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

13th Quarterly Report

1st Quarter Year 2004

Report prepared for EUROCHLOR

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June 1, 2004

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ACRONYMS

| | |
|-------------------|---|
| 16S rRNA | 16S Ribosomal RNA |
| CB | Chlorobenzene |
| CBA | Chlorobenzoic acid |
| CBp | Chlorobiphenyl |
| CDDs | Chlorinated Dibenzo- <i>p</i> -Dioxins |
| CDFs | Chlorinated Dibenzo- <i>p</i> -Furans |
| CF | Chloroform |
| CN | Chloronaphthalenes |
| CPO | Chloroperoxidase |
| CSTR | Continuous Stirred Tank Reactor |
| CT | Carbon Tetrachloride |
| 1,2-DCA | 1,2-Dichloroethane |
| DCB | Dichlorobenzene |
| DCE | Dichloroethene |
| DCM | Dichloromethane |
| DDT | 1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane |
| 2,3-DCDD | 2,3-Dichlorodibenzo- <i>p</i> -dioxin |
| 1,3-DiCB | 1,3-Dichlorobenzene |
| 2,7-DiCDD | 2,7-Dichlorodibenzo- <i>p</i> -dioxin |
| DIRB | Dissimilatory Iron-Reducing Bacterium |
| DNAPL | Dense Non-Aqueous Phase Liquid |
| E-acceptor | Electron Acceptor |
| E-donor | Electron Donor |
| EFs | Enantiomeric Fractions |
| ERs | Enantiomer Ratios |
| ETH | Ethylene, ethene |
| HCB | Hexachlorobenzene |
| HFCs | Hydrochlorofluorocarbons |
| HCH | Hexachlorohexane |
| MCB | Monochlorobenzene |
| MPN | Most Probable Number |
| PBDEs | Polybrominated Diphenyl Ethers |

ACRONYMS (*Continued*)

| | |
|----------------------|---|
| PCBs | Polychlorinated Biphenyls |
| PCB | Pentachlorobenzene |
| PCP | Pentachlorophenol |
| PCE | Tetrachloroethylene |
| PCR | Polymerase Chain Reaction |
| QCB | Pentachlorobenzene |
| RFLP | Restriction Fragment Length Polymorphism |
| TCA | Trichloroacetic Acid |
| 1,1,2,2-TeCA | 1,1,2,2-Tetrachloroethane |
| TCB | Trichlorobenzene |
| TCDF | Trichlorodibenzofuran |
| TCE | Trichlorethylene |
| TeCB | Tetrachlorobenzene |
| TeCBp | Tetrachlorobiphenyl |
| 1,2,3,4-TeCDD | 1,2,3,4-Tetrachlorodibenzo- <i>p</i> -dioxin |
| 1,2,3,4-TeCDF | 1,2,3,4-Tetrachlorodibenzo- <i>p</i> -furan |
| T-RFLP | Terminal Restriction Fragment Length polymorphism |
| VC | Vinyl Chloride |
| ZVI | Zero-Valent Iron |

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

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1. INTRODUCTION

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

2. SUMMARY OF MOST IMPORTANT DEVELOPMENTS

2.a. Microbial Dechlorination

The most important findings in this quarter for microbial dechlorination are two reports implicating the halo-respiring bacterium, *Dehalococcoides* in the reductive dehalogenation of polychlorinated dioxins (2, 15). These are among the first publications in which a halo-respiring species has been implicated in dioxin degradation. The finding is very significant since it would suggest that microorganisms benefit (and thus grow) at the expense of dioxin dechlorination, which means in time the biocatalyst levels increase in the environments resulting in faster degradation of “bioavailable” dioxin.

Another article of interest is a report with numerous empirical data on the rates of chlorinated solvent degradation on landfill cover soil (52).

2.b. Microbial Chlorination

The most important highlights for microbial chlorination are two articles concerned with organohalogen formation during the decomposition of organic matter. The first article evaluated peat bogs in Chile (5). The authors quantified fluxes and accumulation of halogens in the bog. To evaluate influences of peat decomposition processes on halogen enrichment, halogen concentrations were compared to carbon/nitrogen ratios (C/N). The results indicate that up to 95% of chlorine in peat exist in an organically bound form. A second paper utilized Cl K-edge x-ray spectroscopic methods to investigate organohalogen formation during the decomposition of plant litter using a model system in which redwood needles (leaves) were exposed to added chloroperoxidase (CPO) (47). The dominant form of Cl in fresh, unreacted plant material was found to be inorganic Cl^- , which was partially converted to organochlorine in the presence of CPO. The organochlorines produced in the laboratory investigations closely resemble those produced during the natural weathering of redwood needles pointing to a possible involvement of CPO in nature.

3. MICROBIAL DECHLORINATION

3.a. General Reviews

In this quarter, 3 review articles on biological dechlorination were published. One review reports on the degradation of polychlorinated dibenzo-*p*-dioxins/furans (58). The review covers 72 papers dealing with photodegradation, thermal dechlorination, biodegradation, chemical dechlorination and heterogeneously catalytic hydrodechlorination. We were not able to access a copy of the paper. A second review compares the strategies used by different dehalogenases to cleave to carbon-halogen bond (10). The structurally characterized haloalkane dehalogenases, haloacid dehalogenases and 4-chlorobenzoate-coenzyme A dehalogenases, use substitution mechanisms that proceed via a covalent aspartyl intermediate. Recent X-ray crystallographic analysis of a haloalcohol dehalogenase and a *trans*-3-chloroacrylic acid dehalogenase has provided detailed insight into a different intramolecular substitution mechanism and a hydratase-like mechanism, respectively. The available information on the various dehalogenases supports different views on the possible evolutionary origins of their activities. A third review considers the enantioselective biotransformation of chiral pollutants, including chlorinated pollutants (42).

The data show that it is difficult to predict which enantiomer may be enriched and that accumulation of an enantiomer is dependent on the environmental system, the species, and the organ (of animals). Enantioselective degradation implies that the enzymes involved in the conversion of such compounds are able to differentiate between the enantiomers. "Enzyme pairs" have evolved which exhibit almost identical overall folding. Only subtle differences in their active site determine their enantio-selectivities. At the other extreme, there are examples of non-homologous "enzyme pairs" that have developed through convergent evolution to enantio-selectively turn over the enantiomers of a chiral compound.

3.b. Microbial Dechlorination

Vinyl chloride and Other Chlorinated Ethenes

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

Vinyl Chloride (VC) and Dichloroethenes (DCE). In this quarter, there were four studies that directly investigated the biodegradation of lower chlorinated ethenes as parent compounds. The kinetics of the reductive dechlorination VC and *cis*-DCE to ethane was evaluated using variable electron donor (e-donor) concentration with the unidentified bacterium strain VS (9). Competition between VC and *cis*-DCE degradation was observed. The half velocity constant for H₂ as an e-donor supporting dechlorination was found to be 7 nM. A threshold concentration of 4 μM combined VC+DCE is required to have net growth of dehalogenating microorganisms at field concentrations of H₂ (4 nM). An absolute threshold of 0.7 μM combined VC+DCE was found for higher H₂ concentration. Another study provides field evidence for the involvement of the Fe(II) and Fe(III)-bearing mineral, magnetite, as an abiotic catalyst in the reductive dechlorination of *cis*-DCE and 1,1-DCE (17). In groundwater sediments, which were autoclaved to prevent biological activity, the first order rate constants calculated for *cis*-DCE degradation ranged 0.31-2.29 y⁻¹ and for 1,1-DCE was 1.37 y⁻¹. Magnetite was identified in the sediment by X-ray diffraction and optical microscopy. Published rates of transformation of *cis*-DCE by magnetite are consistent with the rates of removal in the microcosm study.

The potential for natural attenuation of VC and other chlorinated organics (e.g. DCE, TCE) in landfill covers was investigated in soil microcosms incubated with methane and air, simulating the gas composition in landfill soil covers (51). VC, *trans*-DCE, *cis*-DCE, 1,1-DCE and TCE were degraded at rates of 1.46, 1.12, 0.32, 0.05 and 0.06 $\mu\text{g g}^{-1}$ soil per h during the oxidation of methane, respectively. Transformation capacities for chlorinated ethenes were measured in the methanotroph, *Methylosinus trichosporium* OB3b. The transformation capacity refers to the quantity of chlorinated solvent degraded per unit weight of cells, prior to the complete inactivation of the cells. The transformation can be used to predict the amount of cells that should be stimulated in *in-situ* bioremediation. The transformation capacity values of 0.58 and 0.80 $\mu\text{mol mg}^{-1}$ cell were obtained for TCE and DCE (either *cis*- or *trans*), respectively, regardless of their concentrations.

Perchloroethylene (PCE) and Trichloroethene (TCE). In this quarter, there were 14 publications reporting on either PCE or TCE microbial degradation. Two of the papers were already addressed above in the section about lower chlorinated ethenes. Two of the articles describe the role of cobalt-containing metalloporphyrin enzyme cofactors in PCE degradation (32, 40) and will be discussed below in Section “3.c. *In Vitro Degradation of Chlorinated Compounds*”. One of the articles describes a new technique for monitoring a TCE degrading population in a mixed culture (7) and will be discussed in Section “3.d. *New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds: Detection of Microbial Populations*”.

Of the remaining eight articles, four were concerned with the aerobic cooxidation of TCE. The first of these involves a description of horizontal biosparge wells applied to the *in situ* bioremediation of TCE contaminated sites [Fournier, 2003 #1]. The second aerobic article considers the use of cane molasses and sludge cake as primary substrates to support the TCE cooxidation in biobarrier systems (27). Both of these primary substrates supported TCE cooxidation utilizing either aquifer sediments or sludge as inocula. The third aerobic article describes the gas phase treatment of TCE in a two-stage continuous stirred tank reactor (CSTR)/trickling biofilter system with the bacterium *Bukholderia cepacia* (34). Phenol was used as the primary substrate to support TCE cooxidation. The *Bukholderia cepacia* cells were cultivated in the CSTR and were subsequently recirculated through the trickling biofilter. The maximum TCE elimination capacity was 28.0 mg TCE $\text{l}^{-1} \text{d}^{-1}$, and complete removal of TCE was obtained for inlet loading below 25.3 mg TCE $\text{l}^{-1} \text{d}^{-1}$. The reactor system was stably operated for more than 3 months. The final aerobic article describes a new TCE cooxidizing bacterial isolate

(35). The isolate named, *Pseudonocardia chloroethenivorans* sp nov, utilizes phenol as its primary substrate.

Three of the articles focus on the anaerobic degradation of PCE or TCE. The first of these evaluated the phylogenetic make-up of two anaerobic enrichment cultures, actively dechlorinating TCE or *cis*-DCE using hydrogen as e-donor with a clone library of bacterial 16S rRNA genes (20). The clones were screened into different groups by restriction fragment length polymorphism (RFLP) analysis. A total of 12 sequence types were identified by phylogenetic analysis of nearly complete 16S rDNA sequences. The sequences were affiliated with six recognized phyla of the domain Bacteria: *Firmicutes* (low G+C Gram-positives), *Chloroflexi* (green non-sulphur bacteria), *Actinobacteria* (high G+C Gram-positives), *Bacteroidetes* (Cytophaga-Flexibacter-Bacteroides), *Nitrospira* and *Spirochaetes*. The results led to the identification of an organism closely related to *Dehalococcoides ethenogenes* to be the presumptive dechlorinator in both enrichments. Most of the sequences identified in the dechlorinating enrichments shared high similarities with sequences previously obtained from other enriched dechlorinating cultures and chlorinated-compound-contaminated sediments or aquifers. The second anaerobic article evaluated the threshold levels of H₂ used as an e-donor for PCE dechlorination in several halo-respiring bacterial cultures (39). H₂ threshold values during PCE dechlorination were found to be 0.05, 0.08 and 0.06 nM for *Sulfurospirillum halorespirans*, *S. multivorans* and *Dehalobacter restrictus*, respectively. The last anaerobic article describes the fate of PCE in a constructed wetland (28). PCE was removed by 65.4% in the wetland system. Most of the losses were accounted for by model considering volatilization losses.

Finally one article considered the combined anaerobic-aerobic degradation of TCE in a single bioreactor (56). The reactor was seeded with a mixed anaerobic sludge and packed with granular peat for biofilm development. The reactor was continuously fed with ethanol as the e-donor to promote reductive dechlorination and methane production by methanogens. Also the reactor was continuously fed with hydrogen peroxide as a source of O₂ to promote cooxidation of partially dechlorinated compounds (e.g. DCE). Extensive characterization of reactor populations using activity tests and polymerase chain reaction (PCR) analysis revealed the development of methanotrophic and methanogenic microorganisms. This consortium was shown to degrade TCE by a combination of reductive and oxidative pathways. A near complete degradation of TCE at a load of 18 mg l⁻¹ d⁻¹ was evidenced by a stoichiometric release of inorganic chloride.

Carbon Tetrachloride (CT) and Chloroform (CF)

The zero-order degradation rates of various chlorinated methanes in landfill soil covers under methane oxidizing conditions were measured (52). Chloroform (CF) and dichloromethane (DCM) were degraded at rates of 0.028, and 0.686 $\mu\text{g g}^{-1}\text{soil h}^{-1}$, respectively. Carbon tetrachloride (CT) was not degraded under methane-oxidizing conditions in the landfill cover.

A second study evaluated the genetic regulation of the synthesis of the bacterial metabolite and transition metal chelator, pyridine-2,6-dithiocarboxylic acid (PDTC), which catalyzes a novel and effective means of dechlorination of CT (37). PDTC production by the bacterium *Pseudomonas putida* strain DSM 3601 was coordinated with production of the well-characterized siderophore pyoverdine; exogenously added pyoverdine led to decreased PDTC production, and added PDTC led to decreased pyoverdine production. Positive regulation of a chromosomal *pdtI-xyleE* transcriptional fusion, and of a 66 kDa outer membrane protein (IROMP), was seen in response to exogenous PDTC. Tests with transition metal chelators indicated that PDTC could provide a benefit under conditions of metal limitation. The loss of PDTC biosynthetic capacity caused by a transformation (*pdtI* transposon insertion) resulted in increased sensitivity to 1,10-phenanthroline, a chelator that has high affinity for a range of divalent transition metals (e.g. Fe^{2+} , Cu^{2+} , Zn^{2+}). Measurement of Fe-59 incorporation showed uptake from Fe-59:PDTC by strain DSM 3601 grown in low-iron medium, but not by cells grown in high iron medium, or by the *pdtI* transformant. The results taken as a whole verify a siderophore function for PDTC, indicating its involvement in the uptake of iron and possibly other transition metals.

Three reports were found during the period of review on the abiotic degradation of CT by iron-containing minerals including biogenic magnetite particles (41) and ferrous iron (Fe(II)) on goethite (12). A related study concerned with the reactivity of Fe(II) on a wide array of iron minerals toward reductive transformation of hexachloroethane and 4-chloronitrobenzene (13) is discussed in *Section 3.b: Microbial dechlorination: Miscellaneous chlorinated compounds*.

One citation on the degradation of atmospheric CT in soils (38) was also found but a full copy of the publication could not be obtained before the completion of this report.

Natural attenuation processes of chlorinated solvents in soils and ground waters are increasingly considered as options to manage contaminated sites. Under anoxic conditions, reactions with ferrous iron (Fe(II)) sorbed at iron(hydr)oxides may dominate the overall transformation of CT and other chlorinated aliphatic hydrocarbons (12). The major products and pathways associated with the abiotic transformation of CT by nano-scale biogenic magnetite/maghemite particles produced by the dissimilatory iron-reducing bacterium (DIRB) *Geobacter metallireducens* were examined (41). CT transformation was shown to occur via three parallel

pathways. CT can undergo hydrogenolysis, via a trichloromethyl free radical ($\cdot\text{CCl}_3$) and possibly a trichloromethyl carbanion ($:\text{CCl}_3^-$), leading to the formation of chloroform (45-50%). In addition, CT can be transformed into a dichlorocarbene intermediate ($:\text{CCl}_2$). The carbene can undergo hydrolysis to form CO (similar to 38%) or undergo further reduction to yield methane (8-10%). The authors conclude that the large fraction of relatively benign products formed by the carbene-mediated pathways suggests that magnetite/maghemite particles may have a beneficial application in the remediation of CT contaminated environments.

The mechanisms and products of surface-mediated reductive dehalogenation of CT by Fe(II) on goethite were investigated by Elsner *et al.* (12). Surface-stabilized intermediates were shown to facilitate abiotic mineralization of CT, whereas the presence of H radical donors, such as natural organic matter, enhanced formation of chloroform (CF), a toxic product that is fairly persistent under anoxic conditions. CF yields were shown to increase slightly with pH at constant Fe(II) sorption density, suggesting that pH-dependent surface processes direct product branching ratios.

CF was not degraded aerobically in experiments with a microbial enrichment originating from a pristine aquifer (44). Results from a 48-h acute toxicity test with the freshwater invertebrate *Ceriodaphnia dubia* showed that CF caused 40% mortality when present at a concentration of 20 mg l⁻¹. A second toxicity assay, examining the effect of mixed pollutants on biodegradation activity, indicated that the presence chloroform had no effect on either benzene or toluene biodegradation.

Chloromethane (CM) and Dichloromethane (DCM)

Genetically transformed strains of dichloromethane (DCM) degrading bacteria *Methylobacterium dichloromethanicum* DM4 and of *Methylobacterium extorquens* AM1 (that express the *dcm A* gene of dichloromethane dehalogenase) undergo lysis when incubated in the presence of dichloromethane and are sensitive to acidic shock (18). The lysis of the transformants was found to be due to a loss in DNA repair mechanisms. The transformed cells are likely to undergo lysis due to the relatively inefficient repair of DNA lesions that are induced in response to the alkylating action of S-chloromethylglutathione, an intermediate product of DCM degradation. The data obtained suggest that the bacterial mineralization of dichloromethane requires an efficient DNA repair system.

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

In this quarter there were two reports on chloroethane degradation. In the first publication 5 bacteria were isolated from African soils that could degrade 1,2-dichloroethane (1,2-DCA) as a

sole source of energy and carbon (45). The isolates were identified as species of *Bacillus*, *Burkholderia*, *Corynebacterium*, *Micrococcus* and *Pseudomonas*. The growth rates of the bacterial isolates varied from 1.1 to 1.7 d⁻¹. The release of organic chlorine as inorganic chloride ranged from 59 to 86%. The zero-order degradation rates of various chlorinated ethanes in landfill soil covers under methane oxidizing conditions were measured (52). 1,1,2-trichloroethane (1,1,2-TCA), 1,1-dichloroethane (1,1-DCA) and 1,2-DCA were degraded at rates of 0.136, 0.169 and 1.716 µg g⁻¹soil h⁻¹, respectively. 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA) and 1,1,1-trichloroethane (1,1,1-TCA) were not degraded under methane oxidizing conditions in the landfill cover. An additional study examined the enzymatic degradation of 1,2-DCA by haloalkane dehalogenases (43) and it will be discussed in Section “3.c. *In Vitro Degradation of Chlorinated Compounds*”.

Chlorobenzenes (CB)

Five reports were found regarding the microbial degradation of chlorobenzene compounds, *i.e.*, two of which examined the reductive dechlorination of chlorobenzenes by anaerobic cultures containing *Dehalococcoides spp.* (2, 15); another the biomineralisation of 1,2,4-trichlorobenzene (1,2,4-TCB) in soils by an adapted microbial population (53), and two other which are concerned with the microbial degradation of chlorobenzene-contaminated groundwater from the Bitterfeld aquifer in Saxonia-Anhalt, Germany, utilizing *in situ* reactors (57) and by natural attenuation reactions (21).

Dehalococcoides ethenogenes strain 195 was shown to exhibit dechlorination activity with hexa-, penta-, and tetrachlorinated benzene congeners as sole electron acceptors (e-acceptor) via the pathways shown in Figure 1 (15). This is suggestive of growth with these compounds. The authors note the significance of these findings given the prevalence of *Dehalococcoides*-like organisms in the environment and its relatedness to *Dehalococcoides sp.* strain CBDB1, which was reported to carry out dehalorespiration with a variety of chlorinated compounds. Cell extracts of strain CBDB1 have been shown earlier to be capable of converting hexachlorobenzene to pentachlorobenzene (Höllscher *et al.* 2003. *Appl. Environ. Microbiol.* 69:2999-3001). Furthermore, the organism exhibited growth with TeCB, 1,2,3-TCB, or 1,2,4-TCB (Bunge *et al.* 2003. *Nature* 421:357-360; Adrian *et al.* 2000. *Nature* 408:580-583).

An anaerobic mixed enrichment culture obtained from river sediments reductively dehalogenated 1,2,3-TCB (60 µM) (2). A molar conversion of approx. 60% to 1,3-dichlorobenzene (1,3-DCB) was observed within 56 days. Dechlorination started after a lag phase of 14 days and reached a rate of 1.4 µmol l⁻¹ day⁻¹ between days 42 and 56.

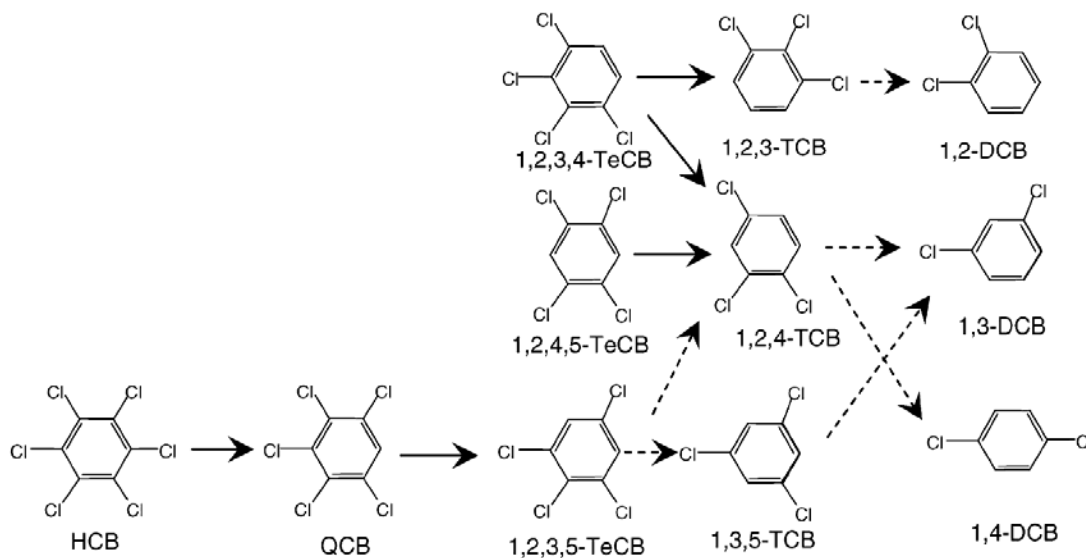


Figure 1. Observed pathway of reductive dechlorination of chlorobenzenes by *Dehalococcoides ethenogenes* strain 195. Solid arrows indicate successful transfer with that chlorobenzene as sole electron acceptor; dashed arrows indicate observed dechlorination but the process did not occur when this chlorinated benzene was supplied as the sole electron acceptor. HCB, hexachlorobenzene; QCB, pentachlorobenzene; TeCB, tetrachlorobenzene; TCB, trichlorobenzene; DCB, dichlorobenzene (15).

Biominingalisation of ^{14}C -labelled 1,2,4-TCB by indigenous microorganisms in an agricultural soil and in a soil from a contaminated site were compared (53). Up to 62% of the initially applied amount of 1,2,4-TCB was mineralised within 23 days by the adapted microbial population in the contaminated soil. In contrast, very low mineralisation of 1% was measured in the agricultural soil within the same time period. High amounts of non-extractable ^{14}C -residues were formed in the soil from the contaminated site.

Bioremediation of ground water contaminated by monochlorobenzene (MCB, up to 450 mM) using in situ reactive barrier technologies was tested nearby a local aquifer in Bitterfeld, (57). Reactive barriers are considered more efficient and cost-effective than traditional pump-and-treat methods. MCB degradation was examined in a reactor packed with original aquifer sediment under two different remediation variants, namely, anoxic conditions in the presence of nitrate; or mixed e-acceptor conditions (oxygen + nitrate) using hydrogen peroxide (H_2O_2) as O_2 -releasing compound. MCB was not removed under anoxic denitrifying conditions, although

MCB disappearance under denitrifying conditions has been reported in the literature. However, MCB (ca. 150 mM) was removed in the lower part of the reactor when hydrogen peroxide (concentrations ranging from 0.88 to 2.94 mM) and nitrate (2 mM) were supplied. Nitrate was uniformly reduced during the flow path in the reactor leading to the formation of low nitrite concentrations. Nitrate consumption was attributed to the activity of autotrophic iron-oxidizing nitrate reducers. MCB degradation mediated by hydrogen peroxide was attributed to microbial activity. This conclusion is supported by the strong increase of the number of cultivable aerobic MCB degrading bacteria observed in reactor water and sediment samples, and by the resistance of MCB to chemical degradation by H₂O₂. Most of the MCB degrading strains isolated were identified as Rhodococci and Pseudomonads, genera which are well known for including strains with the capability to grow on MCB. Two strains were classified as *Acidovorax* species and one strain was identified as *Paenibacillus polymyxa*, none of which are known for MCB degradation to date. In all the isolates tested, chlorocatechol 1,2-dioxygenase activity was detected after aerobic growth on MCB, indicating that the cells might use the modified *ortho* pathway for CB degradation under this conditions. The MCB degradation completely collapsed after reducing the H₂O₂ concentration to 0.44mM. Subsequent increase of the H₂O₂ concentrations to up to 2.94 mM indicated that the oxygen demand for CB degradation was higher than observed before, probably due to a shift in the bacterial population.

The predominant contaminants in the Bitterfeld aquifer are chlorinated hydrocarbons (e.g. PCE, TCE, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane) and BTEX. Analyses of field data from monitoring wells at a contaminated industrial site in Bitterfeld indicated that several microbial processes occurred, including methanogenesis, sulfate and iron reduction as well as reductive dechlorination of the chlorinated hydrocarbons (21). Direct evidence for the latter degradation reaction was observed along the flow path due to the appearance of intermediates (e.g. less chlorinated aliphatic hydrocarbons such as *cis*-DCE, *trans*-DCE, 1,1-DCE, VC and the chlorinated benzene MCB) and an increase in the degree of dechlorination (from nearly 20% in the source area of chlorinated aliphatics to about 80% near the end of the search area).

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

In this quarter, two studies report on the degradation of chlorinated dibenzo-*p*-dioxins (CDDs) by anaerobic cultures containing *Dehalococcoides* spp. (2, 15). An additional review paper on the microbial degradation of CDDs and polychlorinated dibenzo-*p*-furans (CDFs) (58) is discussed in Section “3.a. General Reviews”.

Dehalococcoides ethenogenes strain 195 was shown to reductively dechlorinate diverse chlorinated aromatic pollutants, in addition to its known chloroethene respiratory e-acceptors.

The compounds tested included PCE, various chlorobenzenes, 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD), 2,3,7,8-TeCDD, 2,3-dichlorodibenzo-*p*-dioxin (2,3-DCDD), 1,2,3,4-tetrachlorodibenzo-*p*-furan (1,2,3,4-TeCDF), 2,3,4,5,6-pentachlorobiphenyl, 1,2,3,4-tetrachloronaphthalene, or a mixture of 2-, 3-, and 4-chlorophenol. The results obtained with PCE and chlorobenzenes were discussed earlier. *D. ethenogenes* strain 195 dechlorinated 1,2,3,4-TeCDD, 1,2,3,4-TeCDF, 2,3,4,5,6-pentachlorobiphenyl and 1,2,3,4-tetrachloronaphthalene within 40 to 249 days. Observed pathways of reductive dechlorination of the latter chlorinated aromatic compounds are shown in Figure 2. Dechlorination daughter products were not detected from monochlorophenols, 2,3-DCDD or 2,3,7,8-TeCDD after 249 days, although the cultures were shown to be viable. The ability of *D. ethenogenes* to gain energy for growth on CDDs, dibenzofurans, biphenyls, or naphthalenes was not investigated. However, the authors provided evidence suggesting that the strain could utilize highly chlorinated benzenes as sole growth substrate. *Dehalococcoides* sp. strain CBDB1, a close relative of *D. ethenogenes* strain 195 (98% identity over 1422 nucleotides of 16S rRNA gene sequence), was also reported to carry out dehalorespiration with selected polychlorinated CDD congeners in a recent study (Bunge *et al.* 2003. *Nature* 421: 357-360).

An anaerobic mixed enrichment culture obtained from river sediments reductively dehalogenated 1,2,4- and 1,2,3-TCDD to di- and monochlorinated congeners in the presence of pyruvate (or lactate) and fumarate as cosubstrates (2). Besides TrCDD, PCE and 1,2,3-TCB were dechlorinated. 1,2,4-TrCDD was consistently dechlorinated after 16 transfers via 1,3-DCDD to 2-MCDD. Dechlorination proceeded via two subsequent steps of chlorine removal from the peri-positions 1 and 4. The rate of 1,3-dichlorodibenzo-*p*-dioxin formation increased with rising initial concentrations of 1,2,4-TrCDD (1-250 μM) from 0.05 to 5.4 $\mu\text{mol l}^{-1} \text{ day}^{-1}$. The dechlorination products from 1,2,3-TrCDD indicated chlorine removal from a lateral and peripheral position leading to 1,3-DCDD and 2,3-DCDD, respectively. Dioxin dehalogenation was sensitive to pasteurization, but was not influenced by inhibitors of methanogens, sulfate-reducing bacteria or Gram-positive bacteria. *Dehalococcoides* was proposed as the putative dechlorinating species based on nested PCR indicated the presence of *Dehalococcoides* species in highest most probable number (MPN) dilutions that were positive for dioxin dechlorination. Physiological properties of the dioxin-dechlorinating mixed culture, e.g. ability to dechlorinate TCB, sensitivity to heat, and resistance to vancomycin were similar to those of other *Dehalococcoides* strains. Other species detected in the sediments included *Sulfurospirillum*, *Trichococcus*, *Acetobacterium*, *Desulfitobacterium* and *Desulfuromonas*. This is the first description of an anaerobic dioxin-dechlorinating enrichment culture, which could be successfully transferred without additions of sterilized sediment.

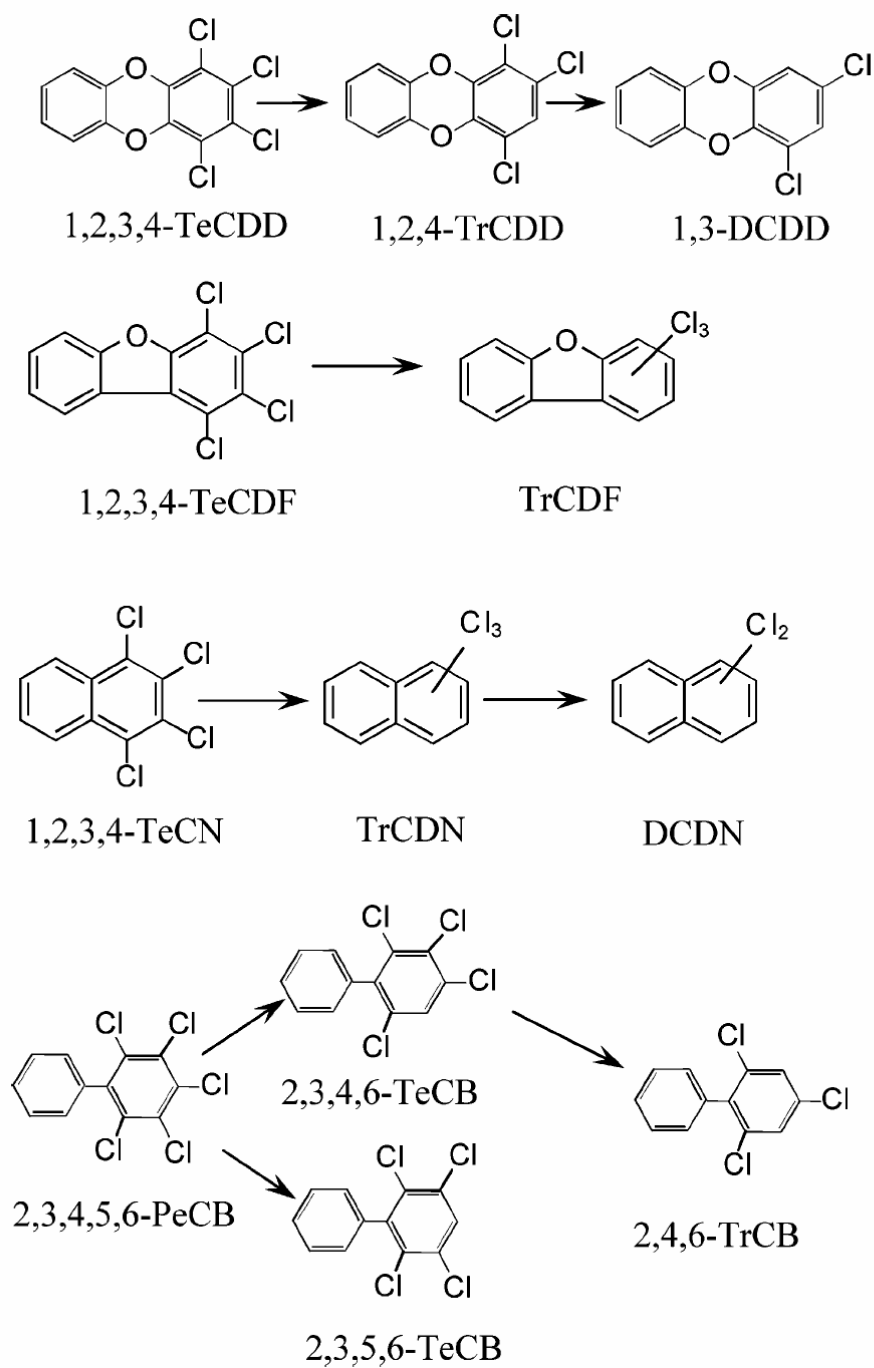


Figure 2. Observed pathways of reductive dechlorination of chlorinated aromatic compounds by *Dehalococcoides ethenogenes* strain 195 (15).

Hexachlorobutadiene and Octachlorostyrene

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

Polychlorinated Biphenyls (PCBs)

In this quarter, six publications reported on the microbial degradation of polychlorinated biphenyls (PCBs). Three of these publications address anaerobic degradation of PCBs (8, 11, 19), with the other three focusing on PCB attack under aerobic conditions (29, 30, 48).

Threshold concentration and dechlorination kinetics of individual congeners in Aroclor 1248 was investigated in laboratory assays using river sediment microorganisms (8). No dechlorination was observed at Aroclor concentrations below 40 ppm [137 nmol (g of sediment)⁻¹]. Above this threshold, congeners could be divided into three categories: group A, congeners that dechlorinated above 40 ppm; group B, congeners that dechlorinated only at high concentrations above 60 ppm [206 nmol (g of sediment)⁻¹]; and group C, lower chlorinated congeners that increased in concentration.

The composition of persistent organochlorine compounds in soils and sediments from two high altitude European mountain lakes, Redon in the Pyrenees and Ladove in the Tatra mountains, and from two lakes in the Tatra mountains was examined (19). DDTs (1.7-13 ng g⁻¹) were the most abundant organochlorine pollutants in soils followed by PCBs (0.41-1.5 ng g⁻¹) and hexachlorobenzene (HCB; 0.15-0.91 ng g⁻¹). In sediments, the dominant organochlorine pollutants were also DDTs (3.3-28 ng g⁻¹) and PCBs (2.3-15 ng g⁻¹). Significant qualitative changes in the soil PCB distributions were observed which could be attributed to anaerobic dechlorination. High molecular weight congeners were dominant in the top core sections and lower weight (*i.e.* less chlorinated) in the bottom.

Chlorine isotopic signatures were shown to remain unaltered during microbial reductive dechlorination of 2,3,4,5-tetrachlorobiphenyl to 2,3,5-trichlorobiphenyl in laboratory cultures over a 90 day incubation period (11).

Four strains belonging to the genus *Bacillus* were shown to be capable of degrading PCBs in culture media and in model soil samples (29). Consumption and degradation of PCBs were studied using tritium-labeled PCBs.

PCB decomposition by a hybrid method of photodechlorination and biodegradation – was examined in a pilot-scale study utilizing commercial PCBs from a high voltage transformer (30). Extensive degradation of Kaneclor 1000 was observed within 8 d using the combined treatment method. Dioxins including dibenzofuran and co-planar PCBs in Kaneclor sample were also

degraded completely. The concentration of PCBs in the final effluent was acceptable by the Japanese standard (0.003 mg l^{-1}) on wastewater.

Measurement of enantiomeric fractions (EFs) of chiral PCBs 95, 136, and 149 in samples of topsoil and outdoor air at one urban and one rural location in the U.K. West Midlands between early 2001 and early 2002 indicated that appreciable enantioselective degradation of the monitored PCBs in topsoil occurs (48). EFs in air were essentially racemic. In contrast, EFs in topsoil indicated appreciable enantio-enrichment of the second eluting enantiomer for PCB 95 and the H enantiomer for PCBs 136 and 149. This is one of only two reports of enantioselective degradation of PCBs in soil worldwide and is particularly noteworthy as it is occurring at PCB concentrations (e.g., 5.9 pg g^{-1} for PCB 136) that are typical of the U.K. and other industrialized countries. The extent of enantioselective degradation in this study for PCBs 95 and 136 is consistent with those reported for soils in the Greater Toronto area. In contrast, enantioselective degradation of PCB 149 observed in this study, while consistent with that reported for U.K. lake sediments, is in excess of that observed in either the Greater Toronto area soil study or in U.S. lake sediments.

Miscellaneous Chlorinated Compounds

The search query used is specifically designed to review literature on the target compounds listed in the *Introduction* section. Interesting publications concerned with compounds outside of the target list that are found in the search process are briefly discussed below. This quarter our search retrieved 14 reports on the biodegradation of miscellaneous chlorinated pollutants, including, hydrochlorofluorocarbons (51); 1-chlorobutane (14); chloronaphthalenes (24); halophenols (6); 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) (23, 26) (1); ethyl 2-chloroacetoacetate (25); hexachlorohexane (HCH) (33, 46) (31, 49); polybrominated diphenyl ethers (22) and toxaphene (50).

An additional report examined the reactivity of ferrous iron (Fe(II))-bearing minerals toward reductive transformation of hexachloroethane and 4-chloronitrobenzene, compounds representing two classes of environmentally relevant transformation reactions of pollutants, *i.e.*, dehalogenation and nitroaryl reduction (13). The study utilized aqueous suspensions of siderite (FeCO_3), nontronite (ferruginous smectite SWa-1), hematite ($\alpha\text{-Fe}_2\text{O}_3$), lepidocrocite ($\gamma\text{-FeOOH}$), goethite ($\alpha\text{-FeOOH}$), magnetite (Fe_3O_4), sulfate green rust ($(\text{Fe}_4\text{Fe}_2\text{II})\text{-Fe-II}(\text{OH})_{12}\text{SO}_4\cdot 4\text{H}_2\text{O}$), pyrite (FeS_2), and mackinawite (FeS) under similar conditions. The reactivities of the different Fe(II) mineral systems varied greatly and systematically both within and between the two data sets obtained with the two probe compounds. The results indicate that

abiotic reactions with surface-bound Fe(II) may affect or even dominate the long-term behavior of reducible pollutants in the subsurface, particularly in the presence of Fe(III) bearing minerals.

Rapid oxidation of the hydrochlorofluorocarbons HCFC-21 (dichlorofluoromethane) and HCFC-22 (chlorodifluoromethane) in landfill cover soils was reported (51). Hydrochlorofluorocarbon (HFC) oxidation occurred in parallel with the oxidation of methane. The most important parameters controlling HFC oxidation in landfill cover soil were found to be temperature, soil moisture, and methane and oxygen supply. Haloalkane hydrolysis of halogenated compounds (such as 1-chlorobutane) by lyophilized *Rhodococcus erythropolis* cells was compared in a novel solid/ gas biofilter and in the aqueous phase (14).

Concentrations and fluxes of 63 congeners of chloronaphthalenes (CN) in sediment from Lake Kitaura in Japan in past 15 centuries were quantified (24). The total CNs content normalized to dry weight of sediment peaked out in the layer dated on 1984-1985, and next nearly two-fold decreased with further gradually decreasing concentration in 1987-2000. The annual flux of CNs into sediments of the Lake Kitaura was 0.073-0.31 pg cm⁻² before 1926, increased to 5.5-14 pg cm⁻² in 1963-1970 and next sharply increased to 50-107 pg cm⁻² in 1971-1985, and after that decreased to 17 Pg cm⁻² in 1997-2000.

Transformation of halophenols, *i.e.*, 4-substituted fluoro-, chloro-, bromo- and iodo-phenol, by crude cell extracts of *Pseudomonas putida* F6 without the exogenous addition of cofactors was reported (6). The rate of substrate consumption decreased with increasing substituent size (F > Cl > Br > I). Cell extracts of *P. putida* F6 also showed activity towards 3-fluorophenol.

Sodium (Na⁺) application was shown to enhance DDT transformation in a long-term contaminated soil (26). Sodium ion is known to disperse clays, which would potentially release and/or expose physically protected DDT thereby enhancing DDT bioavailability. A non-competitive immunoassay for monitoring DDT, its metabolites and analogues in water samples was described (1)

Fungal aerobic reductive dechlorination of the β -keto ester ethyl 2-chloroacetoacetate by *Saccharomyces cerevisiae* was examined and the mechanism of a novel type of glutathione-mediated microbial dehalogenation described (25). *S. cerevisiae* reduces ethyl 2-chloroacetoacetate to the respective chiral *cis*- and *trans*- β -hydroxy esters. In the course of chiral reduction, competing dehalogenation of the xenobiotic substrate to ethyl acetoacetate occurs, in a reaction mediated by cytosolic glutathione.

Enantioselective degradation of α -hexachlorocyclohexane (α -HCH) in groundwater under anaerobic conditions was reported at a contaminated site beneath an active pesticide reformulating and packaging facility in coastal northeastern Florida (33). Investigation of the

stability of bacterial populations in tropical soil showed that exposure to γ -HCH (lindane) at concentrations of 100 mg lindane kg⁻¹ soil did not affect the metabolic versatility and genetic diversity in these soils (49). Lindane was not degraded after 70 days. The fate of lindane in the activated sludge treatment process was investigated (31). Sorption on primary sludge solids was concluded to be the major removal mechanism. Only 0.1-2.8% of lindane inputs was concentrated in activated sludge. A regionally segmented multimedia fate model was described that considers the fate of persistent organic pollutants in Europe (46). A case study examining the fate of γ -HCH (lindane) based on 1998 emission data is presented.

Concentrations of polybrominated diphenyl ethers (PBDEs), compounds widely used as fire retardants, in several environmental media, concentration trends, and congener profiles were reviewed and analyzed (22). The results reviewed show that the environment and people from North America are very much more contaminated with PBDEs as compared to Europe and that these PBDE levels have doubled every 4-6 yr.

Anaerobic transformation of key compounds of technical toxaphene (2-exo,3-endo,6-exo,8,9,10-hexachlorobornane [B6-923] and 2-endo,3-exo,5-endo,6-exo, 8,9,10-heptachlorobornane [B7-1001]) by the gram-negative bacterium *Dehalospirillum multivorans* was reported (50).

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

The corrinoid (cobalamin) cofactor of a reductive PCE dehalogenase from the halorespiring bacterium, *Dehalospirillum multivorans*, was isolated and characterized (32). The isolated cobalamin was found to be in the cobeta-cyano form. The identified structure was found to be non-identical to a variety of known natural vitamin B-12 derivatives. The second publication reports on the halogen and haloalkane dehalogenase activities of five new bacterial isolates recovered from African soils capable of growth on 1,2-DCA as sole source of energy and carbon (45). All the bacterial isolates produced two different dehalogenases, one which is heat labile and specific for halogenated alkanes with optimum activity at a pH of 7.5 and the other which is more heat stable with a higher pH optimum of 9.0 and specific for halogenated alkanic acids. The two enzyme types demonstrated wide substrate specificities. Crude extracts of the cells displayed dehalogenase activity with DCM, CF, CT, TCE and mono- as well as trichloroacetic acid at both pH 7.5 and 9 in addition to the growth substrate, 1,2-DCA. The third publication was a theoretical evaluation of the dynamics of the nucleophilic substitution reaction of a carboxylic group with 1,2-DCA in a dehalogenase protein compared to that of a free carboxylic

acid group in water (43). The evaluation indicates that protein dynamics accelerates the reaction rate by a factor of 2 over the uncatalyzed reaction in water.

A mechanistic study was conducted evaluating the degradation of PCE using cobalamin (vitamin B12) as a catalyst and Ti(III)-citrate as a reducing agent (40). A novel finding of the study was that during degradation there is a covalent bond formed between vitamin B12 and the chlorinated ethene. An intermediate in the process, *cis*-chlorovinylcobalamin, was successfully chemically synthesized to confirm the presence of the covalently bound intermediates.

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

Labeling of Organochlorine Contaminants

A method is described for labeling PCBs with tritium (29). In addition, a radiochemical approach for the investigation of PCBs microbial degradation was developed and its application was demonstrated in experiments utilizing tritium-labeled PCBs to characterize the biodegradation of these chlorinated pollutants by soil bacteria.

Detection of Microbial Populations

Terminal restriction fragment length polymorphism (T-RFLP) fingerprinting of 16S rRNA was applied as a tool for the detection and characterization of a dehalogenating microorganism by in a sulfidogenic, 2-bromophenol-degrading consortium (16)

A laser integrated microarray scanner was used as a new method to quantify and compare the biomass of *Burkholderia cepacia* G4 alone, and mixed with a TCE and phenol degrading community (7). Linearity and sensitivity of the scanner for biomass quantification were established, and a lower detection limit of 2-5 mg l⁻¹ (10³-10⁴ cells ml⁻¹) was calculated. Results suggest that the microarray scanner can be used to quantify the biomass of a single population in a dense mixed microbial community.

Mathematical Tools

A modified polytopic vector analysis was developed to identify and quantify a dioxin dechlorination signature in sediments (3) (4). PVA is a multivariate technique based on a linear mixing model, used to resolve chemical fingerprints and suited for forensic investigations of environmental contamination. The authors developed a modified algorithm to resolve a dioxin dechlorination fingerprint, indicative of biotic/abiotic transformations in field samples of

sediments. The technique was applied to 351 sediment core-derived dioxin samples from the lower reach of the Passaic River, New Jersey.

4. MICROBIAL CHLORINATION

4.a. General Reviews

Review papers concerned with microbial chlorination were not found during the review period.

4.b. Microbial Chlorination in the Environment

Chloromethanes

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

Chlorinated Compounds

Two publications were found during this quarter on the formation of organohalogenes, other than methyl halides, in the terrestrial environment (36, 55). Fluxes and reservoirs of trichloroacetic acid (TCA) were measured for one year at a forest and moorland catchment in Scotland (55). The authors conclude that if the catchment is at steady-state, the flux and reservoir values estimated in their study imply an average catchment residence time for TCA of 6 years if all reservoir TCA is involved in catchment through-flux, or 1-2 years if only the extractable fraction of TCA in soil is considered. Since other evidence indicates the lifetime of TCA in soil and biota is considerably shorter (weeks rather than years), the magnitude of the TCA reservoir is suggested to be strong evidence for net natural TCA production in soils. An alternative explanation is that the majority of TCA in the reservoir is not involved with external fluxes. The first conclusion is supported by reports of TCA formation in laboratory experiments of soil. Additional catchment evidence is provided by observation of enhancement of TCA concentration in the stream as it passes through the organic-rich soil of the forest.

Chlorinated macrolides, haterumalide NA, B and NE, and a new haterumalide X, were produced by the soil bacterium *Serratia plymuthica* (36). These metabolites were biologically

active. The bacterium also produced two other antifungal metabolites: pyrrolnitrin and 1-acetyl-7-chloro-1-H-indole.

Chlorinated Natural Organic Matter

Peat bogs, which are built up exclusively of organic matter and cover approximately 3% of the total continental world area, are potentially significant reservoirs for organohalogen formation. The retention of atmospheric derived halogens and the natural formation of organohalogens in two peat bogs in southernmost Chile were investigated by differential halogen analysis (5). Halogen concentrations were compared to carbon/nitrogen ratios (C/N) to evaluate the impact of peat decomposition processes on halogen enrichment. The study estimated that up to 95% of chlorine, 91% of bromine, and 81% of iodine in peat exist in an organically bound form.

4.c. Chlorination by Marine and Freshwater Organisms

Chloromethanes

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

Other Chlorinated Compounds

The biosynthetic origin of the dichloroimine functional group in terpene metabolites stylotellanes A and B formed by the tropical marine sponge *Stylotella aurantium* was investigated using ¹⁴C-labelled precursor experiments (54).

4.d. Chlorinating Enzymes

The contribution of halocarbons from plant weathering to the total organohalogen budget of terrestrial systems is gaining recognition. Enzymatic formation of organochlorines in weathering plant material (*Sequoia sempervirens* (redwood) needles) was examined in the presence of an external chloroperoxidase (CPO) enzyme using Cl K-edge X-ray absorption spectroscopy (47). The dominant form of Cl in fresh, unreacted plant material was found to be inorganic chloride, which was partially converted to organochlorine in the presence of CPO. Chlorination was affected by the nature of reactant (CPO, H₂O₂) addition, reaction time, and temperature. The

organochlorines produced in these laboratory investigations closely resemble those produced during the natural weathering of redwood needles. A striking consistency in chlorine speciation observed among the various sample types suggests that CPO produced by terrestrial organisms could play a vital role in the generation of organochlorines associated with the degradation of plant material. Furthermore, the results suggest that initial targets of enzymatic chlorination might include lignin-like macromolecules rich in aromatic character and hydroxyl groups. The authors note that their results together with a recent study demonstrating the presence of organochlorines in degrading senescent leaves still attached to the tree (Myneni. 2002. *Formation of stable chlorinated hydrocarbons in weathering plant material. Science, 295:1039-1041*) suggest that a potent chlorinating agent, most likely an enzyme exhibiting haloperoxidase activity, is present not only in soils but throughout the forest environment.

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6. ANNEX

Anfossi, L., G. Giraudi, et al. (2004). "Development of a non-competitive immunoassay for monitoring DDT, its metabolites and analogues in water samples." *Analytica Chimica Acta* 506(1): 87-95.

This report highlights the characteristics of a general method of performing non-competitive immunoassays for low-molecular-mass analytes, which was developed and applied to 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) determination in aqueous samples. The method is based on the separation of the analyte-bound antibody from the excess of the free antibody by a chromatographic step, followed by the dissociation of the complex and the capture of the previously bound antibody on a solid phase. The measured signal is linearly correlated to the concentration of the complex and, consequently, to the analyte concentration. The 3sigma limit of detection (LOD, 8 ng l(-1)) obtained by the above method enabled us to decidedly improve the sensitivity of the corresponding enzyme-linked immunosorbant assay (ELISA) and of all reported immunoassays for DDT. In addition, by applying this new format, even if a very selective antibody was used, a broad selectivity was observed, which allowed DDT + DDD + DDE to be determined instead of only p,p'-DDT as in the ELISA performed with the same antibody. In addition, real water samples were validated in a percentage recovery test. Very good recovery rates were obtained, highlighting the validity of the proposed method to accurately determine the total DDT content in water. (C) 2003 Elsevier B.V. All rights reserved.

Ballerstedt, H., J. Hantke, et al. (2004). "Properties of a trichlorodibenzo-p-dioxin-dechlorinating mixed culture with a Dehalococcoides as putative dechlorinating species." *Fems Microbiology Ecology* 47(2): 223-234.

An anaerobic mixed culture enriched over 16 transfers (1/10) from Saale river sediment reductively dehalogenated 1,2,4- and 1,2,3-trichlorodibenzo-p-dioxin (TrCDD) to di- and monochlorinated congeners in the presence of pyruvate (or lactate) and fumarate as cosubstrates. Besides TrCDD, tetrachloroethene and 1,2,3-trichlorobenzene were dechlorinated. Dioxin dehalogenation was sensitive to pasteurization, but was not remarkably influenced by inhibitors of methanogens, sulfate-reducing bacteria or Gram-positive bacteria. The rate of 1,3-dichlorodibenzo-p-dioxin formation increased with rising initial concentrations of 1,2,4-TrCDD (1-250 µM) from 0.05 to 5.4 µmol l(-1) day(-1). Two isolates, belonging to *Sulfurospirillum* and *Trichococcus*, did not show reductive dehalogenation. 16S rDNA-targeted methods further revealed the presence of *Acetobacterium*, *Desulfitobacterium*, *Desulfuromonas* and *Dehalococcoides*. Nested polymerase chain reaction (PCR) indicated the presence of *Dehalococcoides* in highest most probable number (MPN) dilutions that were positive for dioxin dechlorination. (C) 2003 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.

Barabas, N., P. Adriaens, et al. (2004). "Modified polytopic vector analysis to identify and quantify a dioxin dechlorination signature in sediments. 1. Theory." *Environmental Science & Technology* 38(6): 1813-1820.

Risk-based sediment management decisions require the characterization of contamination sources and fate processes in the field. Polytopic vector analysis (PVA) is a multivariate technique based on a linear mixing model, used to resolve chemical fingerprints and suited for forensic investigations of environmental contamination. The traditional algorithm is constrained to positive fingerprint (end-member) components and cannot resolve fingerprints with both positive and negative values required for a reactive end-member. We developed a modified algorithm (MPVA) to resolve a dioxin dechlorination fingerprint, indicative of biotic/abiotic transformations in field samples of sediments. The new procedure isolates from the dioxin pattern net compositional changes due to dechlorination in a separate end-member. Using two artificial data sets for which the composition and sample contribution of all end-members are known, the dechlorination fingerprint was reproduced with a root mean square error of 28-41%. The dechlorination end-member contribution to the total variability (set at 4.0 and 10.0%, respectively) was overestimated 1-5-fold. The ability of M-PVA to reproduce the dechlorination pattern and its variability contribution depends on the actual contribution of dechlorination to variability. At an actual contribution of 4.0%, the model outcome deviates more strongly from the original than is the case for a contribution of 10.0%. As such, application of M-PVA to environmental data should include an uncertainty analysis to distinguish variability due to

dechlorination from variability due to error. The development of the modified PVA procedure is an important step toward the field characterization of fate processes in dioxin-impacted sediments.

Barabas, N., P. Goovaerts, et al. (2004). "Modified polytopic vector analysis to identify and quantify a dioxin dechlorination signature in sediments. 2. Application to the Passaic river." *Environmental Science & Technology* 38(6): 1821-1827.

Persistent contaminants such as dioxins have been documented to undergo dechlorination reactions in the laboratory; however, little is known about the importance of these reactions in the field. Polytopic vector analysis (PVA) is a statistical pattern recognition technique for multivariate data traditionally used to identify fingerprints of contaminant sources. A modified PVA algorithm with uncertainty analysis was used to model dechlorination fingerprints and sources. The technique was applied to 351 sediment core-derived dioxin samples from the lower reach of the Passaic River, New Jersey. A dechlorination fingerprint was identified with a highly positive 2,3,7,8-tetraCDD component and a highly negative heptaCDD component. The most important industrial source of 2,3,7,8-tetraCDD is a fingerprint related to 2,4,5-trichlorophenoxyacetic acid production. The dechlorination contribution to the data variance is 3.00 +/- 1.00%, corresponding to an average of 1.2 mug/kg of 2,3,7,8-tetraCDD per sample at the expense of heptaCDD. The possible occurrence of dechlorination was validated by comparing the local dechlorination contribution in the results to the value of the ratio 2,3,7,8-tetra CDD/total 1,2,3,7,8-PCDD, which indicates dechlorination in the laboratory. Bootstrap uncertainty analysis yielded the same dechlorination EM in 40% of the realizations. The results indicated that bootstrapping is an important statistical tool to quantify uncertainties with respect to the dechlorination EM and some of the source EMs.

Biester, H., F. Keppler, et al. (2004). "Halogen retention, organohalogen, and the role of organic matter decomposition on halogen enrichment in two Chilean peat bogs." *Environmental Science & Technology* 38(7): 1984-1991.

Natural formation of organohalogen compounds can be shown to occur in all natural environments. Peat bogs, which are built up exclusively of organic matter and cover approximately 3% of the total continental world area, are potentially significant reservoirs for organohalogen formation. Up to now, fluxes and retention rates of halogens and organohalogen formation in peat bogs were mostly unquantified. In our study, we investigated the retention of atmospheric derived halogens and the natural formation of organohalogen by differential halogen analysis in two peat bogs in southernmost Chile. Atmospheric wet deposition rates of chlorine, bromine, and iodine range between 600 and 36000, 6 and 160, and 1 and 3 mg m⁻¹ yr⁻¹, respectively. Mean annual net accumulation rates of these halogens in peat are calculated to be 12-72 mg of Cl m⁻², 1.7-12 mg of Br m⁻², and 0.4-1.2 mg of I m⁻². Retention rates are similarly high for iodine (36-46%) and bromine (7.5-50%), and substantially lower for chlorine (0.22%). To evaluate influences of peat decomposition processes on halogen enrichment, halogen concentrations were compared to carbon/nitrogen ratios (C/N). Our results indicate that up to 95% of chlorine, 91% of bromine, and 81% of iodine in peat exist in an organically bound form. The results also indicate that the concentrations of halogens, especially of bromine and iodine, in peat are largely determined by peat decomposition processes and that halogens are not conservative in bogs.

Brooks, S. J., E. M. Doyle, et al. (2004). "Biotransformation of halophenols using crude cell extracts of *Pseudomonas putida* F6." *Applied Microbiology and Biotechnology* 64(4): 486-492.

Crude cell extracts of *Pseudomonas putida* F6 transformed 4-substituted fluoro-, chloro-, bromo- and iodo-phenol without the exogenous addition of cofactors. The rate of substrate consumption decreased with increasing substituent size (F>Cl>Br>I). Biotransformations resulted in greater than 95% utilisation of the halogenated substrate. Product accumulation was observed in incubations with 4-chloro, 4-bromo- and 4-iodo-phenol. These products were identified as the corresponding 4-substituted catechols. Transformation of 4-fluorophenol did not result in the accumulation of the corresponding catechol; however, manipulation of the reaction conditions by incorporation of ascorbic acid culminated in the formation of 4-fluorocatechol. Cell extracts of *P. putida* F6 also showed activity towards a 3-substituted phenol, namely 3-fluorophenol, resulting in the formation of a single product, 4-fluorocatechol.

Callister, S. J., H. L. Ayala-del-Rio, et al. (2003). "Quantification of a single population in a mixed microbial community using a laser integrated microarray scanner." *Journal of Environmental Engineering and Science* 2(4): 247-253.

A laser integrated microarray scanner was used to quantify and compare the biomass of *Burkholderia cepacia* G4 alone, mixed with *Afipia* sp., and mixed with a trichloroethylene and phenol degrading community. Samples containing *B. cepacia* G4 were placed in 3-mm diameter wells on gelatin coated glass slides then fixed and immunofluorescently labeled using an IgG conjugate with an attached Alexa(TM) 546 fluorophore. Linearity and sensitivity of the scanner for biomass quantification were established, and a lower detection limit of 2-5 mg L⁻¹ (10³-10⁴) cells mL⁻¹ was calculated. Growth of *B. cepacia* G4 alone and in the presence of the TCE degrading community was measured using the scanner. Results suggest that the microarray scanner can be used to quantify the biomass of a single population in a dense mixed microbial community.

Cho, Y. C., R. C. Sokol, et al. (2003). "Reductive dechlorination of polychlorinated biphenyls: Threshold concentration and dechlorination kinetics of individual congeners in aroclor 1248." *Environmental Science & Technology* 37(24): 5651-5656.

Reductive dechlorination of individual PCB congeners in Aroclor 1248 was investigated using sediment microorganisms from the St. Lawrence River (NY). No dechlorination was observed at Aroclor concentrations below 40 ppm [137 nmol (g of sediment)⁻¹]. Above this threshold, congeners could be divided into three categories: group A, congeners that dechlorinated above 40 ppm; group B, congeners that dechlorinated only at high concentrations above 60 ppm [206 nmol (g of Sediment)⁻¹]; and group C, lower chlorinated congeners that increased in concentration. The dechlorination rate of congeners in groups A and B was a linear function of their initial sediment concentration. For group A congeners, the concentration intercepts of this linear function were the same as their concentrations in the Aroclor at the threshold concentration, and these therefore represented the threshold values. However, the intercepts of group B congeners were significantly higher than their levels at the threshold Aroclor concentration and were equivalent to their concentrations in Aroclor 1248 at about 75 ppm [258 nmol (g of sediment)⁻¹]. The final concentrations of group A and group B congeners at the end of dechlorination were the same, regardless of their initial concentrations. These final concentrations were significantly lower than their threshold values. The accumulation rate of group C congeners was a linear function of their initial concentrations, and the total accumulation was greater at higher Aroclor concentrations in sediments.

Cupples, A. M., A. M. Spormann, et al. (2004). "Vinyl chloride and cis-dichloroethene dechlorination kinetics and microorganism growth under substrate limiting conditions." *Environmental Science & Technology* 38(4): 1102-1107.

The reductive dechlorination of tetrachloroethene (PCE) and trichloroethene (TCE) at contaminated sites often results in the accumulation of cis-1,2-dichloroethene (DCE) and vinyl chloride (VC), rather than the nonhazardous end product ethene. This accumulation may be caused by the absence of appropriate microorganisms, insufficient supply of donor substrate, or reaction kinetic limitations. Here, we address the issue of reaction kinetic limitations by investigating the effect of limiting substrate concentrations (electron donor and acceptor) on DCE and VC dechlorination kinetics and microorganism growth by bacterium VS. For this, a model based on Monod kinetics, but also accounting for competition between electron acceptors and the effect of low electron donor and acceptor concentrations (dual-substrate kinetics), was examined. Competitive coefficients for VC (7.8 +/- 1.5 μM) and DCE (3.6 +/- 1.1 μM) were obtained and included in the model. The half velocity coefficient for hydrogen, the electron donor, was experimentally determined (7 +/- 2 nM) through investigating dechlorination over different substrate concentrations. This complete model was then used, along with experimental data, to determine substrate concentrations at which the dechlorinating microorganisms would be in net decay. Notably, the model indicates net decay will result if the total electron acceptor concentration (DCE plus VC) is below 0.7 μM, regardless of electron donor levels. The ability to achieve sustainable bioremediation to acceptable levels can be greatly influenced by this threshold level.

de Jong, R. M. and B. W. Dijkstra (2003). "Structure and mechanism of bacterial dehalogenases: different ways to cleave a carbon-halogen bond." *Current Opinion in Structural Biology* 13(6): 722-730.

The dehalogenases make use of fundamentally different strategies to cleave carbon-halogen bonds. The structurally characterized haloalkane dehalogenases, haloacid dehalogenases and 4-chlorobenzoate-coenzyme A dehalogenases use substitution mechanisms that proceed via a covalent aspartyl intermediate. Recent X-ray crystallographic analysis of a haloalcohol dehalogenase and a trans-3-chloroacrylic acid dehalogenase has provided detailed insight into a different intramolecular substitution mechanism and a hydratase-like mechanism, respectively. The available information on the various dehalogenases supports different views on the possible evolutionary origins of their activities.

Drenzek, N. J., T. I. Eglinton, et al. (2004). "Invariant chlorine isotopic signatures during microbial PCB reductive dechlorination." *Environmental Pollution* 128(3): 445-448.

In order to develop more robust insight into the natural attenuation of polychlorinated biphenyls (PCBs), the chlorine isotopic composition of residual 2,3,4,5-tetrachlorobiphenyl (2,3,4,5-CB) was monitored as it underwent microbial reductive dechlorination to 2,3,5-trichlorobiphenyl (2,3,5-CB) in laboratory cultures. Reverse-phase high performance liquid chromatography (HPLC) was employed to isolate the former compound from the experimental matrix for $\delta(37)\text{Cl}$ measurement. No detectable isotopic fractionation was observed over the 90 day incubation with sterile control, standard, and inoculated samples all exhibiting $\delta(37)\text{Cl}$ values with a range of similar to 0.5‰. These results show that this type of biological activity can be discriminated from other transformations by the absence of a measurable isotope effect during microbial reductive dechlorination. The utility of HPLC isolation for compound-specific $\delta(37)\text{Cl}$ analyses of environmentally relevant species is also demonstrated. (C) 2003 Elsevier Ltd. All rights reserved.

Elsner, M., S. B. Haderlein, et al. (2004). "Mechanisms and products of surface-mediated reductive dehalogenation of carbon tetrachloride by Fe(II) on goethite." *Environmental Science & Technology* 38(7): 2058-2066.

Natural attenuation processes of chlorinated solvents in soils and groundwaters are increasingly considered as options to manage contaminated sites. Under anoxic conditions, reactions with ferrous iron sorbed at iron(hydro)oxides may dominate the overall transformation of carbon tetrachloride (CCl_4) and other chlorinated aliphatic hydrocarbons. We investigated mechanisms and product formation of CCl_4 reduction by Fe(II) sorbed to goethite, which may lead to completely dehalogenated products or to chloroform (CHCl_3), a toxic product which is fairly persistent under anoxic conditions. A simultaneous transfer of two electrons and cleavage of two C-Cl bonds of CCl_4 would completely circumvent chloroform production. To distinguish between initial one- or two-bond cleavage, C-13-isotope fractionation of CCl_4 was studied for reactions with Fe(II)/ goethite (isotopic enrichment factor $\epsilon = -26.5$ parts per thousand) and with model systems for one C-Cl bond cleavage and either single-electron transfer (Fe(II) porphyrin, $\epsilon = -26.1$ parts per thousand) or partial two-electron transfer (polysulfide, $\epsilon = -22.2$ parts per thousand). These values differ significantly from calculations for simultaneous cleavage of two C-Cl bonds (ϵ approximate to 50 parts per thousand), indicating that only one C-Cl bond is broken in the critical first step of the reaction. At pH 7, reduction of CCl_4 by Fe(II)/ goethite produced similar to 33% CHCl_3 , 20% carbon monoxide (CO), and up to 40% formate (HCOO^-). Addition of 2-propanol-d(8) resulted in 33% CDCl_3 and only 4% CO, indicating that both products were generated from trichloromethyl radicals ($(\text{CCl}_3)\text{-C}\cdot$), chloroform by reaction with hydrogen radical donors and CO by an alternative pathway likely to involve surface-bound intermediates. Hydrolysis of CO to HCOO^- was surface-catalyzed by goethite but was too slow to account for the measured formate concentrations. Chloroform yields slightly increased with pH at constant Fe(II) sorption density, suggesting that pH-dependent surface processes direct product branching ratios. Surface-stabilized intermediates may thus facilitate abiotic mineralization of CCl_4 , whereas the presence of H radical donors, such as natural organic matter, enhances formation of toxic CHCl_3 .

Elsner, M., R. P. Schwarzenbach, et al. (2004). "Reactivity of Fe(II)-bearing minerals toward reductive transformation of organic contaminants." *Environmental Science & Technology* 38(3): 799-807.

Fe(II) present at surfaces of iron-containing minerals can play a significant role in the overall attenuation of reducible contaminants in the subsurface. As the chemical environment, i.e., the type and arrangement of ligands,

strongly affects the redox potential of Fe(II), the presence of various mineral sorbents is expected to modulate the reactivity of surficial Fe(II)-species in aqueous systems. In a comparative study we evaluated the reactivity of ferrous iron in aqueous suspensions of siderite (FeCO₃), nontronite (ferruginous smectite SWa-1), hematite (alpha-Fe₂O₃), lepidocrocite (gamma-FeOOH), goethite (alpha-FeOOH), magnetite (Fe₃O₄), sulfate green rust ((Fe₄Fe₂II)-Fe-II(OH)(12)SO₄.4H(2)O), pyrite (FeS₂), and mackinawite (FeS) under similar conditions (pH 7.2, 25 m(2) mineral/L, 1 mM Fe(II)(aq), O₂(aq) < 0.1 g/L). Surface-area-normalized pseudo first-order rate constants are reported for the reduction of hexachloroethane and 4-chloronitrobenzene representing two classes of environmentally relevant transformation reactions of pollutants, i.e., dehalogenation and nitroaryl reduction. The reactivities of the different Fe(II) mineral systems varied greatly and systematically both within and between the two data sets obtained with the two probe compounds. As a general trend, surface-area-normalized reaction rates increased in the order Fe(II) + siderite < Fe(II) + iron oxides < Fe(II) + iron sulfides. 4-Chloronitrobenzene was transformed by mineral-bound Fe(II) much more rapidly than hexachloroethane, except for suspensions of hematite, pyrite, and nontronite. The results demonstrate that abiotic reactions with surface-bound Fe(II) may affect or even dominate the long-term behavior of reducible pollutants in the subsurface, particularly in the presence of Fe(III) bearing minerals. As such reactions can be dominated by specific interactions of the oxidant with the surface, care must be taken in extrapolating reactivity data of surface-bound Fe(II) between different compound classes.

Erable, B., I. Goubet, et al. (2004). "Haloalkane hydrolysis by *Rhodococcus erythropolis* cells: Comparison of conventional aqueous phase dehalogenation and nonconventional gas phase dehalogenation." *Biotechnology and Bioengineering* 86(1): 47-54.

Biofiltration of air polluted by volatile organic compounds is now recognized by the industrial and research communities as an effective and viable alternative to standard environmental technologies. Whereas many studies have focused on solid/liquid/gas biofilters, there have been fewer reports on waste air treatment using other biological processes, especially in a solid/gas biofilter. In this study, a comparison was made of the hydrolysis of halogenated compounds (such as 1-chlorobutane) by lyophilized *Rhodococcus erythropolis* cells in a novel solid/gas biofilter and in the aqueous phase. We first determined the culture conditions for the production of *R. erythropolis* cells with a strong dehalogenase activity. Four different media were studied and the amount of 1-chlorobutane was optimized. Next, we report the possibility to use *R. erythropolis* cells in a solid/gas biofilter in order to transform halogenated compounds in corresponding alcohols. The effect of experimental parameters (total flow into the biofilter, thermodynamic activity of the substrates, temperature, carbon chain length of halogenated substrates) on the activity and stability of lyophilized cells in the gas phase was determined. A critical water thermodynamic activity (a(w)) of 0.4 is necessary for the enzyme to become active and optimal dehalogenase activity for the lyophilized cells is obtained for an a(w) of 0.9. A temperature of reaction of 40degreesC represents the best compromise between stability and activity. Activation energy of the reaction was determined and found equal to 59.5 KJ/mol. The pH effect on the dehalogenase activity of *R. erythropolis* cells was also studied in the gas phase and in the aqueous phase. It was observed that pH 9.0 provided the best activity in both systems. We observed that in the aqueous phase *R. erythropolis* cells were less sensitive to the variation in pH than *R. erythropolis* cells in the gas phase. Finally, the addition of volatile Lewis base (triethylamine) in the gaseous phase and the action of the lysozyme in order to permeabilize the cells was found to be highly beneficial to the effectiveness of the biofilter. (C) 2004 Wiley Periodicals, Inc.

Fennell, D. E., F. Liu, et al. (2003). "Dehalogenation of Polychlorinated dioxins in cultures and sediments: Results and biokinetic modeling." *Abstracts of Papers of the American Chemical Society* 226: U496-U496.

Fennell, D. E., I. Nijenhuis, et al. (2004). "Dehalococcoides ethenogenes strain 195 reductively dechlorinates diverse chlorinated aromatic pollutants." *Environmental Science & Technology* 38(7): 2075-2081.

Dehalococcoides ethenogenes strain 195 dechlorinates tetra chloroethene to vinyl chloride and ethene, and its genome has been found to contain up to 17 putative dehalogenase gene homologues, suggesting diverse dehalogenation ability. We amended pure or mixed cultures containing *D. ethenogenes* strain 195 with 1,2,3,4-tetrachlorodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3-dichlorodibenzo-p-dioxin, 1,2,3,4-tetrachlorodibenzofuran, 2,3,4,5,6-pentachlorobiphenyl, 1,2,3,4-tetra chloronaphthalene, various chlorobenzenes, or a mixture of 2-, 3-, and 4-chlorophenol to determine the dehalogenation ability. *D. ethenogenes* strain 195

dechlorinated 1,2,3,4-tetrachlorodibenzo-p-dioxin to a mixture of 1,2,4-trichlorodibenzo-p-dioxin and 1,3-dichlorodibenzo-p-dioxin. 2,3,4,5,6- Pentachlorobiphenyl was dechlorinated to 2,3,4,6- and/or 2,3,5,6-tetrachlorobiphenyl and 2,4,6-trichlorobiphenyl. 1,2,3,4-Tetrachloronaphthalene was dechlorinated primarily to an unidentified dichloro-naphthalene congener. The predominant end products from hexachlorobenzene dechlorination were 1,2,3,5-tetrachlorobenzene and 1,3,5-trichlorobenzene. We did not detect dechlorination daughter products from monochlorophenols, 2,3-dichlorodibenzo-p-dioxin or 2,3,7,8- tetrachlorodibenzo-p-dioxin. *D. ethenogenes* strain 195 has the ability to dechlorinate many different types of chlorinated aromatic compounds, in addition to its known chloroethene respiratory electron acceptors. Remediation of sediments contaminated with aromatic halogenated organic pollutants such as polychlorinated biphenyls and polychlorinated dibenzo-p-dioxins could require billions of dollars in the coming years. Harnessing microorganisms such as *Dehalococcoides* spp. that detoxify these compounds via removal of halogens may lead to cost-effective biotechnological approaches for remediation.

Fennell, D. E., S. K. Rhee, et al. (2004). "Detection and characterization of a dehalogenating microorganism by terminal restriction fragment length polymorphism fingerprinting of 16S rRNA in a sulfidogenic, 2-bromophenol-utilizing enrichment." *Applied and Environmental Microbiology* 70(2): 1169-1175.

Terminal restriction fragment length polymorphism analysis of reverse-transcribed 16S rRNA during periods of community flux was used as a tool to delineate the roles of the members of a 2-bromophenol-degrading, sulfate-reducing consortium. Starved, washed cultures were amended with 2-bromophenol plus sulfate, 2-bromophenol plus hydrogen, phenol plus sulfate, or phenol with no electron acceptor and were monitored for substrate use. In the presence of sulfate, 2-bromophenol and phenol were completely degraded. In the absence of sulfate, 2-bromophenol was dehalogenated and phenol accumulated. Direct terminal restriction fragment length polymorphism fingerprinting of the 16S rRNA in the various subcultures indicated that phylotype 2BP-48 (a *Desulfovibrio*-like sequence) was responsible for the dehalogenation of 2-bromophenol. A stable coculture was established which contained predominantly 2BP-48 and a second *Desulfovibrio*-like bacterium (designated BP212 based on terminal restriction fragment length polymorphism fingerprinting) that was capable of dehalogenating 2-bromophenol to phenol. Strain 2BP-48 in the coculture could couple reductive dehalogenation to growth with 2-bromophenol, 2,6-dibromophenol, or 2-iodophenol and lactate or formate as the electron donor. In addition to halophenols, strain 2BP-48 appears to use sulfate, sulfite, and thiosulfate as electron acceptors and is capable of simultaneous sulfidogenesis and reductive dehalogenation in the presence of sulfate.

Ferrey, M. L., R. T. Wilkin, et al. (2004). "Nonbiological removal of cis-dichloroethylene and 1,1-dichloroethylene in aquifer sediment containing magnetite." *Environmental Science & Technology* 38(6): 1746-1752.

The U.S. EPA Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater emphasizes biological reductive dechlorination as the primary mechanism for destruction of chlorinated solvents. However, biological reductive dechlorination could not explain the removal of cis-dichloroethylene (cis-DCE) and 1,1-DCE from a plume of contaminated groundwater in Minnesota. Several recent laboratory studies have demonstrated that common iron minerals such as magnetite can also transform chlorinated alkenes. Laboratory microcosms were constructed with sediment from three depth intervals in the aquifer near the source of the plume. The microcosms were autoclaved to prevent biological transformations. In these autoclaved sediments, the rates of removal of cis-DCE in samples from the shallow, intermediate, and deeper depth intervals in the aquifer were 0.58 +/- 0.09, 2.29 +/- 0.26, and 0.31 +/- 0.08 per year at 95% confidence. The rate of removal of 1,1-DCE in sediment from the shallow interval was 1.37 +/- 0.50 per year. The rates of removal in the microcosms are similar to the rates of attenuation observed in the field. Magnetite was identified in the sediment by X-ray diffraction and optical microscopy. Published rates of transformation of cis-DCE by magnetite are consistent with the rates of removal in the microcosm study.

Firsova, Y. E., N. V. Doronina, et al. (2004). "Physiological and biochemical analysis of the transformants of aerobic methylobacteria expressing the dcmA gene of dichloromethane dehalogenase." *Microbiology* 73(1): 24-29.

Transformants of *Methylobacterium dichloromethanicum* DM4 (DM4-2cr(-)/pME 8220 and DM4-2cr(-)/pME822 1) and of *Methylobacterium extorquens* AM1 (AM1/pME8220 and AM1/pME8221) that express the dcm A gene of dichloromethane dehalogenase undergo lysis when incubated in the presence of dichloromethane and are sensitive to acidic shock. The lysis of the transformants was found to be related neither to the accumulation of Cl⁻ ions, CH₂O, or HCOOH, nor to the impairment of glutathione synthesis or to the disturbance of intracellular pH homeostasis. The (exo(-)) Klenow fragment-mediated incorporation of [α -P-32]dATP into the DNA of the transformants DM4-2cr(-)/pME8220 and AM1/pME8220 was considerably greater when the transformed cells were incubated with CH₂Cl₂ than when they were incubated with CH₃OH, indicating the occurrence of a significant increase in the total length of gaps. At the same time, the strain AM1 (which lacks dichloromethane dehalogenase) and the dichloromethane-degrading strain DM4 incubated with CH₂Cl₂ showed an insignificant increase in the total length of the gaps. The transformed cells are likely to lyse due to the relatively inefficient repair of DNA lesions that are induced in response to the alkylating action of S-chloromethylglutathione, an intermediate product of CH₂Cl₂ degradation. The data obtained suggest that the bacterial mineralization of dichloromethane requires an efficient DNA repair system.

Grimalt, J. O., B. L. van Drooge, et al. (2004). "Persistent organochlorine compounds in soils and sediments of European high altitude mountain lakes, PDF." *Chemosphere* 54(10): 1549-1561.

The composition of persistent organochlorine compounds (OC) in soils and sediments from two high altitude European mountain lakes, Redon in the Pyrenees and Ladove in the Tatra mountains, has been studied. Sediment cores from two additional lakes in the Tatra mountains, Starolesnianske Pleso and Dlugi Staw, have also been examined. DDTs (1.7-13 ng g⁻¹) were the most abundant OC in soils followed by total polychlorobiphenyls (PCBs; 0.41-1.5 ng g⁻¹) and hexachlorobenzene (HCB; 0.15-0.91 ng g⁻¹). In sediments, the dominant OC were also DDTs (3.3-28 ng g⁻¹) and PCBs (2.3-15 ng g⁻¹). These concentrations are low, involving absence of major pollution sources in these high mountain regions. The downcore OC profiles in soils and sediments were similar but higher concentrations and steeper vertical gradients were observed in the latter. Radiometric determinations showed absence of significant OC transport from catchment to lake. The sediment-soil difference points therefore to a better retention of the OC load in sediments than soils which may be related to the low temperatures that are currently encountered at the bottom of the lake water column and the depletion of sediment bioturbation in these cold environments. Significant qualitative changes in the soil PCB distributions are observed downcore. These involve a dominance of the high molecular weight congeners in the top core sections and those of lower weight (i.e. less chlorinated) in the bottom. Anaerobic dechlorination of higher molecular weight congeners occurring in microsites, e.g. as observed in flooded or poorly drained soils, could be responsible for these changes. This process could be concurrent to bioturbation. (C) 2003 Elsevier Ltd. All rights reserved.

Gu, A. Z., B. P. Hedlund, et al. (2004). "Analysis and comparison of the microbial community structures of two enrichment cultures capable of reductively dechlorinating TCE and cis-DCE." *Environmental Microbiology* 6(1): 45-54.

In order to study the effect of different chloroethenes (electron acceptors) on the bacterial composition of dechlorinating communities, two reductive dechlorinating enrichment cultures were developed that were able to reduce trichloroethene (TCE) and cis-1,2-dichloroethene (cis-DCE) to ethene using hydrogen as electron donor, respectively. The inoculum for the cultures was material from a methanogenic fluidized bed reactor (FBR), which was originally seeded with digester sludge and showed a stable capacity for tetrachloroethene (PCE) reduction to ethene for over six years. Molecular methods were used to determine and compare the microbial communities of these two enrichment cultures. A clone library of bacterial 16S rRNA genes was generated for each enrichment. The clones were screened into different groups by restriction fragment length polymorphism (RFLP) analysis using two different four base pair recognition restriction enzymes. A total of 12 sequence types were identified by phylogenetic analysis of nearly complete 16S rDNA sequences (similar to 1450 bp). The sequences were affiliated with six recognized phyla of the domain Bacteria: Firmicutes (low G+C Gram-positives), Chloroflexi (green non-sulphur bacteria), Actinobacteria (high G+C Gram-positives), Bacteroidetes (Cytophaga-Flexibacter-Bacteroides), Nitrospira and Spirochaetes. The results led to the identification of an organism closely related to Dehalococcoides

ethenogenes to be the presumptive dechlorinator in both enrichments. Different electron acceptors affected the bacterial diversity and the community profiles of the two enrichments. Most of the sequences identified in our dechlorinating enrichments shared high similarities with sequences previously obtained from other enriched dechlorinating cultures and chlorinated-compound-contaminated sediments or aquifers, suggesting these bacteria may have direct or indirect roles in reductive dechlorination.

Heidrich, S., H. Weiss, et al. (2004). "Attenuation reactions in a multiple contaminated aquifer in Bitterfeld (Germany)." *Environmental Pollution* 129(2): 277-288.

Large-scale contaminated sites with multiple contaminants in the groundwater present a challenge to risk assessment and remediation. Attenuation reactions take place in the subsurface and act to contain contaminants, but must be thoroughly investigated on a site-specific basis. Field data from monitoring wells at a contaminated industrial site in Bitterfeld, Germany, are presented and analyzed for evidence of the prevalent biodegradation reactions. The groundwater in the Tertiary aquifer is contaminated with large quantities of chlorinated aliphatic compounds, in addition to chlorobenzenes and BTEX. In this strictly anaerobic environment, geochemical indications for several microbial processes were found, including methanogenesis, sulfate and iron reduction as well as reductive dechlorination of the chlorinated hydrocarbons. Direct evidence for the latter degradation reaction was observed along the flowpath due to the appearance of intermediates and an increase in the degree of dechlorination. (C) 2003 Elsevier Ltd. All rights reserved.

Hites, R. A. (2004). "Polybrominated Diphenyl Ethers in the Environment and in People: A Meta-Analysis of Concentrations." *Environmental Science & Technology* 38(4): 945-956.

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in many types of consumer products. Perhaps as a result of their widespread use and their lipophilicity, these compounds have become ubiquitous in the environment and in people. This review summarizes PBDE concentrations measured in several environmental media and analyzes these data in terms of relative concentrations, concentration trends, and congener profiles. In human blood, milk, and tissues, total PBDE levels have increased exponentially by a factor of ~100 during the last 30 yr; this is a doubling time of ~5 yr. The current PBDE concentrations in people from Europe are ~2 ng/g lipid, but the concentrations in people from the United States are much higher at ~35 ng/g lipid. Current PBDE concentrations in marine mammals from the Canadian Arctic are very low at ~5 ng/g lipid, but they have increased exponentially with a doubling time of ~7 yr. Marine mammals from the rest of the world have current PBDE levels of ~1000 ng/g lipid, and these concentrations have also increased exponentially with a doubling time of ~5 yr. Some birds' eggs from Sweden are also highly contaminated (at ~2000 ng/g lipid) and show PBDE doubling times of ~6 yr. Herring gull eggs from the Great Lakes region now have PBDE concentrations of ~7000 ng/g lipid, and these levels have doubled every ~3 yr. Fish from Europe have ~10 times lower PBDE concentrations than fish from North America. From these and other data, it is clear that the environment and people from North America are very much more contaminated with PBDEs as compared to Europe and that these PBDE levels have doubled every 4-6 yr. Analyses of the relative distributions of the most abundant PBDE congeners (using category averages and principal component analysis) indicated that these patterns cannot yet be used to assign sources to these pollutants.

Hong, J. Y., J. H. Kim, et al. (2003). "Production and characterization of DDT antibodies and its application to enzyme immunoassay: Relation of response and affinity to coating ligand." *Bulletin of the Korean Chemical Society* 24(11): 1605-1608.

To development an immunodetection method for DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDT) and its metabolites (p,p'-DDA, p,p'-DDE, p,p'-DDD), five derivatives of DDT haptens have been synthesised and characterized as coating ligands for antibody evaluation. The appropriate lengths of linkers were introduced to investigate a matching pair of coating ligand and antibody. Among these hapten derivatives, 2,2-bis(4-chlorophenyl)acetic acid (DDA), 5,5-bis(4-chlorophenyl)-5-hydroxypentanoic acid (DDHP) and 5,5-bis(4-chlorophenyl)-5-chloropentanoic acid (DDCP) were conjugated with keyhole limpet hemocyanin (KLH) for its use as an immunogen. The bovine serum albumin (BSA) conjugates of these derivatives were prepared as a coating ligand for monoclonal antibody screening. Fifteen monoclonal antibody clones were screened using these probes. 6,6-Bis(4-chlorophenyl)-6-hydroxyhexanoic acid (DDHH) and 3-[6,6-Bis(4-chlorophenyl)-6-hydroxyhexanoylamino]propanoic acid (DDHHAP), in addition to the above hapten derivatives, were conjugated to

ovalbumin (OVA) and bovine serum albumin (BSA) for their use as coating ligands to measure the titration level of the antibody and the displacement of free analytes. The indirect competitive ELISA results indicate that the titration level and free analyte displacement were greatly influenced by the DDT derivatives and carrier proteins used. Three matching pairs of monoclonal antibodies and coating ligands were selected for the DDT immunoassay: antibody clone 1A3 and coating ligand DDA-OVA, 1A1 and DDHAP-BSA, and 1A4 and DDHP-OVA.

Jorg, G. and M. Bertau (2004). "Fungal aerobic reductive dechlorination of ethyl 2-chloroacetoacetate by *Saccharomyces cerevisiae*: Mechanism of a novel type of microbial dehalogenation." *Chembiochem* 5(1): 87-92.

Saccharomyces cerevisiae reduces the beta-keto ester ethyl 2-chloro acetoacetate to the respective chiral cis- and trans-beta-hydroxy esters. In the course of chiral reduction, competing dehalogenation of the xenobiotic substrate to ethyl acetoacetate occurs, in a reaction mediated by cytosolic glutathione (GSH). Mechanistically, the dechlorination is a novel type of glutathione-dependent dehalogenation catalysed by an as yet unidentified glutathione-dependent dehalogenase. The first step consists of a nucleophilic replacement of the chloride substituent by glutathione. In the subsequent enzyme catalysed step a second glutathione molecule liberates the dehalogenation product by thiolytic attack at the thioether bridge, resulting in a net transfer of two electrons to the substrate and in the formation of glutathione disulfide (GSSG). Being effective under aerobic conditions and catalysed by a fungus, this reductive dechlorination of an aliphatic substrate is an outstanding example of a novel, glutathione-mediated microbial dehalogenation.

Kantachote, D., I. Singleton, et al. (2004). "Sodium application enhances DDT transformation in a long-term contaminated soil." *Water Air and Soil Pollution* 154(1-4): 115-125.

Bioremediation is an economically attractive option to remediate soil contaminated with DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane] and other organochlorine pesticides. However, lack of DDT bioavailability in soil presents one major obstacle to this technology particularly in soils that have been contaminated for long periods. In this work, sodium ion (Na⁺) was applied to a long-term DDT contaminated soil as Na⁺ is known to disperse clays, which would potentially release and/or expose physically protected DDT thereby enhancing DDT bioavailability. Sodium ion addition significantly increased dissolved organic carbon (DOC) levels, anaerobic bacterial numbers and the amount of DDT residues measured in soil solution. DDT transformation ranged from 95% (30-80 mg Na⁺ kg⁻¹ soil) to 72% (no Na⁺ added) with the optimum level of DDT transformation occurring at 30 mg Na⁺ kg⁻¹ soil. Higher Na⁺ levels repressed DDT transformation and this appeared to be related to lower DOC levels and flocculation of soils. The anaerobic incubation conditions employed (high water content) prevented DDE [1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene] production and DDD [1,1-dichloro-2,2-bis(p-chlorophenyl) ethane] was the major breakdown product formed. Overall it appeared that Na⁺ has potential as a cheap and safe alternative to surfactants as a method for increasing DDT transformation in contaminated soil.

Kao, C. M., K. F. Chen, et al. (2004). "Biobarrier system for remediation of TCE-contaminated aquifers." *Bulletin of Environmental Contamination and Toxicology* 72(1): 87-93.

Keefe, S. H., L. B. Barber, et al. (2004). "Fate of volatile organic compounds in constructed wastewater treatment wetlands." *Environmental Science & Technology* 38(7): 2209-2216.

The fate of volatile organic compounds was evaluated in a wastewater-dependent constructed wetland near Phoenix, AZ, using field measurements and solute transport modeling. Numerically based volatilization rates were determined using inverse modeling techniques and hydraulic parameters established by sodium bromide tracer experiments. Theoretical volatilization rates were calculated from the two-film method incorporating physicochemical properties and environmental conditions. Additional analyses were conducted using graphically determined volatilization rates based on field measurements. Transport (with first-order removal) simulations were performed using a range of volatilization rates and were evaluated with respect to field concentrations. The inverse and two-film reactive transport simulations demonstrated excellent agreement with measured concentrations for 1,4-dichlorobenzene, tetrachloroethene, dichloromethane, and trichloromethane and fair agreement for

dibromochloromethane, bromodichloromethane, and toluene. Wetland removal efficiencies from inlet to outlet ranged from 63% to 87% for target compounds.

Kim, A. A., G. T. Djuraeva, et al. (2004). "Investigation of PCBs biodegradation by soil bacteria using tritium-labeled PCBs." *Journal of Radioanalytical and Nuclear Chemistry* 259(2): 301-304.

The method of tritium labeling of polychlorinated biphenyls (PCBs) has been developed. It allows producing of uniformly labeled tritium PCBs. High specific activity permits the tracing all of the tritium labeled PCBs biodegradation products. Radiochemical approach of the investigation of PCBs microbial degradation has been developed and PCB-destructive activity of soil bacteria strains has been studied. It was found that 4 investigated bacteria strains of *Bacillus* sp. has the ability accumulate and destroy PCBs. Use of developed radiochemical methods in complex with other analytical methods in investigation of PCBs biodegradation provide useful additional information. The radiochemical methods developed can be successfully used for wide screening of microorganisms, destructors of PCBs.

Kim, A. A., G. V. Pestsov, et al. (2004). "Microorganisms degrading polychlorinated biphenyls." *Applied Biochemistry and Microbiology* 40(1): 60-62.

Four strains belonging to the genus *Bacillus* capable of degrading polychlorinated biphenyls (PCBs) were isolated by screening collection strains of soil bacteria degrading an organochlorine pesticide, hexachlorocyclohexane (HCH). A method for production of tritium-labeled PCBs was developed. Consumption and degradation of PCBs by the soil bacterial strains selected were studied using tritium-labeled PCBs and GLC. It was demonstrated that PCBs are degradable both in culture media and in model soil samples.

Kimbara, K., T. Ushioji, et al. (2004). "PCB decomposition by a hybrid method of photodechlorination and biodegradation - Review article." *Seibutsu-Kogaku Kaishi* 82(2): 56-62.

Polychlorinated biphenyls (PCBs) are widespread environmental pollutants. Although microbiological PCB degradation has been studied for a few decades, it has not been applied for a large-scale disposal of concentrated PCBs. We had developed a method combining photodechlorination and biological degradation for the complete decomposition of PCBs. Here we examine a pilot scale study on the degradation of commercial PCBs from a high voltage transformer. After optimizing the culture conditions, over 99.9999% of 1300-1800g of Kaneclor 1000 was degraded within 8 d. Mass balance study showed undesirable by-products such as polymerized PCBs were not produced. Dioxins including dibenzofuran and co-planar PCBs in Kaneclor sample were also degraded completely by UV dechlorination and biodegradation. The concentration of PCBs in the final effluent was acceptable by the Japanese standard (0.003 mg/l) on wastewater. Based on the above results, we have demonstrated that biological degradation combined with UV dechlorination is a useful method for the safe disposal of PCBs.

Kipopoulou, A. M., A. Zouboulis, et al. (2004). "The fate of lindane in the conventional activated sludge treatment process." *Chemosphere* 55(1): 81-91.

A pilot-scale treatability study was performed to evaluate the fate of lindane (γ -hexachlorocyclohexane) in wastewater treatment plants operating in the conventional activated sludge mode. Different types of wastewater (industrial and municipal) spiked with variable lindane concentrations were used at different dosing rates in order to determine distribution and removal under various operational conditions. The major amount (67-91%) of lindane inputs to the treatment process was found to concentrate in primary sludge. A significant linear correlation between the compound's partition coefficient ($\log K_p$) and the organic fraction of primary sludge (f_{oc}) was found. Sorption on primary sludge solids was concluded to be the major removal mechanism. Only 0.1-2.8% of lindane inputs was concentrated in activated sludge. Lindane losses in primary treatment were low (4-26%). Higher losses (up to 61%) were observed during the biological treatment probably due to biodegradation. These losses were negatively correlated with the inflow rate of lindane into the aeration tank. Activated sludge aged about 23 d presented the maximum loss of lindane. Increased sludge age was associated with increased percentages of lindane in the final effluent. (C) 2003 Elsevier Ltd. All rights reserved.

Krautler, B., W. Fieber, et al. (2003). "The cofactor of tetrachloroethene reductive dehalogenase of *Dehalospirillum multivorans* is norpseudobeta-B-12, a new type of a natural corrinoid." *Helvetica Chimica Acta* 86(11): 3698-3716.

The corrinoid cofactor of the tetrachloroethene reductive dehalogenase of *Dehalospirillum multivorans* was isolated in its Cobeta-cyano form. This cofactor represents the main corrinoid found in *D. multivorans* cells. Analysis of the isolated cyano-corrinoid by a combination of HPLC and UV/VIS-absorbance spectroscopy revealed it to be nonidentical to a variety of known natural B-12 derivatives. From high-resolution mass-spectrometric analysis, the molecular formula of the corrinoid isolated from *D. multivorans* could be deduced as C₅₈H₈₁CoN₁₇O₁₄P. The sample of the novel corrinoid from *D. multivorans* was further analyzed by UV/VIS, CD, and one- and two-dimensional H-1-, C-13-, and N-15-NMR spectroscopy, which indicated its structure to be closely related to that of pseudovitamin B-12 (Cobeta-cyano-7"-adeninylcobamide). By the same means, the corrinoid could be shown to differ from pseudovitamin B12 only by the lack of the methyl group attached to carbon 176, and, therefore, it was named norpseudovitamin B-12 (or, more precisely, 176-norpseudovitamin B-12). Norpseudovitamin B-12 represents the first example of a 'complete' B-12-cofactor that lacks one of the methyl groups of the cobamide moiety, indicating that the B-12-biosynthetic pathway in *D. multivorans* differs from that of other organisms. X-Ray crystal-structures were determined for norpseudovitamin B-12 from *D. multivorans* and the analogues pseudovitamin B-12 and factor A (Cobeta-cyano-7"-[2-methyl]adeninylcobamide). These first accurate crystal structures of complete corrinoids with an adeninyl pseudonucleotide confirmed the expected coordination properties around Co and corroborated the close conformational similarity of the nucleotide moieties of norpseudovitamin B-12 and its two homologues.

Law, S. A., T. F. Bidleman, et al. (2004). "Evidence of enantioselective degradation of alpha-hexachlorocyclohexane in groundwater." *Environmental Science & Technology* 38(6): 1633-1638.

In the fall of 2000, 34 groundwater samples were collected from beneath an active pesticide reformulating and packaging facility in coastal northeastern Florida to measure the enantiomer fractions (EFs) of alpha-hexachlorocyclohexane (alpha-ACH) as an indicator of biodegradation of this chlorinated pesticide in groundwater. Concentrations of alpha-ACH as high as 500 µg/L were observed beneath the historical source area and decreased with distance downgradient. Seventy-eight percent of the EF values were greater than 0.504 and ranged up to 0.890, indicating that the (-)-alpha-HCH enantiomer is preferentially degraded relative to the (+)-alpha-HCH enantiomer at this site. Samples taken from the groundwater that flows north from the historical disposal facility to a local discharge point at a creek did not indicate enantioselective degradation (EF values ranged from 0.495 to 0.512). The acidity (pH 3.7-4.6) and short flow path to the creek for this lobe of the groundwater plume likely preclude biodegradation of alpha-ACH. In contrast, the neutral lobe of the groundwater plume, which flows eastward from the historical source area, demonstrated enantioselective degradation (EF values ranged from 0.500 to 0.890 and increased with distance from the source area). Groundwater conditions beneath this portion of the site are conducive to biodegradation of HCH owing to anaerobic reducing conditions and lengthy travel times, and the chiral signatures for alpha-HCH provide evidence that biological degradation is occurring beneath this portion of the site.

Lee, E. Y. (2003). "Continuous treatment of gas-phase trichloroethylene by *Burkholderia cepacia* G4 in a two-stage continuous stirred tank reactor/trickling biofilter system." *Journal of Bioscience and Bioengineering* 96(6): 572-574.

A two-stage continuous stirred tank reactor/trickling biofilter system was developed and operated for continuous treatment of gas-phase trichloroethylene (TCE) by *Burkholderia cepacia*. The maximum TCE elimination capacity was 28.0 mg TCE/l(.d), and complete removal of TCE was obtained for inlet loading below 25.3 mg TCE/l(.d). The reactor system was stably operated for more than 3 months.

Lee, E. Y., J. M. Kang, et al. (2003). "Evaluation of transformation capacity for degradation of ethylene chlorides by *Methylosinus trichosporium* OB3b." *Biotechnology and Bioprocess Engineering* 8(5): 309-312.

The transformation capacity (T-c) of *Methylosinus trichosporium* OB3b in the degradation of ethylene chlorides was determined by measuring the decrease of soluble methane monooxygenase (sMMO) activity of resting cells in batch experiments. All measurements of sMMO activity were taken in the presence of 20 mM formate to avoid the deficiency of reducing power, and within 2 hrs to avoid the effect of natural inactivation from instability of

the resting cells. The constant T-c values of 0.58 +/- 0.132 and 0.80 +/- 0.17 $\mu\text{mol}/\text{mg}$ cell were obtained for trichloroethylene (TCE) and 1,2-dichloroethylene (cis and trans-1,2-DCE), respectively, regardless of their concentrations. The transformation capacity measured by this method can be used to predict the amount of cells that should be stimulated in in-situ bioremediation.

Lee, S. B., S. E. Strand, et al. (2004). "Pseudonocardia chloroethenivorans sp nov., a chloroethene-degrading actinomycete." *International Journal of Systematic and Evolutionary Microbiology* 54: 131-139.

A bacterial strain, SL-1(T), capable of degrading trichloroethene was isolated from a laboratory enrichment in the Department of Civil and Environmental Engineering, University of Washington, USA. The material in the enrichments was derived from a soil sample from Seattle, WA, USA. Strain SL-1(T) was capable of using phenol as a source of carbon and energy. Chemotaxonomic, morphological, physiological and phylogenetic analyses showed that strain SL-1(T) is a member of the genus *Pseudonocardia*. The ability of strain SL-1(T) to utilize phenol and degrade trichloroethene, as well as other phenotypic properties and the results from a 16S rRNA phylogenetic analysis, led to the proposal of a novel species, *Pseudonocardia chloroethenivorans* sp. nov. The type strain is SL-1(T) (= ATCC BAA-742(T) = DSM 44698(T)). Trichloroethene and other chloroethenes are major pollutants at many environmental sites, and *P. chloroethenivorans* has biodegradation properties that should be of interest to environmental microbiologists and engineers.

Levenfors, J. J., R. Hedman, et al. (2004). "Broad-spectrum antifungal metabolites produced by the soil bacterium *Serratia plymuthica* A 153." *Soil Biology & Biochemistry* 36(4): 677-685.

Chlorinated macrolides, haterumalide NA, B and NE, and a new haterumalide X, were produced by the soil bacterium *Serratia plymuthica*. Haterumalides NA, B and NE caused complete suppression of apothecial formation in sclerotia of *Sclerotinia sclerotiorum* at a concentration of 0.5 μg ml⁻¹. Ascospore germination of this fungus was inhibited in the concentration range 0.8-3.0 μg ml⁻¹. Haterumalides NA, B and NE prevented spore germination of several other filamentous fungi as well as Oomycetes at concentrations ranging from 0.4 to 40 μg ml⁻¹, but did not show any effect against the yeast *Candida albicans*. Inhibition data could not be collected for haterumalide X due to its rapid conversion to haterumalide NA. The bacterium also produced two other antifungal metabolites: pyrrolnitrin and 1-acetyl-7-chloro-1-H-indole, which in contrast to the haterumalides, did not inhibit the apothecial formation on sclerotia. Pyrrolnitrin, and haterumalide NA, B and NE effectively inhibited spore germination of tested filamentous fungi at concentrations ranging from 0.06 to 50 μg ml⁻¹, whereas 1-acetyl-7-chloro-1-H-indole inhibited spore germination only at concentrations above 50 μg ml⁻¹. The minimal inhibitory concentrations of the respective compounds needed for total inhibition of spore germination varied for the fungal species tested. (C) 2004 Elsevier Ltd. All rights reserved.

Lewis, T. A., L. Leach, et al. (2004). "Physiological and molecular genetic evaluation of the dechlorination agent, pyridine-2,6-bis(monothiocarboxylic acid) (PDTC) as a secondary siderophore of *Pseudomonas*." *Environmental Microbiology* 6(2): 159-169.

The bacterial metabolite and transition metal chelator pyridine-2,6-dithiocarboxylic acid (PDTC), promotes a novel and effective means of dechlorination of the toxic and carcinogenic pollutant, carbon tetrachloride. Pyridine-2,6-dithiocarboxylic acid has been presumed to act as a siderophore in the *Pseudomonas* strains known to produce it. To explore further the physiological function of PDTC production, we have examined its regulation, the phenotype of PDTC-negative (pdt) mutants, and envelope proteins associated with PDTC in *P. putida* strain DSM 3601. Aspects of the regulation of PDTC production and outer membrane protein composition were consistent with siderophore function. Pyridine-2,6-dithiocarboxylic acid production was coordinated with production of the well-characterized siderophore pyoverdine; exogenously added pyoverdine led to decreased PDTC production, and added PDTC led to decreased pyoverdine production. Positive regulation of a chromosomal *pdtI-xylE* transcriptional fusion, and of a 66 kDa outer membrane protein (IROMP), was seen in response to exogenous PDTC. Tests with transition metal chelators indicated that PDTC could provide a benefit under conditions of metal limitation; the loss of PDTC biosynthetic capacity caused by a *pdtI* transposon insertion resulted in increased sensitivity to 1,10-phenanthroline, a chelator that has high affinity for a range of divalent transition metals (e.g. Fe²⁺, Cu²⁺, Zn²⁺). Exogenously added PDTC could also suppress a phenotype of pyoverdine-negative (Pvd(-)) mutants, that of sensitivity to EDDHA, a chelator with higher affinity and specificity for Fe³⁺. Measurement of Fe-59 incorporation

showed uptake from Fe-59:PDTC by DSM 3601 grown in low-iron medium, but not by cells grown in high iron medium, or by the pdtI mutant, which did not show expression of the 66 kDa envelope protein. These data verified a siderophore function for PDTC, and have implicated it in the uptake of transition metals in addition to iron.

McCormick, M. L. and P. Adriaens (2004). "Carbon tetrachloride transformation on the surface of nanoscale biogenic magnetite particles." *Environmental Science & Technology* 38(4): 1045-1053.

Iron-reducing conditions in subsurface environments promote dechlorination reactions via both biotic and abiotic pathways, the latter often mediated via biologically activated minerals formed by dissimilatory iron-reducing bacteria (DIRB). Here we report the major products and pathways associated with the abiotic transformation of carbon tetrachloride (CT) by nanoscale biogenic magnetite/maghemite particles produced by the DIRB *Geobacter metallireducens*. Product formation and free radical/carbene trapping studies indicate that CT transformation occurs via three parallel pathways. The first pathway (hydrogenolysis) results in the formation of chloroform (45-50%) via a trichloromethyl free radical ($\cdot\text{CCl}_3$) and possibly a trichloromethyl carbanion ($:\text{CCl}_3^-$). The second and third pathways involve a dichlorocarbene intermediate ($:\text{CCl}_2$) which either hydrolyzes to form CO (similar to 38%) (carbene hydrolysis), or undergoes further reduction to yield methane (8-10%) (carbene reduction). The mechanism of methane formation from $:\text{CCl}_2$ is not known, but is speculated to involve a sequence of surface coordinated carbenoid and free radical complexes. The large fraction of relatively benign products formed by the carbene-mediated pathways suggests that magnetite/maghemite particles may have a beneficial application in the remediation of CT contaminated environments.

Muller, T. A. and H. P. E. Kohler (2004). "Chirality of pollutants - effects on metabolism and fate." *Appl. Microbiol. Biot.* 64(3): 300-316.

In most cases, enantiomers of chiral compounds behave differently in biochemical processes. Therefore, the effects and the environmental fate of the enantiomers of chiral pollutants need to be investigated separately. In this review, the different fates of the enantiomers of chiral phenoxyalkanoic acid herbicides, acetamides, organochlorines, and linear alkylbenzenesulfonates are discussed. The focus lies on biological degradation, which may be enantioselective, in contrast to non-biotic conversions. The data show that it is difficult to predict which enantiomer may be enriched and that accumulation of an enantiomer is dependent on the environmental system, the species, and the organ. Racemization and enantiomerization processes occur and make interpretation of the data even more complex. Enantioselective degradation implies that the enzymes involved in the conversion of such compounds are able to differentiate between the enantiomers. "Enzyme pairs" have evolved which exhibit almost identical overall folding. Only subtle differences in their active site determine their enantioselectivities. At the other extreme, there are examples of non-homologous "enzyme pairs" that have developed through convergent evolution to enantioselectively turn over the enantiomers of a chiral compound. For a better understanding of enantioselective reactions, more detailed studies of enzymes involved in enantioselective degradation need to be performed.

Nam, K., X. Prat-Resina, et al. (2004). "Dynamics of an enzymatic substitution reaction in haloalkane dehalogenase." *Journal of the American Chemical Society* 126(5): 1369-1376.

Reactive flux molecular dynamics simulations have been carried out using a combined QM/MM potential to study the dynamics of the nucleophilic substitution reaction of dichloroethane by a carboxylate group in haloalkane dehalogenase and in water. We found that protein dynamics accelerates the reaction rate by a factor of 2 over the uncatalyzed reaction. Compared to the thermodynamic effect in barrier reduction, protein dynamic contribution is relatively small. However, analyses of the friction kernel reveal that the origins of the reaction dynamics in water and in the enzyme are different. In aqueous solution, there is significant electrostatic solvation effect, which is reflected by the slow reorganization relaxation of the solvent. On the other hand, there is no strong electrostatic coupling in the enzyme and the major effect on reaction coordinate motion is intramolecular energy relaxation.

Nunes-Halldorson, V. D., R. L. Steiner, et al. (2004). "Residual toxicity after biodegradation: interactions among benzene, toluene, and chloroform." *Ecotoxicology and Environmental Safety* 57(2): 162-167.

A microbial enrichment originating from a pristine aquifer was found to aerobically biodegrade benzene and toluene, but not chloroform. This enrichment culture was used to study changes in pollutant toxicity as affected by biodegradative activity. Two assays for toxicity were used: (1) a 48-h acute toxicity test using the freshwater invertebrate *Ceriodaphnia dubia* and (2) microbial biodegradation activity as affected by the presence of mixed pollutants. At 20-ppm concentrations, toluene was significantly more toxic (99% mortality) to *C. dubia* than benzene (48% mortality) or chloroform (40% mortality). Also at 20-ppm concentrations, but before biodegradation, toluene was significantly more toxic (88% mortality) to *C. dubia* than benzene (33% mortality). After biodegradation of 98% of toluene and benzene, significant residual toxicity still remained in the bacterial supernatant: toluene-degraded supernatant caused 33% mortality in *C. dubia* and benzene-degraded supernatant caused 24% mortality. In the second toxicity assay, examining the effect of mixed pollutants on biodegradation activity, the presence of benzene slowed the biodegradation of toluene, but chloroform had no effect on either benzene or toluene biodegradation. Results indicate that significant toxicity remain, after biodegradation and that halogenated aliphatic hydrocarbons may have little or no effect on aromatic hydrocarbon biodegradation at sites impacted by mixed pollutants. (C) 2003 Elsevier Science (USA). All rights reserved.

Olaniran, A. O., D. Pillay, et al. (2004). "Haloalkane and haloacid dehalogenases from aerobic bacterial isolates indigenous to contaminated sites in Africa demonstrate diverse substrate specificities." *Chemosphere* 55(1): 27-33.

Five bacteria were isolated from contaminated sites in Nigeria and South Africa using the culture enrichment technique. They were subjected to standard cultural, biochemical and microbiological techniques and identified to be species of *Bacillus*, *Burkholderia*, *Corynebacterium*, *Micrococcus* and *Pseudomonas*. Axenic cultures of the bacterial isolates utilized 1,2-dichloroethane (1,2-DCE) as the sole carbon source up to a final substrate concentration of 10 mM. Their mean generation time in 1,2-DCE ranged significantly ($P < 0.05$) from 9.77 to 15.72 h with the maximum chloride release ranging between 59% and 86%. All the bacterial isolates produced two different dehalogenases, viz. one which is heat labile and specific for halogenated alkanes with optimum activity at a pH of 7.5 and the other which is more heat stable with a higher pH optimum of 9.0 and specific for halogenated alkanic acids. However, the two enzyme types when tested demonstrated wide substrate specificities. It is therefore adjudged that these organisms may play a vital role in the bioremediation of sites polluted with chlorinated hydrocarbons. (C) 2003 Elsevier Ltd. All rights reserved.

Prevedouros, K., M. MacLeod, et al. (2004). "Modelling the fate of persistent organic pollutants in Europe: parameterisation of a gridded distribution model." *Environmental Pollution* 128(1-2): 251-261.

A regionally segmented multimedia fate model for the European continent is described together with an illustrative steady-state case study examining the fate of gamma-HCH (lindane) based on 1998 emission data. The study builds on the regionally segmented BETR North America model structure and describes the regional segmentation and parameterisation for Europe. The European continent is described by a 5degrees x 5degrees grid, leading to 50 regions together with four perimetric boxes representing regions buffering the European environment. Each zone comprises seven compartments including; upper and lower atmosphere, soil, vegetation, fresh water and sediment and coastal water. Inter-regions flows of air and water are described, exploiting information originating from GIS databases and other georeferenced data. The model is primarily designed to describe the fate of Persistent Organic Pollutants (POPs) within the European environment by examining chemical partitioning and degradation in each region, and inter-region transport either under steady-state conditions or fully dynamically. A test case scenario is presented which examines the fate of estimated spatially resolved atmospheric emissions of lindane throughout Europe within the lower atmosphere and surface soil compartments. In accordance with the predominant wind direction in Europe, the model predicts high concentrations close to the major sources as well as towards Central and Northeast regions. Elevated soil concentrations in Scandinavian soils provide further evidence of the potential of increased scavenging by forests and subsequent accumulation by organic-rich terrestrial surfaces. Initial model predictions have revealed a factor of 5-10 underestimation of lindane concentrations in the atmosphere. This is explained by an underestimation of source strength and/or an underestimation of European background levels. The model presented can further be used to predict deposition fluxes and chemical inventories, and it can also be adapted

to provide characteristic travel distances and overall environmental persistence, which can be compared with other long-range transport prediction methods. (C) 2003 Elsevier Ltd. All rights reserved.

Reina, R. G., A. C. Leri, et al. (2004). "ClK-edge x-ray spectroscopic investigation of enzymatic formation of organochlorines in weathering plant material." *Environmental Science & Technology* 38(3): 783-789.

The contribution of halocarbons from plant weathering to the total organohalogen budget of terrestrial systems is gaining recognition. To evaluate the formation of such halocarbons, speciation of chlorine in *Sequoia sempervirens* (redwood) needles was examined in the presence of an external chloroperoxidase (CPO) enzyme using Cl K-edge X-ray absorption spectroscopy. The Cl forms in fresh and naturally weathered needles and in model laboratory reactions were compared. To provide a straightforward analogue to the enzymatic chlorination in plants, chlorination reactions were conducted for phenol, a common moiety of plant macromolecules. Plant material chlorination was also examined in the presence of hypochlorite in an ancillary mechanistic investigation. The dominant form of Cl in fresh, unreacted plant material was found to be inorganic Cl⁻, which was partially converted to organochlorine in the presence of CPO. Chlorination is affected by the nature of reactant (CPO, H₂O₂) addition, reaction time, and temperature. The organochlorines produced in these laboratory investigations closely resemble those produced during the natural weathering of redwood needles. A striking consistency in chlorine speciation observed among the various sample types suggests that (i) CPO produced by terrestrial organisms could play a vital role in the generation of organochlorines associated with the degradation of plant material and (ii) initial targets of enzymatic chlorination might include lignin-like macromolecules rich in aromatic character and hydroxyl groups. These findings lend further credibility to a significant biogenic contribution to the global organohalogen burden by elucidating a probable route of enzymatic chlorination of natural organic matter in terrestrial systems.

Robson, M. and S. Harrad (2004). "Chiral PCB signatures in air and soil: Implications for atmospheric source apportionment." *Environmental Science & Technology* 38(6): 1662-1666.

Enantiomeric fractions (EFs) of chiral PCBs 95, 136, and 149 were measured in samples of topsoil and outdoor air at one urban and one rural location in the U.K. West Midlands between early 2001 and early 2002. While EFs in air were essentially racemic, those in topsoil indicated appreciable enantioenrichment of the second eluting enantiomer for PCB 95 and the H enantiomer for PCBs 136 and 149. This suggests (i) that essentially all atmospheric PCBs at both sites arise from racemic (i.e., primary) sources, rather than volatilization from soil and (ii) that appreciable enantioselective degradation of the monitored PCBs in topsoil occurs. This is one of only two reports of enantioselective degradation of PCBs in soil worldwide and is particularly noteworthy as it is occurring at PCB concentrations (e.g., 5.9 pg g⁻¹) for PCB 136 that are typical of the U.K. and other industrialized countries. The extent of enantioselective degradation in this study for PCBs 95 and 136 is consistent with those reported for soils in the Greater Toronto area (GTA). In contrast, enantioselective degradation of PCB 149 observed in this study is while consistent with that reported for U.K. lacustrine sediments-in excess of that observed in either the GTA soil study or in U.S. lake sediments.

Rodriguez, R. A. and G. A. Toranzos (2003). "Stability of bacterial populations in tropical soil upon exposure to Lindane." *International Microbiology* 6(4): 253-258.

The effect of the pesticide Lindane on microbial populations was analyzed in soil with a history of contamination with various chemicals, including this pesticide. Soil microcosms were amended with 100 mg Lindane/kg soil and microbial populations were monitored for 70 days. Bacterial cell concentrations, metabolic versatility (whole community Biolog), and genetic diversity (16S rDNA/denaturing gradient gel electrophoresis) were used to monitor microbial communities. Results show the persistence of Lindane in the soil environment; at the end of the experiment, 70% of the added Lindane remained undegraded. A reduction of 50% in bacterial cell concentration was observed in Lindane-amended microcosms during the 2nd week of the experiment. This reduction was correlated with a reduction in the rate of substrate utilization as observed by Biolog. Overall, no effect of Lindane was observed on the metabolic versatility and genetic diversity in these soils, demonstrating the ability of these bacterial populations to tolerate the pressure caused by the addition of pesticides.

Ruppe, S., A. Neumann, et al. (2004). "Anaerobic transformation of compounds of technical toxaphene. 2. Fate of compounds lacking geminal chlorine atoms." *Environmental Toxicology and Chemistry* 23(3): 591-598.

The major toxaphene metabolites in sediment and soils (2-exo,3-endo,6-exo,8,9,10-hexachlorobornane [B6-923] and 2-endo,3-exo,5-endo,6-exo,8,9, 10-heptachlorobornane [B7-1001]) were incubated with the isolated gram-negative bacterium *Dehalospirillum multivorans*. Within 14 d, biotransformation of B7-1001 was nearly quantitative, resulting in two penta- and six hexachlorobornanes, as well as one unsaturated hexachloro compound of technical toxaphene. The major transformation product (similar to 50% of all metabolites) was identified as 2-exo,3-endo,5-exo,8,9,10-hexachlorobornane (B6-903). Abiotic dehydrochlorination of B7-1001 with methanolic KOH resulted in the formation and subsequent identification of the lone unsaturated compound as 2,5-endo,6-exo,8,9,10-hexachloroborn-2-ene. Thus, dehydrochlorination was found to be a minor process of the anaerobic transformation of toxaphene. Biotransformation of 70% of amended B6-923 within 14 d demonstrated that reductive dechlorination was not exclusively associated with geminal Cl atoms, as previously suggested. Three pentachlorobornanes were identified as transformation products, one of which was identical with a transformation product of B7-1001. This commonality unequivocally proves this metabolite to be 2-exo,3-endo,8,9,10-pentachlorobornane. Fifteen previously unknown metabolites of B6-923, B7-1001, and other toxaphene compounds identified in this study were detected in sediment from Lake Ontario (Canada), underscoring the importance of microbial toxaphene transformation in natural, aquatic environments.

Scheutz, C. and P. Kjeldsen (2004). "Environmental Factors Influencing Attenuation of Methane and Hydrochlorofluorocarbons in Landfill Cover Soils." *Journal of Environmental Quality* 33(1): 72-79.

The influence of different environmental factors on methane oxidation and degradation of hydrochlorofluorocarbons (HCFCs) was investigated in microcosms containing soil sampled at Skellingsted Landfill, Denmark. The soil showed a high capacity for methane oxidation resulting in a maximum oxidation rate of 104 $\mu\text{g CH}_4 \text{ g}^{-1} \text{ h}^{-1}$ and a low affinity of methane with a half-saturation constant of 2.0% v/v. The hydrochlorofluorocarbons HCFC-21 (dichlorofluoromethane) and HCFC-22 (chlorodifluoromethane) were rapidly oxidized and the oxidation occurred in parallel with the oxidation of methane. The maximal HCFC oxidation rates were 0.95 and 0.68 $\mu\text{g g}^{-1} \text{ h}^{-1}$ for HCFC-21 and HCFC-22, respectively. Increasing concentrations of HCFCs resulted in decreased methane oxidation rates. However, compared with typical concentrations in landfill gas, relatively high HCFC concentrations were needed to obtain a significant inhibition of methane oxidation. In general, the environmental factors studied influenced the degradation of HCFCs in almost the same way as they influenced methane oxidation. Temperature had a strong influence on the methanotrophic activity giving high Q₁₀ values of 3.4 to 4.1 over the temperature range of 2 to 25°C. Temperature optimum was around 30°C; however, oxidation occurred at temperatures as low as 2°C. A moisture content of 25% w/w yielded the maximum oxidation rate as it allowed good gas transport together with sufficient microbial activity. The optimum pH was around neutrality (pH = 6.5–7.5) showing that the methanotrophs were optimally adapted to the in situ pH, which was 6.9. Copper showed no inhibitory effect when added in relatively high concentrations (up to 60 mg kg⁻¹), most likely due to sorption of copper ions to soil particles. At higher copper concentrations the oxidation rates decreased. The oxidation rates for methane, HCFC-21, and HCFC-22 were unaltered in ammonium-amended soil up to 14 mg kg⁻¹. Higher ammonium concentrations inhibited the oxidation process. The most important parameters controlling oxidation in landfill cover soil were found to be temperature, soil moisture, and methane and oxygen supply.

Scheutz, C., H. Mosbaek, et al. (2004). "Attenuation of methane and volatile organic compounds in landfill soil covers." *Journal of Environmental Quality* 33(1): 61-71.

The potential for natural attenuation of volatile organic compounds (VOCs) in landfill covers was investigated in soil microcosms incubated with methane and air, simulating the gas composition in landfill soil covers. Soil was sampled at Skellingsted Landfill at a location emitting methane. In total, 26 VOCs were investigated, including chlorinated methanes, ethanes, ethenes, fluorinated hydrocarbons, and aromatic hydrocarbons. The soil showed a high capacity for methane oxidation resulting in very high oxidation rates of between 24 and 112 $\mu\text{g CH}_4 \text{ g}^{-1} \text{ h}^{-1}$. All lower chlorinated compounds were shown degradable, and the degradation occurred in parallel with the oxidation of methane. In general, the degradation rates of the chlorinated aliphatics were inversely related to the chlorine to carbon ratios. For example, in batch experiments with chlorinated ethylenes, the highest rates were observed for vinyl chloride (VC) and lowest rates for trichloroethylene (TCE),

while tetrachloroethylene (PCE) was not degraded. Maximal oxidation rates for the halogenated aliphatic compounds varied between 0.03 and 1.7 $\mu\text{g g}^{-1} \text{h}^{-1}$. Fully halogenated hydrocarbons (PCE, tetrachloromethane [TeCM], chlorofluorocarbon [CFC]-11, CFC-12, and CFC-113) were not degraded in the presence of methane and oxygen. Aromatic hydrocarbons were rapidly degraded giving high maximal oxidation rates (0.17-1.4 $\mu\text{g g}^{-1} \text{h}^{-1}$). The capacity for methane oxidation was related to the depth of oxygen penetration. The methane oxidizers were very active in oxidizing methane and the selected trace components down to a depth of 50 cm below the surface. Maximal oxidation activity occurred in a zone between 15 and 20 cm below the surface, as this depth allowed sufficient supply of both methane and oxygen. Mass balance calculations using the maximal oxidation rates obtained demonstrated that landfill soil covers have a significant potential for not only methane oxidation but also cometabolic degradation of selected volatile organics, thereby reducing emissions to the atmosphere.

Schroll, R., F. Brahusi, et al. (2004). "Biomineralisation of 1,2,4-trichlorobenzene in soils by an adapted microbial population." *Environmental Pollution* 127(3): 395-401.

In laboratory experiments the mineralisation of C-14-labelled 1,2,4-trichlorobenzene (1,2,4-TCB) in soils was studied by direct measurement of the evolved (CO₂)-C-14. The degradation capacity of the indigenous microbial population was investigated in an agricultural soil and in a soil from a contaminated site. Very low mineralisation of 1% within 23 days was measured in the agricultural soil. Whereas in the soil from the contaminated site the mineralisation occurred very fast and in high rates; up to 62% of the initially applied amount of 1,2,4-TCB were mineralised within 23 days. The transfer of the adapted microbial population into the agricultural soil significantly enhanced the mineralisation of 1,2,4-TCB in this soil, reflecting, that the transferred microbial population survived and maintained its degradation ability in the new microbial ecosystem. Additional nutrition sources ((NH₄)₂HPO₄) increased the mineralisation rates in the first days significantly in the contaminated soil. In the soil from the contaminated site high amounts of non extractable C-14-residues were formed. (C) 2003 Elsevier Ltd. All rights reserved.

Simpson, J. S., A. Brust, et al. (2004). "Biosynthetic pathways to dichloroimines; precursor incorporation studies on terpene metabolites in the tropical marine sponge *Stylotella aurantium*." *Organic & Biomolecular Chemistry* 2(6): 949-956.

The biosynthetic origin of the dichloroimine functional group in the marine sponge terpene metabolites stylotellanes A (3) and B (4) was probed by the use of [C-14]-labelled precursor experiments. Incubation of the sponge *Stylotella aurantium* with [C-14]-labelled cyanide or thiocyanate resulted in radioactive terpenes in which the radiolabel was shown by hydrolytic chemical degradation to be associated specifically with the dichloroimine carbons. Additionally, label from both precursors was incorporated into farnesyl isothiocyanate (2). A time course experiment with [14C]cyanide revealed that the specific activity for farnesyl isothiocyanate decreases over time, but increases for stylotellane B (4), consistent with the rapid formation of farnesyl isothiocyanate (2) from inorganic precursors followed by a slower conversion to stylotellane B (4). The advanced precursors farnesyl isothiocyanate (2) and farnesyl isocyanide (5) were supplied to *S. aurantium*, and shown to be incorporated efficiently into stylotellane A (3) and B (4). Feeding of [C-14]-farnesyl isothiocyanate (2) resulted in a higher incorporation of label than with [C-14]-farnesyl isocyanide (5). Farnesyl isocyanide was incorporated into farnesyl isothiocyanate in agreement with labelling studies in other marine sponges. Both farnesyl isocyanide and isothiocyanate were further incorporated into axinyssamide A (11) as well as the cyclized dichloroimines (12)-(14), (16) that represent more advanced biosynthetic products of this pathway. These results identify the likely biosynthetic pathway leading to the major metabolites of *S. aurantium*.

Stidson, R. T., C. A. Dickey, et al. (2004). "Fluxes and reservoirs of trichloroacetic acid at a forest and moorland catchment." *Environmental Science & Technology* 38(6): 1639-1647.

The concentrations and input/output fluxes of trichloroacetic acid (TCA) were measured in all relevant media for one year at a 0.86 km² upland conifer plantation and moorland catchment in SW Scotland (n > 380 separate samples analyzed). Annual wet precipitation to the catchment was 2.5 and 0.4 m for rain and cloud, respectively. TCA input to the catchment for the year was 2100 g, predominantly in rainwater ON, with additional input via cloudwater (13%) and gas plus particle dry deposition (1%). There were no seasonal trends in TCA deposition, and cloudwater concentration was not enhanced over rainwater. TCA in precipitation exceeded

concentrations estimated using currently accepted routes of gas-phase oxidation from anthropogenic chlorinated hydrocarbon precursors, in agreement with previous studies. Export of TCA from the catchment in streamwater totalled 1970 g for the year of study. The TCA concentration in streamwater at outflow (median 1.2 µg L⁻¹) was significantly greater than that before the stream had passed through the conifer plantation. To well-within measurement uncertainties, the catchment is currently at steady-state with respect to TCA input/output. The catchment reservoir of TCA was dominated by soils (similar to 90%), with the remainder distributed in forest litter (similar to 9%), forest branchwood and stemwood (similar to 0.7%), forest foliage (similar to 0.5%), and moorland foliage (similar to 0.1%). Although TCA is clearly taken up into foliage, which consequently may be important for the vegetation, this was a relatively minor process for TCA at the catchment scale. If it is assumed, on the basis of laboratory extraction experiments, that only similar to 20% of "whole soil" TCA measured in this work was water extractable, then total mass of TCA in the catchment is reduced from similar to 13 to similar to 3.5 kg. Comparing the latter value with the annual flux yields an average steady-state residence time for TCA in the catchment of similar to 1 similar to 2 y, if all TCA is involved in catchment turnover. Considering that other evidence indicates the lifetime of TCA in soil and biota is considerably shorter than this (weeks rather than years), the magnitude of the TCA reservoir is suggested to be strong evidence for net natural TCA production in soils and/or that the majority of TCA in the reservoir is not involved with external fluxes.

Tartakovsky, B., M. F. Manuel, et al. (2003). "Trichloroethylene degradation in a coupled anaerobic/aerobic reactor oxygenated using hydrogen peroxide." *Environmental Science & Technology* 37(24): 5823-5828.

In this work, trichloroethylene (TCE) degradation under combined anaerobic-aerobic conditions was studied in an ethanol-fed biofilm reactor oxygenated using hydrogen peroxide. The reactor was inoculated with a biomass originating from an anaerobic digester. Granulated peat was added to the reactor as a substratum for biofilm development. Extensive characterization of reactor populations using activity tests and PCR analysis revealed the development of a mutualistic consortium, particularly methanotrophic and methanogenic microorganisms. This consortium was shown to degrade TCE by a combination of reductive and oxidative pathways. A near complete degradation of TCE at a load of 18 mg L⁻¹ day⁻¹ was evidenced by a stoichiometric release of inorganic chloride.

Vogt, C., A. Alfreider, et al. (2004). "Bioremediation of chlorobenzene-contaminated ground water in an in situ reactor mediated by hydrogen peroxide." *Journal of Contaminant Hydrology* 68(1-2): 121-141.

New in situ reactive barrier technologies were tested nearby a local aquifer in Bitterfeld, Saxonia-Anhalt, Germany, which is polluted mainly by chlorobenzene (CB), in concentrations up to 450 µM. A reactor filled with original aquifer sediment was designed for the microbiological remediation of the ground water by indigenous bacterial communities. Two remediation variants were examined: (a) the degradation of CB under anoxic conditions in the presence of nitrate; (b) the degradation of CB under mixed electron acceptor conditions (oxygen + nitrate) using hydrogen peroxide as the oxygen-releasing compound. Under anoxic conditions, no definite degradation of CB was observed. Adding hydrogen peroxide (2.94 mM) and nitrate (2 mM) led to the disappearance of CB (ca. 150 µM) in the lower part of the reactor, accompanied by a strong increase of the number of cultivable aerobic CB degrading bacteria in reactor water and sediment samples, indicating that CB was degraded mainly by productive bacterial metabolism. Several aerobic CB degrading bacteria, mostly belonging to the genera *Pseudomonas* and *Rhodococcus*, were isolated from reactor water and sediments. In laboratory experiments with reactor water, oxygen was rapidly released by hydrogen peroxide, whereas biotic-induced decomposition reactions of hydrogen peroxide were almost four times faster than abiotic-induced decomposition reactions. A clear chemical degradation of CB mediated by hydrogen peroxide was not observed. CB was also completely degraded in the reactor after reducing the hydrogen peroxide concentration to 880 µM. The CB degradation completely collapsed after reducing the hydrogen peroxide concentration to 440 µM. In the following, the hydrogen peroxide concentrations were increased again (to 880 µM, 2.94 mM, and 880 µM, respectively), but the oxygen demand for CB degradation was higher than observed before, indicating a shift in the bacterial population. During the whole experiment, nitrate was uniformly reduced during the flow path in the reactor. (C) 2003 Elsevier B.V. All rights reserved.

Xia, C. H., J. Xu, et al. (2004). "Advance in degradation of polychlorinated dibenzo-p-dioxins/furans." *Progress in Chemistry* 16(1): 123-130.

Polychlorinated dibenzo-p-dioxins/furans (dioxins) is a kind of important persistent organic pollutants (pops) and carcinogen. Dechlorination is an efficient means to eliminate the toxicity of dioxins. A review with 72 references concerning degradation methods was given in this paper. These degradation methods include photodegradation, thermal dechlorination, biodegradation, chemical dechlorination and heterogeneously catalytic hydrodechlorination. The heterogeneously catalytic hydrodechlorination might be developed to an efficient technology to eliminate the toxicity of dioxins.