The authors conclude that isotopic characterization has significant potential as a tool for tracing sources of pollution, and for validating models of transport and fate of pollutants. Published studies on the latter topics are reviewed in the introduction of their paper.

### Metabolic engineering

Haro *et al.* (18) reported on their current efforts to construct *Pseudomonas* strains genetically designed for degradation of the recalcitrant compound 2-chlorotoluene. Two different *P. aeruginosa* strains capable of 2-chlorobenzoate were modified by insertion of two catabolic segments, one encoding a toluene dioxygenase (which affords the bioconversion of 2-chlorotoluene into 2-chlorobenzaldehyde) and other encoding a benzyl alcohol dehydrogenase

and benzaldehyde dehydrogenase (which accept o-chloro-substituted substrates all the way down to 2-chlorobenzoate). The engineered strains were shown to convert 2-chlorotoluene into 2-chlorobenzoate, however, they failed to grow on 2-chlorotoluene as the only carbon source. Based on these results, the authors discussed the challenges and bottlenecks in the metabolic engineering of bacteria destined for environmental bioremediation. They consider that the rate of the metabolic fluxes, the non-productive spill of side-metabolites and the physiological control of degradative pathways are the real bottlenecks for degradation of certain pollutants, rather than the theoretical enzymatic and genetic fitness of the recombinant bacteria to the process.

### **Enzyme characterization**

Comparative binding energy (COMBINE) analysis of the substrate specificity of haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 (DhlA) was performed by Kmuniek *et al.* (25) in order to identify the amino acid residues determining the substrate specificity of this enzyme. This information is essential for identifying suitable targets for enzyme modification for biotechnological applications by site-directed mutagenesis. A total of 18 substrates of DhlA were considered, *i.e.*, 1-chlorobutane, 1-chlorohexane, dichloromethane, 1,2-dichloroethane, 1,2-dichloropropane, 2-chloroethanol, epichlorohydrine, 2-chloroacetonitrile, 2-chloroacetamide, and their brominated analogues. Complexes of the enzyme with these substrates were modeled and then refined by molecular mechanics energy minimization. The COMBINE results revealed that the amino acid residues contributing most significantly to the substrate specificity of DhlA were Phe164, Phe172, Trp175, Phe222, Pro223, and Leu263.

## **4. MICROBIAL CHLORINATION**

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

Review articles on microbial dehalogenation were not available in the 2<sup>nd</sup> quarter of the current year. The first quarterly report discussed a review article written by Van Pee (46) on the microbial biosynthesis of halometabolites (then still in press) that has recently been published.

### **4.b. Microbial Chlorination in Soils**

#### **Halomethanes**

Dimmer (12) provided new evidence indicating that peatland ecosystems, make a significant contribution to the global budgets of several important halocarbons. High emission of

chloromethanes (*i.e.*,  $\text{CHCl}_3$ ,  $\text{CH}_3\text{Cl}$ ) and other halomethanes ( $\text{CH}_3\text{Br}$ ,  $\text{CH}_3\text{I}$ ) was recorded from different Irish peatland sites monitored, with minor flux of other halocarbons ( $\text{CH}_2\text{Cl}_4$  and  $\text{CH}_2\text{Br}_2$ ) at certain sites. A diurnal cycle in halocarbon emissions was observed at all open peatland sites, suggesting that solar radiation intensity is a critical factor influencing the extent of flux. Furthermore,  $\text{CHCl}_3$  demonstrated a 180% higher production at night. In contrast, methyl halides showed a small decrease in production at night, supporting a different  $\text{CHCl}_3$  production mechanism (haloperoxidase enzyme activity) to the halomethanes (methyl transferase activity). Global annual fluxes of 4.7 (0.1-151.9), 5.5 (0.9-43.4), 0.9 (0.1-3.3), and 1.4 (0.1-12.8) Gg/yr for  $\text{CHCl}_3$ ,  $\text{CH}_3\text{Cl}$ ,  $\text{CH}_3\text{Br}$  and  $\text{CH}_3\text{I}$ , respectively, were estimated for peatland ecosystems. In the paper, these estimates are compared with annual fluxes reported elsewhere for other biogenic sources of halomethanes, *e.g.*, terrestrial, wetlands, and oceans. In the concluding remarks, the authors emphasize the possibility of an indirect anthropogenic effect on levels of halomethanes through man's impact on land use, *e.g.*, global scale deforestation and wetland destruction, or global warming resulting in increasing microbial halomethane oxidation.

#### **Other Chlorinated Compounds**

The fungus *Caldariomyces fumago* was shown to halogenate the flavanones, naringenin and hesperetin, at the C-6 and C-8 ring positions in the presence of either chloride or bromide (49). Additional results presented by the authors on the chlorination of flavonoids by chloroperoxidase (CPO) of *C. fumago* are discussed in heading 4.d Microbial "Chlorinating enzymes".

Biologically active chlorinated metabolites (*i.e.*, 3-chloro-4-hydroxy-5-(3,7,11-trimethyl-dodeca- 2,6,10-trienyl)-benzamide and strobilurin B) were isolated from an unidentified South African soil fungus (27).

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

##### **Halomethanes**

A freshwater lake (Lake Washington) was shown to be a net sink for the atmospheric methyl halides  $\text{CH}_3\text{Cl}$  and  $\text{CH}_3\text{Br}$  in the winter, probably because of unspecified microbiological processes (19). In the summer, the surface waters were apparently a sink for  $\text{CH}_3\text{Cl}$  but a net source of  $\text{CH}_3\text{Br}$ . The authors hypothesized that enhanced biological production of  $\text{CH}_3\text{Cl}$  at the higher temperatures prevailing during the summer season, was compensated by larger combined microbial and chemical sinks terms. Based on the results from this study, freshwater lakes are suggested to imply a negligible contribution (<0.05%) to global budgets of  $\text{CH}_3\text{Cl}$  and  $\text{CH}_3\text{Br}$ .

### Other Chlorinated Compounds

A study of the tropospheric concentrations of chlorinated ethenes suggested possible emissions of TCE in the sub-tropical ocean (11). Oceanic TCE sources were hypothesized to result from, either production by ocean microalgae, or release from supersaturated water, originating from high latitudes during the winter months. Biogenic production of TCE by coastal algae has been reported earlier (1, 2).

Several new polychlorinated acetamidoalkynes and alkanes were isolated from the organic extract of the cyanobacterium *Microcoleus lyngbyaceus* (33). A number of halogenated metabolites from the Malaysian red algae *Laurencia pannosa* (42) were isolated, including two novel halogenated sesquiterpenoids (*i.e.*, monochloro-monobromo-C<sub>15</sub>-terpenoids), named pannosanol and pannosane, along with a known halogenated C<sub>15</sub>-acetogenin, chlorofucin. These metabolites displayed inhibitory effects against marine bacteria. Novel polyhalogenated homosesquiterpenic acids (*i.e.*, dichloro-monobromo-C<sub>15</sub>-terpenoid fatty acids) were also isolated from the red alga *Plocamium cartilagineum* (34).

### 4.d. Chlorinating Enzymes

A recent study provides the first evidence for the chloroperoxidase-catalyzed halogenation of flavonoid compounds (49). Flavonoids are very common as secondary metabolites found in nature. Whole cells and the CPO enzyme of *Caldariomyces fumago* were capable of halogenating the flavanones, naringenin and hesperetin at nucleophilic C-6 and C-8 positions (in the A-rings) in the presence of either Cl or Br, but were inactive toward the test flavones (*i.e.*, flavone, flavonol, 7-hydroxy-flavone, 5,7-dihydroxyflavone, quercetin, and 6-chloro-flavone). The inertness of these flavonoids to the enzymatic reaction was suggested to result from their characteristic, strongly conjugated aromatic ring system. The biohalogenated products of naringenin and hesperetin were isolated and found to be identical to those obtained from chemical halogenation using molecular halogen and hypohalous acid.

The *C. fumago* chloroperoxidase was successfully expressed in *Aspergillus niger* (10). The catalytic properties of the recombinant enzyme (rCPO) (*i.e.*, pH optimum, specific chlorination activity, substrate specificity and yields) were similar to those of native CPO.

In a recent study, Woggon *et al.* (48) proposed reaction mechanisms of CPO based on complementary studies of enzyme models and CPO from *C. fumago*. This protein has been known for about 30 years, however, no detailed information on the reaction mechanism of CPO was available until recently. The authors propose a mechanism of CPO which includes H<sub>2</sub>O<sub>2</sub>

cleavage, subsequent formation of compound I, and the identification of two elusive intermediates. The HOCl adduct of the iron(III)-porphyrin is the catalytically competent  $\text{Cl}^+$  donor chlorinating activated C-H bonds of substrates bound to the enzyme. The electric field of the protein is suggested to play a role in stabilizing the low-spin state of the cofactor of the enzyme, triggering the reactivity of compound I such that both concerted and two-step reactions are feasible.

Vanadium haloperoxidases (VHP) from several brown algae *Laminaria* species were isolated and shown to require vanadium (V) to restore activity in vitro (3). The three VHP forms characterized were moderately thermostable and presented good stability in different to organic solvents.

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## 6. ANNEX

**Almeida, M., S. Filipe, et al. (2001). "Vanadium haloperoxidases from brown algae of the Laminariaceae family." *Phytochemistry* 57(5): 633-642.**

Vanadium haloperoxidases were extracted, purified and characterized from three different species of Laminariaceae - *Laminaria saccharina* (Linne) Lamouroux, *Laminaria hyperborea* (Gunner) Foslie and *Laminaria ochroleuca* de la Pylaie. Two different forms of the vanadium haloperoxidases were purified from *L. saccharina* and *L. hyperborea* and one form from *L. ochroleuca* species. Reconstitution experiments in the presence of several metal ions showed that only vanadium(V) completely restored the enzymes activity. The stability of some enzymes in mixtures of buffer solution and several organic solvents such as acetone, ethanol, methanol and I-propanol was noteworthy; for instance, after 30 days at least 40% of the initial activity for some isoforms remained in mixtures of 3:1 buffer solution/organic solvent. The enzymes were also moderately thermostable, keeping full activity up to 40 degreesC. Some preliminary steady-state kinetic studies were performed and apparent Michaelis-Menten kinetic parameters were determined for the substrates iodide and hydrogen peroxide. Histochemical studies were also performed in fresh tissue sections from stipe and blade of *L. hyperborea* and *L. saccharina*, showing that haloperoxidase activity was concentrated in the external cortex near the cuticle, although some activity was also observed in the inner cortical region. (C) 2001 Elsevier Science Ltd. All rights reserved.

**Andrews, E. J. and P. J. Novak (2001). "Influence of ferrous iron and pH on carbon tetrachloride degradation by *Methanosarcina thermophila*." *Water Research* 35(9): 2307-2313.**

The influence of environmental conditions on the biological transformation of a contaminant must be well understood to optimize remediation processes. One factor that impacts the biological transformation of carbon tetrachloride (CT) is elemental iron (Fe<sup>0</sup>). Previous research has shown that Fe<sup>0</sup> increases the methanogenic CT degradation rate by providing H<sub>2</sub> for cell growth and dechlorination. As Fe<sup>0</sup> oxidizes it also increases the pH and Fe<sup>2+</sup> levels, which may also impact the biological transformation of CT. Experiments were performed with *Methanosarcina thermophila* to investigate the influence of these factors on CT degradation. The transformation of CT and CF was greatly influenced by pH, with the rate of CT and CF degradation increasing with increasing pH. After 6 h, > 90% of the CT had been degraded in the treatments containing cells at a pH of 8.5, whereas only about 51% of the CT had been degraded in similar treatments at a pH of 5.5. Fe<sup>2+</sup> did not significantly influence the degradation of CT; however, 60% less CF was formed in systems containing cells + Fe<sup>2+</sup> than in systems containing cells only. In addition, Fe<sup>2+</sup> prolonged rapid transformation of CF when added to treatments containing cells. The product distribution after 9 days in all systems containing cells was very similar, with 98.04 +/- 5.46% (two-sided 95% confidence interval) of the originally fed CT present as soluble products. These results show that pH and Fe<sup>2+</sup> influence the degradation of CT and CF, although transiently. Because the residence time of contaminants in Fe<sup>0</sup> barriers varies with the thickness of the barrier, it is likely that this influence will be important for some flow-through systems. This implies that a combined Fe<sup>0</sup>/organism remediation system may have previously unrealized advantages (due to pH and Fe<sup>2+</sup> changes). (C) 2001 Elsevier Science Ltd. All rights reserved.

**Bae, J., M. A. Mousa, et al. (2001). "Stimulation of contraction of pregnant rat uterus in vitro by non-dechlorinated and microbially dechlorinated mixtures of polychlorinated biphenyls." *Environmental Health Perspectives* 109(3): 275-282.**

A previous study of six polychlorinated biphenyl (PCB) congeners showed that DCBs with four or fewer chlorines and ortho substitution stimulate uterine contraction frequency in vitro, whereas congeners with a greater number of chlorines or non-ortho substitution are inactive in vitro. We tested the hypothesis that PCB mixtures stimulate uterine contractions in a manner inversely related to the degree of chlorination and the presence of chlorines in the ortho-position of the biphenyl constituents of the mixtures. Uterine strips from pregnant rats were suspended in standard muscle baths and analyzed for changes in isometric contractions in response to in vitro exposure to commercial PCB mixtures (Aroclors) and their dechlorinated products after microbial degradation. The PCB mixtures Aroclor 1242, 1248, and 1254 significantly stimulated uterine contraction frequency, and the least chlorinated mixture, Aroclor 1242, was the most potent stimulant. Microbes from Hudson River sediment dechlorinated Aroclor 1242 and Aroclor 1254 under reducing conditions to produce mixtures with an increased proportion of ortho-substituted congeners with one or two chlorine substitutions. The PCB mixtures that had undergone microbial reductive dechlorination stimulated uterine contraction frequency to a significantly greater extent than the parent mixtures. These results show

that increased uterotonic activity was associated with decreased chlorination and increased ortho substitution of the biphenyl constituents of the mixtures.

**Bunge, M., H. Ballerstedt, et al. (2001). "Regiospecific dechlorination of spiked tetra- and trichlorodibenzo-p-dioxins by anaerobic bacteria from PCDD/F-contaminated Spittelwasser sediments." *Chemosphere* 43(4-7): 675-681.**

Samples were taken from sediment of the River Spittelwasser (district Bitterfeld, Germany), which is highly polluted with PCDD/Fs and other chloroorganic compounds. The sediment cores were separated into 10-20 cm thick layers, spiked with 50  $\mu\text{M}$  of 1,2,3,4-tetrachlorodibenzo-p-dioxin and incubated for 8 months under anaerobic conditions in the presence of cosubstrates. Reductive dechlorination of the tetrachlorinated congener and formation of tri- and dichlorinated products were observed in all biologically active incubations. Analysis of subcultures spiked with 1,2,3- and 1,2,4-trichlorodibenzo-p-dioxin, respectively, revealed two different dechlorination pathways within the sediment cores. Pathway M was characterized by the simultaneous dechlorination of peri- and lateralchlorine atoms, whereas sequence SP was restricted to the dechlorination at positions flanked by chlorine atoms on both sides.

**Chen, I. M., F. C. Chang, et al. (2001). "Comparisons of PCBs dechlorination occurrences in various contaminated sediments." *Chemosphere* 43(4-7): 649-654.**

A comparison was made of reductive dechlorination occurrences of polychlorinated biphenyls (PCBs) by microorganisms collected from contaminated sediments including Er-Jen River (Tainan, Taiwan), Hudson River (Ft. Edward, NY), Silver Lake (Pittsfield, MA) and Puget Sound (Washington State). Comparisons were made in terms of chromatographic data (referring to the biological activity, including microbial availability) and thermodynamic data (demonstrating the selectivity of anaerobic microorganisms in the dechlorination of chlorinated compounds). Chromatographic data was established in terms of difference in relative retention time ( $\Delta\text{ln RRT}$ ) and thermodynamic data was estimated as heat of reaction ( $\Delta\text{Hr0}$ ). Both were calculated and correlated to occurrences of dechlorination reactions. Observed dechlorination reactions for individually introducing PCB congener had  $\Delta\text{ln RRT}$  levels measured as  $>0.47$  (Er-Jen River),  $>0.29$  (Hudson River),  $>0.36$  (Silver Lake) and  $>0.45$  (Puget Sound, for Aroclor 1254 dechlorination). Critical of  $\Delta\text{Hr0}$  and  $\Delta\text{ln RRT}$  values showed that Hudson River and Silver Lake microorganisms were capable of dechlorinating PCBs through reactions with larger  $\text{Hr0}$  value (lower levels of released energy) and smaller  $\Delta\text{ln RRT}$  value compared with those found in Er-Jen River and Puget Sound sediments. Differences in the critical  $\Delta\text{ln RRT}$  values of these sediments may be due to differences in their levels of PCB contamination.

**Chiu, P. C. and M. Lee (2001). "2-Bromoethanesulfonate affects bacteria in a trichloroethene - dechlorinating culture." *Applied and Environmental Microbiology* 67(5): 2371-2374.**

Long-term exposure to 2-bromoethanesulfonate (BES), an agent known to inhibit methanogenesis, altered the bacterial community structure of an anaerobic enrichment culture that reductively dechlorinated trichloroethene (TCE). BES did not hinder the dechlorination of TCE or other chlorinated ethenes as previously reported, although different intermediates and end products were observed.

**Cho, Y.-C., O.-S. Kwon, et al. (2001). "Microbial PCB dechlorination in dredged sediments and the effect of moisture." *Chemosphere* 43(8): 1119-1126.**

Evidence of reductive dechlorination of polychlorinated biphenyls (PCBs) in sediments was investigated in Hudson River sediments dredged and encapsulated in 1978 at Moreau, NY. The effect of different moisture contents in dredged sediments on dechlorination and dechlorinating microorganisms was also determined using PCB-spiked sediments in which the moisture level was adjusted by simulating a dewatering process. The congener pattern of PCBs indicated that the dechlorination in the dredged sediments was far less advanced than that in the river sediments collected from the general area of the dredged site (Ft. Edward site). Dechlorination in encapsulated sediments at the Moreau site appeared to have stopped soon after dredging. When microorganisms eluted from the encapsulated sediments were inoculated in clean sediments spiked with Aroclor 1242, an extensive dechlorination was observed, indicating that the encapsulated sediments still harbored dechlorinating microorganisms. However, the same inoculum failed to further dechlorinate residual congeners in the dredged sediments. On the other hand, an inoculum obtained in 1990 from the dredged site in the Hudson River dechlorinated the residual congeners further. In simulated dredged sediments, the maximum level of dechlorination was lower at reduced moisture contents. The population size of dechlorinating microorganisms, as determined by the most probable number (MPN) technique,

was also smaller at the lower moisture levels. There was a significant correlation between the maximum extent of dechlorination and the specific death rate of dechlorinating populations. These results indicate that the underlying mechanism of the moisture-dependent maximum dechlorination is the moisture-dependence of the death rate of dechlorinating microorganisms.

**Conesa, A., F. van-De-Velde, et al. (2001). "Expression of the *Caldariomyces fumago* chloroperoxidase in *Aspergillus niger* and characterization of the recombinant enzyme." *J Biol Chem* 276(21): 17635-40.**

The *Caldariomyces fumago* chloroperoxidase was successfully expressed in *Aspergillus niger*. The recombinant enzyme was produced in the culture medium as an active protein and could be purified by a three-step purification procedure. The catalytic behavior of recombinant chloroperoxidase (rCPO) was studied and compared with that of native CPO. The specific chlorination activity (47 units/nmol) of rCPO and its pH optimum (pH 2.75) were very similar to those of native CPO. rCPO catalyzes the oxidation of various substrates in comparable yields and selectivities to native CPO. Indole was oxidized to 2-oxindole with 99% selectivity and thioanisole to the corresponding R-sulfoxide (enantiomeric excess >98%). Incorporation of (<sup>18</sup>O) from labeled H(<sub>2</sub>)<sup>18</sup>O(<sub>2</sub>) into the oxidized products was 100% in both cases.

**Dimmer, C. H., A. McCulloch, et al. (2001). "Tropospheric concentrations of the chlorinated solvents, tetrachloroethene and trichloroethene, measured in the remote northern hemisphere." *Atmospheric Environment* 35: 1171-1182.**

A fully automated twin ECD gas chromatograph system with sample enriching adsorption-desorption primary stage was deployed on two field campaigns } Ny-Aslesund, Svalbard, Arctic Norway (July-September 1997), and the RRS Discovery CHAOS cruise of the northeast Atlantic (April-May 1998). Concentrations of an extensive set of halocarbons were detected at hourly intervals at pptv levels. We present here the results obtained for the chlorinated solvents, tetrachloroethene (PCE) and trichloroethene (TCE). Average baseline PCE and TCE concentrations of 1.77 and 0.12 pptv, respectively, were recorded in Ny-Aslesund. During pollution incidences, concentrations rose to 5.61 (PCE) and 3.18 pptv (TCE). The cruise data showed average concentrations ranging from 4.26 (PCE) and 1.66 pptv (TCE) for air masses originating over the North Atlantic and Arctic open oceans, to maxima of 15.59 (PCE) and 17.51 pptv (TCE) for polluted air masses from Northern Europe. The data sets emphasise the difficulties in defining remote sites for background tropospheric halocarbon measurements, as Ny-Aslesund research station proved to be a source of tetrachloroethene. The data also suggest possible oceanic emissions of TCE in the sub-tropical ocean.

**Dimmer, C. H., P. G. Simmonds, et al. (2001). "Biogenic fluxes of halomethanes from Irish peatland ecosystems." *Atmospheric Environment* 35(2): 321-330.**

Irish peatland ecosystems have been shown to be important sources of low molecular weight halocarbons. Emission of CH<sub>3</sub>Br, CH<sub>3</sub>Cl, CH<sub>3</sub>I and CHCl<sub>3</sub>, was recorded from all peatland sites monitored, with minor flux of other halocarbons at certain sites. Fluxes were found to be highly linked to incident light, with strong diurnal cycles recorded at all open peatland sites. Estimates of halomethane emissions, particularly from coastal peatland and conifer plantation forest floor sites, suggests that these ecosystems may make a significant contribution to the global budgets of several important halocarbons. Global annual fluxes of 4.7 (0.1-151.9), 0.9 (0.1-3.3), 5.5 (0.9-43.4), and 1.4 (0.1-12.8) Gg yr<sup>-1</sup> for CHCl<sub>3</sub>, CH<sub>3</sub>Br, CH<sub>3</sub>Cl, and CH<sub>3</sub>I, resp., were determined for peatland ecosystems.

**Drzyzga, O., J. Gerritse, et al. (2001). "Coexistence of a sulphate-reducing *Desulfovibrio* species and the dehalorespiring *Desulfitobacterium frappieri* TCE1 in defined chemostat cultures grown with various combinations of sulphate and tetrachloroethene." *Environmental Microbiology* 3(2): 92-99.**

A two-member co-culture consisting of the dehalorespiring *Desulfitobacterium frappieri* TCE1 and the sulphate-reducing *Desulfovibrio* sp. strain SULF1 was obtained via anaerobic enrichment from soil contaminated with tetrachloroethene (PCE). In this co-culture, PCE dechlorination to cis-dichloroethene was due to the activity of the dehalorespiring bacterium only. Chemostat experiments with lactate as the primary electron donor for both strains along with varying sulphate and PCE concentrations showed that the sulphate-reducing strain outnumbered the dehalogenating strain at relatively high ratios of sulphate/PCE. Stable co-cultures with both organisms present at similar cell densities were observed when both electron accepters were supplied in the reservoir medium in nearly equimolar amounts. In the presence of low sulphate/PCE ratios, the *Desulfitobacterium* sp. became the numerically dominant strain within the chemostat co-culture. Surprisingly, in the absence of sulphate, strain SULF1 did not

disappear completely from the co-culture despite the fact that there was no electron acceptor provided with the medium to be used by this sulphate reducer. Therefore, we propose a syntrophic association between the sulphate-reducing and the dehalorespiring bacteria via interspecies hydrogen transfer. The sulphate reducer was able to sustain growth in the chemostat co-culture by fermenting lactate and using the dehalogenating bacterium as a 'biological electron acceptor'. This is the first report describing growth of a sulphate-reducing bacterium in a defined two-member continuous culture by syntrophically coupling the electron and hydrogen transfer to a dehalorespiring bacterium.

**Eguchi, M., M. Kitagawa, et al. (2001). "A field evaluation of in situ biodegradation of trichloroethylene through methane injection." *Water Research* 35(9): 2145-2152.**

A field study of biodegradation of trichloroethylene (TCE) through methane injection was conducted at the yard of a home in Japan. Methane was selected as the safest substrate for injection into groundwater. Methane, oxygen, nitrate, and phosphate were introduced into groundwater contaminated with 220 µg/L of TCE. After a week of biostimulation, methane concentrations gradually decreased below the detection limit. Methane oxidizing bacterial numbers increased from 10 to 10(4) cells/mL with methane consumption. During methane injection, 10-20% of TCE removal was observed. The biotransformation yield was 3-13 mgTCE/gCH<sub>4</sub> in this field test. After methane injections were stopped, TCE removal was not observed. These results indicated that bioremediation using methane was useful as a safe technology for a TCE-contaminated area near homes. (C) 2001 Elsevier Science Ltd.

**Fox, W. M., L. Connor, et al. (2001). "The organochlorine contamination history of the Mersey estuary, UK, revealed by analysis of sediment cores from salt marshes." *Marine Environmental Research* 51(3): 213-227.**

Sediment profiles in the Banks, Ince and Widnes Warth salt marshes in Northwest England contain a mappable record of historic pollution. For persistent organochlorine compounds this stretches back over 90 years. The PCB and HCH profiles can be successfully rationalised by dating methods, and they can be related to the dates of initial production and subsequent withdrawal from use of these chemicals as a result of restrictive environmental legislation. HCB has a more complex pollution profile as it has been manufactured in Northwest England, both deliberately as a pesticide and accidentally as a by-product of several chlorination processes, dating back to the start of the 20th century. The concentrations of degradation products of DDT are relatively constant through the sediment profile and are dominated by *op'*- and *pp'*-DDD with only minor contributions from the most toxic species, *pp'*-DDT. The quantities of these compounds resident in the reservoir of pollutants under these marshes have been calculated, and have fallen progressively in the last 30-50 years.

**Fu, Q. S., A. L. Barkovskii, et al. (2001). "Dioxin cycling in aquatic sediments: The Passaic river estuary." *Chemosphere* 43(4-7): 643-648.**

The contribution of dechlorination reactions to the transformation of dioxins in river sediments was evaluated under a range of geochemical conditions mimicking freshwater, estuarine and marine environments, and interpreted in the light of recent evidence for their accumulation at the air-water interface. The yield of dechlorinated products increased with salinity, but never exceeded 20% of the initial dioxins present. Among the homologue groups in saline conditions, diCDDs were dominant at 32-47 mol% (8-16 nM). The production of 2,3,7,8-TCDD increased with decreasing salinity and in the presence of dissolved organic matter, exhibiting a maximum ratio (2,3,7,8-TCDD:non-2,3,7,8-TCDD) of 0.5. These differences in patterns may be related to the dominant microbial respiratory processes responsible for carbon turnover under the imposed conditions, and are strongly affected by the presence of organic matter. Based on these results, a conceptual dechlorination and carbon turnover model is proposed to help explain the likely reactivity of dioxins in the Passaic River Estuary.

**Futamata, H., S. Harayama, et al. (2001). "Diversity in kinetics of trichloroethylene-degrading activities exhibited by phenol-degrading bacteria." *Applied Microbiology Biotechnology* 55(2): 248-253.**

Whole-cell kinetics of phenol- and trichloroethylene (TCE)-degrading activities expressed by 13 phenol-degrading bacteria were analyzed. The *K<sub>s</sub>* (apparent affinity constant in Haldane's equation) values for TCE were unexpectedly diverse, ranging from 11 µM to over 800 µM. The *V<sub>max</sub>/K<sub>s</sub>* values for phenol were three orders of magnitude higher than the values for TCE in all bacteria analyzed, suggesting that these bacteria preferentially degrade phenol rather than TCE. A positive correlation between *K<sub>s</sub>* for phenol and *K<sub>s</sub>* for TCE was found, i.e., bacteria exhibiting high *K<sub>s</sub>* values for phenol showed high *K<sub>s</sub>* values for TCE, and vice versa. A comparison of the *K<sub>s</sub>* values allowed grouping

of these bacteria into three types, i.e., low-, moderate- and high-Ks types. Pseudo-first-order degradation-rate constants for TCE at 3.8  $\mu\text{M}$  were found to be adequate to rapidly discriminate among the three types of bacteria. When bacteria were grown on phenol at the initial concentration of 2 mM, *Comamonas testosteroni* strain R5, a representative of low-Ks bacteria, completely degraded TCE at 3.8  $\mu\text{M}$ , while strain P-8, a representative of high-Ks bacteria, did not. A mixed culture of these two bacteria poorly degraded TCE under the same conditions, where P-8 outgrew R5. These results suggest that low-Ks bacteria should be selectively grown for effective bioremediation of TCE-contaminated groundwater.

**Haro, M. A. and V. de Lorenzo (2001). "Metabolic engineering of bacteria for environmental applications: Construction of Pseudomonas strains for biodegradation of 2-chlorotoluene." *Journal of Biotechnology* 85(2): 103-113.**

In this article, we illustrate the challenges and bottlenecks in the metabolic engineering of bacteria destined for environmental bioremediation, by reporting current efforts to construct *Pseudomonas* strains genetically designed for degradation of the recalcitrant compound 2-chlorotoluene. The assembled pathway includes one catabolic segment encoding the toluene dioxygenase of the TOD system of *Pseudomonas putida* F1 (todC1C2BA), which affords the bioconversion of 2-chlorotoluene into 2-chlorobenzaldehyde by virtue of its residual methyl-monoxygenase activity on *o*-substituted substrates. A second catabolic segment encoded the entire upper TOL pathway from pWW0 plasmid of *P. putida* mt-2. The enzymes, benzyl alcohol dehydrogenase (encoded by xylB) and benzaldehyde dehydrogenase (xylC) of this segment accept *o*-chloro-substituted substrates all the way down to 2-chlorobenzoate. These TOL and TOD segments were assembled in separate mini-Tn5 transposon vectors, such that expression of the encoded genes was dependent on the toluene-responsive Pu promoter of the TOL plasmid and the cognate XylR regulator. Such gene cassettes (mini-Tn5 (UPP2) and mini-Tn5 (TOD2)) were inserted in the chromosome of the 2-chlorobenzoate degraders *Pseudomonas aeruginosa* PA142 and *P. aeruginosa* JB2. GC-MS analysis of the metabolic intermediates present in the culture media of the resulting strains verified that these possessed, not only the genetic information, but also the functional ability to mineralise 2-chlorotoluene. However, although these strains did convert the substrate into 2-chlorobenzoate, they failed to grow on 2-chlorotoluene as the only carbon source. These results pinpoint the rate of the metabolic fluxes, the non-productive spill of side-metabolites and the physiological control of degradative pathways as the real bottlenecks for degradation of certain pollutants, rather than the theoretical enzymatic and genetic fitness of the recombinant bacteria to the process.

**Huang, W. L., Bu, X., Nguyen, L., Gammon, R. H., and J. L. Bullister (2000). "Production and consumption of methyl halides in a freshwater lake." *Limnology Oceanography* 45(7): 1537-1545.**

The concentrations of the methyl halides CH<sub>3</sub>Cl and CH<sub>3</sub>Br in Lake Washington and its tributary streams were measured during the period 1994-1998. Strong seasonal variation was observed. In summer, the surface mixed layer in the middle of the lake was found to be generally undersaturated for CH<sub>3</sub>Cl but consistently supersaturated for CH<sub>3</sub>Br relative to air-water gas equilibrium; meanwhile, the deep waters (*z*(max) = 65 m) of the summertime lake were found to be depleted to near zero levels for both compounds. In winter, the methyl halides were observed to be vertically well mixed throughout the water column and strongly undersaturated in both species. A simple mass-balance box model indicates that the wintertime lake (*T* = 8 degreesC) is a net sink for atmospheric methyl halides with estimated loss rates of similar to 2.6-3.2 pM d<sup>-1</sup> for CH<sub>3</sub>Cl (1 pM = 10(-12) mol L<sup>-1</sup>) and 0.09-0.13 pM d<sup>-1</sup> for CH<sub>3</sub>Br, probably because of unspecified microbiological processes. In summer, the mass balance model indicates that there is a net source of CH<sub>3</sub>Br in the surface mixed layer (0.17-0.25 pM d<sup>-1</sup>), as required to maintain the observed strong supersaturation. However, the summer surface waters are apparently a sink for CH<sub>3</sub>Cl, (1.9-2.9 pM d<sup>-1</sup>), with enhanced biological production compensated by larger combined microbial and chemical sinks terms. In summer, methyl halide concentrations in the deep waters continue to decrease exponentially at the observed wintertime rate, while both production and loss rates in the surface waters are likely much faster than in winter, resulting in much shorter turnover timescales. An order-of-magnitude extrapolation of these Lake Washington methyl halide fluxes to a global source/sink from freshwater lakes implies a negligible contribution (<0.05%) with respect to the currently estimated global budgets of these gases.

**Janssen, D. B., J. E. Oppentocht, et al. (2001). "Microbial dehalogenation." *Current Opinion in Biotechnology* 12(3): 254-258.**

Novel dehalogenases have been identified recently in various bacteria that utilise halogenated substrates. X-ray studies and sequence analysis have revealed insight into the molecular mechanisms of hydrolytic dehalogenases.

Furthermore, genetic and biochemical studies have indicated that reductive dehalogenases are extra-cytoplasmic corrinoid-containing iron-sulphur proteins. Sequence analysis and mutagenesis studies indicate that several dehalogenases are homologous to enzymes that carry out transformations on non-halogenated substrates.

**Jendrzewski, N., H. G. M. Eggenkamp, et al. (2001). "Characterisation of chlorinated hydrocarbons from chlorine and carbon isotopic compositions: scope of application to environmental problems." *Applied Geochemistry* 16(9-10): 1021-1031.**

A set of chlorinated hydrocarbons (TCE, PCE, DCM, 1,1,1-TCA, chloroform) provided by four manufacturers has been isotopically characterised for both C and Cl, using a new sensitive method. A very large range of  $\delta$  C-13 (from -51.66 to -24.07‰/PDB) associated with a very large range of  $\delta$  Cl-37 (from -2.7 to +3.4‰/SMOC) was obtained. This range of  $\delta$  Cl-37 is much larger than that of inorganic Cl (+/-1‰ SMOC) and most individual solvents show a very distinct  $\delta$  Cl-37 compared to inorganic Cl isotopic signatures. Moreover,  $\delta$  Cl-37/ $\delta$  C-13 pairs are distinct from one solvent/manufacturer to another. In a  $\delta$  C-13 versus  $\delta$  Cl-37 diagram,  $\delta$  Cl-37/ $\delta$  C-13 pairs show different trends for the products of a single manufacturer compared to another. This suggests that Cl isotopic compositions are probably highly fractionated during organic synthesis. The  $\delta$  Cl-37 values can be interpreted in terms of the probable manufacturing processes. Unlike the data published previously, with one exception, all the new results for samples reported here have positive  $\delta$  Cl-37 values which might differentiate natural Cl from that derived from degradation. This method has significant potential as a tool for investigating environmental pollution problems; in particular, it offers the possibility for validating models of transport and fate of pollutants. (C) 2001 Elsevier Science Ltd. All rights reserved.

**Kalin, R. M., J. T. Hamilton, et al. (2001). "Continuous flow stable isotope methods for study of  $\delta$ 13C fractionation during halomethane production and degradation." *Rapid Commun Mass Spectrom* 15(5): 357-363.**

GC/mass spectrometry/isotope ratio mass spectrometry (GC/MS/IRMS) methods for  $\delta$ (13)C measurement of the halomethanes CH(3)Cl, CH(3)Br, CH(3)I and methanethiol (CH(3)SH) during studies of their biological production, biological degradation, and abiotic reactions are presented. Optimisation of gas chromatographic parameters allowed the identification and quantification of CO(2), O(2), CH(3)Cl, CH(3)Br, CH(3)I and CH(3)SH from a single sample, and also the concurrent measurement of  $\delta$ (13)C for each of the halomethanes and methanethiol. Precision of  $\delta$ (13)C measurements for halomethane standards decreased (+/-0.3, +/-0.5 and +/-1.3 per thousand) with increasing mass (CH(3)Cl, CH(3)Br, CH(3)I, respectively). Given that carbon isotope effects during biological production, biological degradation and some chemical (abiotic) reactions can be as much as 100 per thousand, stable isotope analysis offers a precise method to study the global sources and sinks of these halogenated compounds that are of considerable importance to our understanding of stratospheric ozone destruction. Copyright 2001 John Wiley.

**Kao, C. M., S. C. Chen, et al. (2001). "Development of a biobarrier for the remediation of PCE-contaminated aquifer." *Chemosphere* 43(8): 1071-1078.**

The industrial solvent tetrachloroethylene (PCE) is among the most ubiquitous chlorinated compounds found in groundwater contamination. The objective of this study was to develop a biobarrier system, which includes a peat layer to enhance the anaerobic reductive dechlorination of PCE in situ. Peat was used to supply primary substrate (electron donor) continuously. A laboratory-scale column experiment was conducted to evaluate the feasibility of this proposed system or PCE removal. This experiment was performed using a series of continuous-flow glass columns including a soil column, a peat column, followed by two consecutive soil columns. Anaerobic acclimated sludges were inoculated in all three soil columns to provide microbial consortia for PCE biodegradation. Simulated PCE-contaminated groundwater with a flow rate of 0.25 l/day was pumped into this system. Effluent samples from each column were analyzed for PCE and its degradation byproducts (trichloroethylene (TCE), cis-dichloroethylene (cis-DCE), vinyl chloride (VC), ethylene (ETH), and ethane). Results show that the decrease in PCE concentrations and production of PCE byproducts were observed over a 65-day operating period. Up to 98% of PCE removal efficiency was obtained in this passive system. Results indicate that the continuously released organics from peat column enhanced PCE biotransformation. Thus, the developed biobarrier treatment scheme has the potential to be developed into a cost-effective in situ PCE-remediation technology, and can be utilized as an interim step to aid in system scale-up.

**Kim, S. and F. Picardal (2001). "Microbial growth on dichlorobiphenyls chlorinated on both rings as a sole carbon and energy source." *Applied and Environmental Microbiology* 67(4): 1953-1955.**

We have isolated bacterial strains capable of aerobic growth on ortho-substituted dichlorobiphenyls as sole carbon and energy sources. During growth on 2,2'-dichlorobiphenyl and 2,4'-dichlorobiphenyl strain SK-4 produced stoichiometric amounts of 2-chlorobenzoate and 4-chlorobenzoate, respectively. Chlorobenzoates were not produced when strain SK-3 was grown on 2,4'-dichlorobiphenyl.

**Kmuniek, J., S. Luengo, et al. (2001). "Comparative binding energy analysis of the substrate specificity of haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10." *Biochemistry* 407.(30): 8905-8917.**

Comparative binding energy (COMBINE) analysis was conducted for 18 substrates of the haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 (DhlA): 1-chlorobutane, 1-chlorohexane, dichloromethane, 1,2-dichloroethane, 1,2-dichloropropane, 2-chloroethanol, epichlorohydrine, 2-chloroacetonitrile, 2-chloroacetamide, and their brominated analogues. The purpose of the COMBINE analysis was to identify the amino acid residues determining the substrate specificity of the haloalkane dehalogenase. This knowledge is essential for the tailoring of this enzyme for biotechnological applications. Complexes of the enzyme with these substrates were modeled and then refined by molecular mechanics energy minimization. The intermolecular enzyme-substrate energy was decomposed into residue-wise van der Waals and electrostatic contributions and complemented by surface area dependent and electrostatic desolvation terms. Partial least-squares projection to latent structures analysis was then used to establish relationships between the energy contributions and the experimental apparent dissociation constants. A model containing van der Waals and electrostatic intermolecular interaction energy contributions calculated using the AMBER force field explained 91% (73% cross-validated) of the quantitative variance in the apparent dissociation constants. A model based on van der Waals intermolecular contributions from AMBER and electrostatic interactions derived from the Poisson-Boltzmann equation explained 93% (74% cross-validated) of the quantitative variance. COMBINE models predicted correctly the change in apparent dissociation constants upon single-point mutation of DhlA for six enzyme-substrate complexes. The amino acid residues contributing most significantly to the substrate specificity of DhlA were identified; they include Asp124, Trp125, Phe164, Phe172, Trp175, Phe222, Pro223, and Leu263. These residues are suitable targets for modification by site-directed mutagenesis.

**Koons, B. W., J. L. Baeseman, et al. (2001). "Investigation of cell exudates active in carbon tetrachloride and chloroform degradation." *Biotechnology and Bioengineering* 74(1): 12-17.**

Contamination of groundwater by chlorinated solvents such as carbon tetrachloride (CCl<sub>4</sub>) and chloroform (CHCl<sub>3</sub>) is a widespread problem. The cell exudates from the methanogen *Methanosarcina thermophila* are active in the degradation of CCl<sub>4</sub> and CHCl<sub>3</sub>. This research was performed to characterize these exudates. Examination of the influence of pH indicated that activity was greater under alkaline conditions. Rapid CCl<sub>4</sub> degradation occurred from 35-65 degreesC, with first-order degradation rate coefficients increasing as temperature increased. It was found that proteins were not responsible for CCl<sub>4</sub> degradation. The active agents in the cell exudates were <10 kDa in size, with degradation activity present in both 1-10 kDa and <1 kDa size ranges. Upon purification of the <10 kDa size range of the cell exudates on a C-18 chromatography column, 17 fractions (out of 100) degraded >50% of the added CCl<sub>4</sub> in 8 h. These 17 fractions were pooled into three samples based on their elution time from the C-18 column. One of these pooled samples contained elevated levels of cobalt, zinc, and iron, at 2, 3, and 13 times the levels measured in similarly fractionated and pooled samples of medium, respectively. The UV-visible spectrum of this pooled sample had an absorption maximum at 560-580 nm, which is similar to the absorption maxima of heme (approximately 550 and 575 nm). The two other pooled samples contained elevated levels of zinc at 11 and 22 times the concentration measured in similarly fractionated and pooled samples of medium, respectively, and also contained very low levels of nickel, cobalt, and iron. This research suggests that the cell exudates from *M. thermophila* contain porphyrinogen-type molecules capable of dechlorination, possibly excreted corrinoids, hemes, and zinc-containing molecules. (C)

**Krohn, K., M. John, et al. (2001). "Biologically active metabolites from fungi 14 - 3-chloro-4-hydroxy-5-(3,7,11-trimethyldodeca-2,6,10-trienyl)benzamide, a new antibacterial agent from a soil fungus." *Natural Product Letters* 15(1): 9-12.**

The antibacterial 3-chloro-4-hydroxy-5-(3,7,11-trimethyldodeca-2,6,10-trienyl)-benzamide, and the antifungal strobilurins B and D were isolated from an unidentified South African soil fungus.

**Lewandowicz, A., Rudziski, J., L. Tronstad, et al. (2001). "Chlorine kinetic isotope effects on the haloalkane dehalogenase reaction." *Journal of the American Chemical Society* 123(19): 4550-4555.**

We have found chlorine kinetic isotope effects on the dehalogenation catalyzed by haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 to be  $1.0045 \pm 0.0004$  for 1,2-dichloroethane and  $1.0066 \pm 0.0004$  for 1-chlorobutane. The latter isotope effect approaches the intrinsic chlorine kinetic isotope effect for the dehalogenation step. The intrinsic isotope effect has been modeled using semiempirical and DFT theory levels using the ONIOM QM/QM scheme. Our results indicate that the dehalogenation step is reversible; the overall irreversibility of the enzyme-catalyzed reaction is brought about by a step following the dehalogenation.

**Mihopoulos, P. G., M. T. Suidan, et al. (2001). "Complete remediation of PCE contaminated unsaturated soils by sequential anaerobic-aerobic bioventing." *Water Science Technology* 43(5): 365-372.**

Bioventing principles have been applied to completely dechlorinate tetrachloroethylene vapors in the unsaturated zone in a sequential anaerobic-aerobic pattern. The anaerobic step yields trans-DCE and VC as PCE reductive dechlorination byproducts, while TCE and cis-DCE are observed as intermediates. The aerobic step results in rapid oxidation of the VC and trans-DCE to carbon dioxide. Hydrogen was delivered in the gas phase as a reducing agent for the anaerobic step at levels of 1%, and oxygen at 4.2% was used as an electron acceptor in the aerobic step. PCE and VC half lives in the anaerobic and aerobic steps respectively, where less than 10 min.

**Miller, L. G., R. M. Kalin, et al. (2001). "Large carbon isotope fractionation associated with oxidation of methyl halides by methylotrophic bacteria." *Proc Natl Acad Sci U S A* 98(10): 5833-5837.**

The largest biological fractionations of stable carbon isotopes observed in nature occur during production of methane by methanogenic archaea. These fractionations result in substantial (as much as approximately 70 per thousand) shifts in  $\delta(13)C$  relative to the initial substrate. We now report that a stable carbon isotopic fractionation of comparable magnitude (up to 70 per thousand) occurs during oxidation of methyl halides by methylotrophic bacteria. We have demonstrated biological fractionation with whole cells of three methylotrophs (strain IMB-1, strain CC495, and strain MB2) and, to a lesser extent, with the purified cobalamin-dependent methyltransferase enzyme obtained from strain CC495. Thus, the genetic similarities recently reported between methylotrophs, and methanogens with respect to their pathways for C(1)-unit metabolism are also reflected in the carbon isotopic fractionations achieved by these organisms. We found that only part of the observed fractionation of carbon isotopes could be accounted for by the activity of the corrinoid methyltransferase enzyme, suggesting fractionation by enzymes further along the degradation pathway. These observations are of potential biogeochemical significance in the application of stable carbon isotope ratios to constrain the tropospheric budgets for the ozone-depleting halocarbons, methyl bromide and methyl chloride.

**Okeke, B. C., Y. C. Chang, et al. (2001). "Purification, cloning, and sequencing of an enzyme mediating the reductive dechlorination of tetrachloroethylene (PCE) from *Clostridium bifermentans* DPH-1." *Canadian Journal of Microbiology* 47(5): 448-456.**

An enzyme mediating the reductive dechlorination of tetrachloroethylene (PCE) from cell-free extracts of *Clostridium bifermentans* DPH-1 was purified, cloned, and sequenced. The enzyme catalyzed the reductive dechlorination of PCE to cis-1,2-dichloroethylene via trichloroethylene, at a  $V_{max}$  and  $K_m$  of 73 nmol/mg protein and 12  $\mu M$ , respectively. Maximal activity was recorded at 35°C and pH 7.5. Enzymatic activity was independent of metal ions but was oxygen sensitive. A mixture of propyl iodide and titanium citrate caused a light-reversible inhibition of enzymatic activity suggesting the involvement of a corrinoid cofactor. The molecular mass of the native enzyme was estimated to be approximately 70 kDa. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and matrix-assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/MS) revealed molecular masses of approximately 35 kDa and 35.7 kDa, respectively. A broad spectrum of chlorinated aliphatic compounds (PCE, trichloroethylene, cis-1,2-dichloroethylene, trans-1,2-dichloroethylene, 1,1-dichloroethylene, 1,2-dichloropropane, and 1,1,2-trichloroethane) was degraded. With degenerate primers designed from the N-terminal sequence (27 amino acid residues), a partial sequence (81 bp) of the encoding gene was amplified by polymerase chain reaction (PCR) and sequenced. Southern analysis of *C. bifermentans* genomic DNA using the PCR product as a probe revealed restriction fragment bands. A 5.0 kb *Cla*I fragment, harboring the relevant gene (designated *pceC*) was cloned (*pDEHAL5*) and the complete nucleotide sequence of *pceC* was determined. The gene showed homology mainly with microbial membrane proteins and no homology with any known dehalogenase, suggesting a distinct PCE dehalogenase.

**Orsini, M. A., L. K. Pannell, et al. (2001). "Polychlorinated acetamides from the cyanobacterium *Microcoleus lyngbyaceus*." *Journal of Natural Products* 64(5): 572-577.**

Several new compounds were isolated from the organic extract of the cyanobacterium *Microcoleus lyngbyaceus*, and their structures were determined by spectroscopic means. Polychlorinated acetamidoalkynes and alkanes were the major metabolites. 6-Acetamido-1,1,1-trichloroundecane, a positional isomer of the naturally occurring 5-acetamido-1,1,1-trichloroundecane, was synthesized in six steps from -decanolactone.

**Quensen, J. F. I., J. M. Tiedje, et al. (2001). "Factors controlling the rate of DDE dechlorination to DDMU in Palos Verdes margin sediments under anaerobic conditions." *Environmental Science and Technology* 35(2): 286-291.**

Marine sediments off the coast of the Palos Verdes Peninsula in California have been designated a Superfund site primarily because of the presence of DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethene]. For decades, it was believed that DDE was not microbially transformed, but anaerobic bacteria in the Palos Verdes sediments reductively dechlorinate DDE to DDMU [1-chloro-2,2-bis(p-chlorophenyl)- ethene], which is also found in the sediments. The effects of electron donor to sulfate ratio, available carbon, sampling sites, sediment depth, and temperature on the rate and extent of DDE dechlorination in anaerobic Palos Verdes sediment microcosms were investigated. Dechlorination rates varied, depending on the site and depth from which the sediments were collected, but DDE dechlorination occurred with sediments from all locations studied. Sulfate and low temperatures slowed dechlorination, but in the presence of sulfate and at in situ temperature, the dechlorination rates observed in the microcosms agree well with the observed rate of DDE disappearance from the Palos Verdes margin sediments.

**Rezanka, T. and V. M. Dembitsky (2001). "Polyhalogenated homosesquiterpenic fatty acids from *Plocamium cartilagineum*." *Phytochemistry* 57(4): 607-611.**

We describe the composition of novel polyhalogenated homosesquiterpenic acids from the red alga *Plocamium* collected during summer on Maltese Islands and Corsica. The compounds, predominantly derivatives with unique groups (bromine, chlorine, and diene), were identified by means of <sup>1</sup>H and <sup>13</sup>C NMR, MS, IR and UV spectra.

**Rhee, G. Y., R. C. Sokol, et al. (2001). "Kinetics of polychlorinated biphenyl dechlorination and growth of dechlorinating microorganisms." *Environmental Toxicology and Chemistry* 20(4): 721-726.**

The present study has investigated a correlation between the kinetics of polychlorinated biphenyl (PCB) dechlorination and the growth of dechlorinating microbial populations. Microorganisms were eluted from Aroclor(R) 1248-contaminated St. Lawrence River (NY, USA) sediments and inoculated into clean sediments spiked with Aroclor 1248 at 10 concentrations ranging from 0 to 3.12  $\mu\text{mol/g}$  sediment (0-900 ppm). The time course of PCB dechlorination and population growth were concurrently determined by congener-specific analysis and the most probable number technique, respectively. The specific growth rate was a saturation function of PCB concentrations above the threshold concentration (0.14  $\mu\text{mol/g}$  sediment, or 40 ppm), below which no dechlorination or growth of dechlorinations were observed. The maximum growth rate was 0.20/d with a half-saturation constant of 1.23  $\mu\text{mol/g}$  sediment. The yield of dechlorinating microorganisms showed a peak at 0.70  $\mu\text{mol/g}$  sediment (200 ppm), with a value of  $10.3 \times 10^{12}$  cells/mol Cl removed, and decreased below and above this concentration. The dechlorination rate ( $\mu\text{mol Cl removed/g sediment/d}$ ) was a linear function of Aroclor concentration. Both the log of this rate and the maximum level of dechlorination were significantly correlated with growth rate. The biomass-normalized dechlorination rate ( $\mu\text{mol Cl removed/g sediment/cell/d}$ ) was first order because of the exponential manner of the population growth. The first-order rate constant was a saturation function of Aroclor concentrations, with a maximum of 0.24/d (a half-life of 2.9 d) and a half-saturation constant of 1.18  $\mu\text{mol/g}$  sediment, which are similar to the constants for growth. These results indicate that the dechlorination rate is tightly linked to the population growth of dechlorinating microorganisms.

**Seeger, M., B. Camara, et al. (2001). "Dehalogenation, denitration, dehydroxylation, and angular attack on substituted biphenyls and related compounds by a biphenyl dioxygenase." *Journal of Bacteriology* 183(12): 3548-3555.**

The attack by the bph-encoded biphenyl dioxygenase of *Burkholderia* sp. strain LB400 on a number of symmetrical ortho-substituted biphenyls or quasi ortho-substituted biphenyl analogues has been investigated. 2,2'-Difluoro-, 2,2'-dibromo-, 2,2'-dinitro-, and 2,2'-dihydroxybiphenyl were accepted as substrates. Dioxygenation of all of these compounds showed a strong preference for the semisubstituted pair of vicinal ortho and meta carbons, leading to the

formation of 2'-substituted 2,3-dihydroxybiphenyls by subsequent elimination of HX (X=F, Br, NO<sub>2</sub>, or OH). All of these products were further metabolized by 2,3-dihydroxybiphenyl 1,2-dioxygenases of *Burkholderia* sp. strain LB400 or of *Rhodococcus globerulus* P6. Dibenzofuran and dibenzodioxin, which may be regarded as analogues of doubly ortho-substituted biphenyls or diphenylethers, respectively, were attacked at the "quasi ortho" carbon (the angular position 4a) and its neighbor. This shows that an aromatic ring-hydroxylating dioxygenase of class IIB is able to attack angular carbons. The catechols formed, 2,3,2'-trihydroxybiphenyl and 2,3,2'-trihydroxydiphenylether, were further metabolized by 2,3-dihydroxybiphenyl 1,2-dioxygenase. While angular attack by the biphenyl dioxygenase was the main route of dibenzodioxin oxidation, lateral dioxygenation leading to dihydrodiols was the major reaction with dibenzofuran (DBF). These results indicate that this enzyme is capable of hydroxylating ortho or angular carbons carrying a variety of substituents which exert electron-withdrawing inductive effects. They also support the view that the conversions of phenols into catechols by ring-hydroxylating dioxygenases, such as the transformation of 2,2'-dihydroxybiphenyl into 2,3,2'-trihydroxybiphenyl, are the results of di- rather than of monooxygenations. Lateral dioxygenation of dibenzofuran and subsequent dehydrogenation and extradiol dioxygenation by a number of biphenyl-degrading strains yielded intensely colored dead-end products. Thus, DBF can be a useful chromogenic indicator for the activity of the first three enzymes of biphenyl catabolic pathways.

**Seigneur, C., A. Vuillemin, et al. (2001). "A procedure for production of adapted bacteria to degrade chlorinated aromatics." *Journal of Hazardous Materials* 84(2-3): 265-277.**

Production of biomass adapted to the degradation of a mixture of chlorobenzene (CB) and 1,2-dichlorobenzene (DCB) was investigated in a batch culture with substrates supplied by pulses. CB and o-DCB concentrations which gave the best adapted biomass productivity were determined and found to be 150 and 30 mg/l(-1), respectively. The biomass productivity was 51 mg/l(-1)h(-1). The biomass yield was 0.38g of biomass dry weight per gram of substrate. The pulses of 200 mg/l CB and 40 mg/l o-DCB, were inhibitory to the bacterial culture. Among the metabolites, muconic acid was found in large quantities in the medium and in the cells. At a time between two pulses of 60 min, adding 150 mg/l CB and 30 mg/l o-DCB per each pulse, 7.6 g/l(-1) of biomass was obtained. The produced biomass served as an inoculum for the biotrickling filter which treated industrial waste gases contaminated by CBs. The method of adapted biomass production was described using CBs, but the degradation of any other toxic volatile pollutant can be improved using this technique.

**Shah, J. K., G. D. Sayles, et al. (2001). "Anaerobic bioventing of unsaturated zone contaminated with DDT and DNT." *Water Science and Technology* 43(2): 35-42.**

Initial degradation of highly chlorinated compounds and nitroaromatic compounds found in munition waste streams is accelerated under anaerobic conditions followed by aerobic treatment of the degradation products. The establishment of anaerobic environment in a vadose zone can be accomplished by feeding appropriate anaerobic gas mixture, i.e., "anaerobic bioventing". The gas mixture contains an electron donor for the reduction of these compounds. Lab scale study was conducted to evaluate potential of anaerobic bioventing for the treatment of an unsaturated zone contaminated with 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and 2,4-dinitrotoluene (DNT). Hydrogen was used as the electron donor. Using the soil columns inoculated with anaerobic microorganisms, it was observed that by feeding a gas mixture of 1% hydrogen, 1% carbon dioxide and nitrogen, methanogenic conditions were established and DDT was reductively dechlorinated. 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) accumulated as the intermediate product. The half life of DDT was calculated to be 8.5 months. DNT completely disappeared after six months of operation and no intermediates could be detected.

**Spanelova, M. (2001). "Biodegradation transformations of chlorinated aliphatic hydrocarbons." *Chemicke Listy* 95(3): 169-172.**

The review summarizes the present knowledge of biodegradation of chlorinated aliphatic hydrocarbons in environment. Transformation of these compounds by aerobic, facultative aerobic and anaerobic microorganisms in mixed and pure cultures is discussed. The anaerobic biodegradation under methanogenic conditions was shown to be a successful pathway for dechlorination of the compounds.

**Studer, A., E. Stupperich, et al. (2001). "Chloromethane:tetrahydrofolate methyl transfer by two proteins from *Methylobacterium chloromethanicum* strain CM4." *European Journal of Biochemistry* 268(10): 2931-2938.**

The *cmuA* and *cmuB* genes are required for growth of *Methylobacterium chloromethanicum* strain CM4 with chloromethane as the sole carbon source. While *CmuB* was previously shown to possess methylcobalamin:tetrahydrofolate methyltransferase activity, sequence analysis indicated that *CmuA* represented a novel and so far unique two-domain methyltransferase/corrinoid-binding protein involved in methyl transfer from chloromethane to a corrin moiety. *CmuA* was purified from wild-type *M. chloromethanicum* strain CM4 and characterized as a monomeric, cobalt-containing and zinc-containing enzyme of molecular mass 67 kDa with a bound vitamin B12 cofactor. In combination, *CmuA* and *CmuB* proteins catalyze the *in vitro* transfer of the methyl group of chloromethane to tetrahydrofolate, thus affording a direct link between chloromethane dehalogenation and core C1 metabolism of *Methylobacterium*. Chloromethane dehalogenase activity *in vitro* is limited by *CmuB*, as formation of methyltetrahydrofolate from chloromethane displays apparent Michaelis-Menten kinetics with respect to methylated *CmuA*, with an apparent  $K_m$  of 0.27  $\mu\text{M}$  and a  $V_{max}$  of 0.45  $\mu\text{mol}\cdot\text{min}^{-1}$ . This contrasts with sequence-related systems for methyl transfer from methanogens, which involve methyltransferase and corrinoid protein components in well-defined stoichiometric ratios.

**Suzuki, M., M. Daitoh, et al. (2001). "Novel halogenated metabolites from the Malaysian *Laurencia pannosa*." *Journal of Natural Products* 64: 597-602.**

In connection with our chemotaxonomic studies of Malaysian species of the red algal genus *Laurencia*, the chemical composition of *Laurencia pannosa* Zarardini was examined. Two halogenated sesquiterpenoids, named pannosanol (1) and pannosane (2), have been isolated along with a halogenated C15-acetogenin, (3Z)-chlorofucin (3). The structures of these compounds were determined from their spectroscopic data (IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, 2D NMR, and MS). Pannosanol and pannosane are novel halometabolites with an unusual rearranged chamigrane framework. Antibacterial activities of these metabolites against marine bacteria are also described.

**Tesoriero, A. J., F. E. Loffler, et al. (2001). "Fate and origin of 1,2-dichloropropane in an unconfined shallow aquifer." *Environmental Science and Technology* 35(3): 455-461.**

A shallow aquifer with different redox zones overlain by intensive agricultural activity was monitored for the occurrence of 1,2-dichloropropane (DCP) to assess the fate and origin of this pollutant. DCP was detected more frequently in groundwater samples collected in aerobic and nitrate-reducing zones than those collected from iron-reducing zones. Simulated DCP concentrations for groundwater entering an iron-reducing zone were calculated from a fate and transport model that included dispersion, sorption, and hydrolysis but not degradation. Simulated concentrations were well in excess of measured values, suggesting that microbial degradation occurred in the iron-reducing zone. Microcosm experiments were conducted using aquifer samples collected from iron-reducing and aerobic zones to evaluate the potential for microbial degradation of DCP and to explain field observations. Hydrogenolysis of DCP and production of monochlorinated propanes in microcosm experiments occurred only with aquifer materials collected from the iron-reducing zone, and no dechlorination was observed in microcosms established with aquifer materials collected from the aerobic zones. Careful analyses of the DCP/1,2,2-trichloropropane ratios in groundwater indicated that older fumigant formulations were responsible for the high levels of DCP present in this aquifer.

**Van de Pas, B. A., J. Gerritse, et al. (2001). "Two distinct enzyme systems are responsible for tetrachloroethene and chlorophenol reductive dehalogenation in *Desulfitobacterium* strain PCE1." *Archives of Microbiology* ASAP Article.**

*Desulfitobacterium* strain PCE1 is able to use tetrachloroethene and chloroaromatics as terminal electron acceptors for growth. Cell extracts of *Desulfitobacterium* strain PCE1 grown with tetrachloroethene as electron acceptor showed no dehalogenase activity with 3-chloro-4-hydroxyphenylacetate (Cl-OH-phenylacetate) and other ortho-chlorophenolic compounds in an *in vitro* assay. Extracts of cells that were grown with Cl-OH-phenylacetate as electron acceptor dechlorinated tetrachloroethene at 10% of the dechlorination rate of Cl-OH-phenylacetate. In both cell extracts dechlorination was inhibited by the addition of 1-iodopropane and dinitrogen oxide, inhibitors of cobalamin-containing enzymes. The enzymes responsible for tetrachloroethene and Cl-OH-phenylacetate dechlorination were partially purified. A 100-fold enriched fraction of chlorophenol reductive dehalogenase was obtained that mainly contained a protein with a subunit size of 48 kDa. The characteristics of this enzyme are similar

to that of the chlorophenol reductive dehalogenase of *D. dehalogenans*. After partial purification of the tetrachloroethene reductive dehalogenase, a fraction was obtained that also contained a 48-kDa protein, but the N-terminal sequence showed no similarity with that of the chlorophenol reductive dehalogenase sequence or with the N-terminal amino acid sequence of tetra- and trichloroethene reductive dehalogenase of *Desulfitobacterium* strain TCE1. These results provide strong evidence that two different enzymes are responsible for tetrachloroethene and chlorophenol dechlorination in *Desulfitobacterium* strain PCE1. Furthermore, the characterization of partially purified tetrachloroethene reductive dehalogenase indicated that this enzyme is a novel type of reductive dehalogenase

**Van de Pas, B. A., H. J. M. Harmsen, et al. (2001). "A *Desulfitobacterium* strain isolated from human feces that does not dechlorinate chloroethenes or chlorophenols." *Archives of Microbiology* ASAP Article.**

An anaerobic bacterium, strain DP7, was isolated from human feces in mineral medium with formate and 0.02% yeast extract as energy and carbon source. This rod-shaped motile bacterium used pyruvate, lactate, formate, hydrogen, butyrate, and ethanol as electron donor for sulfite reduction. Other electron acceptors such as thiosulfate, nitrate and fumarate stimulated growth in the presence of 0.02% yeast extract and formate. Acetate was the only product during fermentative growth on pyruvate. Six mol of pyruvate were fermented to 7 mol of acetate. <sup>13</sup>C-NMR labeling experiments showed homoacetogenic <sup>13</sup>C-CO<sub>2</sub> incorporation into acetate. The pH and temperature optimum of fermentative growth on pyruvate was 7.4 and 37 °C, respectively. The growth rate under these conditions was approximately 0.10 h<sup>-1</sup>. Strain DP7 was identified as a new strain of *Desulfitobacterium frappieri* on the basis of 16S rRNA sequence analysis (99% similarity) and DNA-DNA hybridization (reassociation value of 83%) with *Desulfitobacterium frappieri* TCE1. In contrast to described *Desulfitobacterium* strains, the newly isolated strain has not been isolated from a polluted environment and did not use chloroethenes or chlorophenols as electron acceptor.

**van Pee, K. H. (2001). "Microbial biosynthesis of halometabolites." *Archives of Microbiology* 175(4): 250-258.**

Halometabolites are compounds that are commonly found in nature and they are produced by many different organisms. Whereas bromometabolites can mainly be found in the marine environment, chlorometabolites are predominately produced by terrestrial organisms; iodo- and fluorocompounds are only produced infrequently. The halogen atoms are incorporated into organic compounds by enzyme-catalyzed reactions with halide ions as the halogen source. For over 40 years haloperoxidases were thought to be responsible for the incorporation of halogen atoms into organic molecules. However, haloperoxidases lack substrate specificity and regioselectivity, and the connection of haloperoxidases with the *in vivo* formation of halometabolites has never been demonstrated. Recently, molecular genetic investigations showed that, at least in bacteria, a different class of halogenases is involved in halometabolite formation. These halogenases were found to require FADH(2), which can be produced from FAD and NADH by unspecific flavin reductases. In addition to FADH(2), oxygen and halide ions (chloride and bromide) are necessary for the halogenation reaction. The FADH(2)-dependent halogenases show substrate specificity and regioselectivity, and their genes have been detected in many halometabolite-producing bacteria, suggesting that this type of halogenating enzymes constitutes the major source for halometabolite formation in bacteria and possibly also in other organisms.

**Villarante, N. R., P. M. Armenante, et al. (2001). "Dehalogenation of dichloroethene in a contaminated soil: fatty acids and alcohols as electron donors and an apparent requirement for tetrachloroethene." *Applied Microbiology and Biotechnology* 55(2): 239-247.**

Environmental soil contamination at an industrial site in Marion, Ohio (USA) with PCE resulted in residual *cis*-1, 2-dichloroethene (DCE) contamination that had not declined after more than 15 years. Microcosm slurries containing 2.6% soil from this site were supplemented with different electron donors, i.e., individual fatty acids or alcohols. None of the microcosms supported complete DCE dechlorination, unless PCE was added to the microcosm at initiation. The addition of fresh PCE resulted in the dehalogenation of PCE to DCE in the microcosms supplemented with fatty acids having an even number of carbon atoms (acetate, butyrate, and caproate), but not in those with an odd number of carbon atoms (formate, propionate, and valerate), where negligible or no activity was detected. No significant further DCE degradation was observed in any of the microcosms supplied with fatty acids as electron donors. Microcosms supplemented with freshly added PCE bioconverted PCE to DCE and completely dehalogenated both the *ex-novo* and soil-supplied DCE within 60 days, but only if alcohols having an even number of carbon atoms

(ethanol or butanol) were also added as electron donors. Odd-numbered alcohols either did not produce dehalogenation (as with methanol) or only dehalogenated PCE to DCE (as with propanol).

**Woggon, W. D., H. A. Wagenknecht, et al. (2001). "Synthetic active site analogues of heme-thiolate proteins - Characterization and identification of intermediates of the catalytic cycles of cytochrome P450(cam) and chloroperoxidase." *Journal of Inorganic Biochemistry* 83(4): 289-300.**

In view of recent results from different sources, the reaction mechanisms of two heme-thiolate proteins, cytochrome P450(cam) and chloroperoxidase (CPO), are discussed. In this context a mechanism of CPO is proposed which includes H<sub>2</sub>O<sub>2</sub> cleavage, subsequent formation of compound I and the identification of two elusive intermediates. The HOCl adduct of the iron(III)porphyrin is the catalytically competent Cl<sup>+</sup> donor chlorinating activated C-H bonds of substrates bound to the enzyme. Pulse-EPR characterization of an enzyme model of the resting state of P450(cam) suggests a role of the electric field of the protein for stabilizing the low-spin state of the cofactor of the enzyme. It is further suggested that the same effect of the protein may trigger the reactivity of compound I such that both concerted and two-step reactions are feasible within the concept of a Two-State-Reactivity. This review emphasizes the value of synthetic enzyme models complementing investigations of the native proteins. (C) 2001 Elsevier

**Yaipakdee, P. and L. W. Robertson (2001). "Enzymatic halogenation of flavanones and flavones." *Phytochemistry* 57(3): 341-347.**

The whole cells and the chloroperoxidase enzyme of *Caldariomyces fumago* were capable of halogenating the flavanones, naringenin and hesperetin, at C-6 and C-8 in the presence of either Cl<sup>-</sup> or Br<sup>-</sup>. However, they did not act on other test flavones. The biohalogenated products of naringenin and hesperetin were isolated and found to be identical to those obtained from chemical reactions using molecular halogen and hypohalous acid.

**Yeager, C. M., P. J. Bottomley, et al. (2001). "Cytotoxicity associated with trichloroethylene oxidation in *Burkholderia cepacia* G4." *Applied and Environmental Microbiology* 67(5): 2107-2115.**

The effects of trichloroethylene (TCE) oxidation on toluene 2-monooxygenase activity, general respiratory activity, and cell culturability were examined in the toluene-oxidizing bacterium *Burkholderia cepacia* G4. Nonspecific damage outpaced inactivation of toluene 2-monooxygenase in *B. cepacia* G4 cells. Cells that had degraded approximately 0.5 μmol of TCE (mg of cells<sup>-1</sup>) lost 95% of their acetate-dependent O<sub>2</sub> uptake activity (a measure of general respiratory activity), yet toluene-dependent O<sub>2</sub> uptake activity decreased only 35%. Cell culturability also decreased upon TCE oxidation; however, the extent of loss varied greatly (up to 3 orders of magnitude) with the method of assessment. Addition of catalase or sodium pyruvate to the surfaces of agar plates increased enumeration of TCE-injured cells by as much as 100-fold, indicating that the TCE-injured cells were ultrasensitive to oxidative stress. Cell suspensions that had oxidized TCE recovered the ability to grow in liquid minimal medium containing lactate or phenol, but recovery was delayed substantially when TCE degradation approached 0.5 μmol (mg of cells<sup>-1</sup>) or 66% of the cells' transformation capacity for TCE at the cell density utilized. Among *B. cepacia* G4 cells isolated on Luria-Bertani agar plates from cultures that had degraded approximately 0.5 μmol of TCE (mg of cells<sup>-1</sup>), up to 90% were Tol<sup>-</sup> variants, no longer capable of TCE degradation. These results indicate that a toxicity threshold for TCE oxidation exists in *B. cepacia* G4 and that once a cell suspension has exceeded this toxicity threshold, the likelihood of reestablishing an active, TCE-degrading biomass from the cells will decrease significantly.