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

**12th Quarterly Report
4th Quarter Year 2003**

Report prepared for EUROCHLOR

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March 3, 2004

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ACRONYMS

16S rRNA	16S Ribosomal RNA
BPDO	Biphenyl Dioxygenase
CB	Chlorobenzene
CBA	Chlorobenzoic acid
CBp	Chlorobiphenyl
CDDs	Chlorinated Dibenzo- <i>p</i> -Dioxins
CDFs	Chlorinated Dibenzo- <i>p</i> -Furans
CF	Chloroform
CHMs	Chlorinated Hydroquinone Metabolites
COD	Chemical Oxygen Demand
CPO	Chloroperoxidase
CT	Carbon Tetrachloride
2,4-D	2,4-Dichlorophenoxyacetate
1,2-DCA	1,2-Dichloroethane
DCBp	Dichloro Biphenyl
DCE	Dichloroethene
DCM	Dichloromethane
DDT	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane
1,3-DiCB	1,3-Dichlorobenzene
2,7-DiCDD	2,7-Dichlorodibenzo- <i>p</i> -dioxin
DNAPL	Dense Non-Aqueous Phase Liquid
E-acceptor	Electron Acceptor
E-donor	Electron Donor
ERs	Enantiomer Ratios
ETH	Ethylene, ethene
HCB	Hexachlorobenzene
HCH	Hexachlorohexane
LinD	Lindane Dehalogenase
LiP	Lignin Peroxidase
MCBp	Monochloro Biphenyl
MiP	Manganese-Independent Peroxidase
MnP	Manganese Peroxidase
PBDEs	Polybrominated Diphenyl Ethers

ACRONYMS (*Continued*)

PCBs	Polychlorinated Biphenyls
PCBz	Pentachlorobenzene
PCP	Pentachlorophenol
PCE	Tetrachloroethylene
PCR	Polymerase Chain Reaction
PH	Pentachlorophenol Hydroxylase
TBF	Trickling Biofilter
TCA	Trichloroacetic Acid
TCB	Trichlorobenzene
TCBp	Trichloro Biphenyl
2,4,8-TCDF	2,4,8-Trichlorodibenzofuran
TCE	Trichlorethylene
TeCB	Tetrachlorobenzene
TeCBp	Tetrachlorobiphenyl
TeCMP	2,3,5,6-Tetrachloro-4-methoxyphenol
VB	Vinyl Bromide
V-BrPO	Vanadium Bromoperoxidase
VC	Vinyl Chloride
V-CIPO	Vanadium Chloroperoxidase
ZVI	Zero-Valent Iron

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

**12th Quarterly Report
4th Quarter– Year 2003**

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1. INTRODUCTION

This report presents a review of scientific literature published during the last quarter of 2003 (covering November 2003 to January 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 as follows:

- The oxidation of 2-chloroethanol under denitrifying conditions by a pure bacterial culture is reported for the first time (14).
- The anaerobe *Dehalococcoides* sp strain CBDB1 was shown to be capable of halorespiration utilizing hexachlorobenzene (HCB) and pentachlorobenzene (PeCBz) as electron acceptors (*e-acceptors*) with hydrogen as electron donor (*e-donor*) (25). This constitutes the first

report describing reductive dechlorination of HCB and PCBz via halorespiration linked to growth by a pure bacterial culture. Previously, halorespiration of tetrachlorobenzene was known.

- Two reports illustrating that the aerobic TCE and VC cooxidation can be achieved with non-toxic and non-flammable primary substrates (29, 61)

2.b. Microbial Chlorination

The most important highlight for microbial chlorination is one publication reporting on the anaerobic degradation of natural chlorinated hydroquinone metabolites (31). The report demonstrates that there are natural pathways for the dechlorination of natural occurring aromatic organohalogenes. The paper establishes a fundamental role for microbial reductive degradation in the global chlorine cycle.

3. MICROBIAL DECHLORINATION

3.a. General Reviews

In this quarter, 4 review articles on biological dechlorination were published. The first article provides a comprehensive review of xenobiotic compound degradation by white rot fungi (3). The review includes chlorinated pollutants such as pentachlorophenol and trichloroethylene. A second article reviewed information on anaerobic dehalogenation in marine environments, but a copy of the publication was not available for the authors of this report to review (23).

Additionally two review articles were published in the last quarter on the degradation and remediation of higher chlorinated ethenes. One of the publications provides a review on the anaerobic bioremediation of perchloroethylene contaminated sites (9). The review discusses the role of steady state hydrogen levels to control the competition between halorespiring bacteria and methanogens for electron donating substrates applied to contaminated sites to promote dehalogenation. The second publication reviews current knowledge on the microbial degradation of trichloroethylene, with an emphasis on aerobic cooxidation (48).

3.b. Microbial Dechlorination

Vinyl chloride and Other Chlorinated Ethenes

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

Vinyl Chloride (VC) and Dichloroethenes (DCE). In this quarter, there was only one study which directly investigated the biodegradation of lower chlorinated ethenes as a parent compound. Vinyl chloride (VC) cooxidation by a propane-oxidizing strain, *Rhodococcus rhodochrous*, was studied using non-flammable degradation products of propane, acetone and isopropanol as the primary substrates (29). The study demonstrates that isopropanol and acetone can induce the enzymes in *R. rhodochrous* that degrade VC. Cells of *R. rhodochrous* can also be grown on rich microbiological media and, subsequently, the harvested cells can be induced to carry out VC cooxidation with isopropanol or acetone.

Perchloroethylene (PCE) and Trichloroethene (TCE). In this quarter, there were 13 publications reporting on either PCE or TCE microbial degradation. Two of the papers were review articles (9, 48) already discussed above in Section “3.a. General Reviews”. Four of the articles provide mathematical tools to better model PCE or TCE remediation at contaminated sites (7, 10, 13, 54). These will be discussed below in the Section “3.d. New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds”. Two of the articles evaluate the use of metalloporphyrin enzyme cofactors to directly reduce chlorinated ethenes (15, 51). These will also be discussed below in Section “3.c. In Vitro Degradation of Chlorinated Compounds”. One article reports on the development of primers for the detection of dehalogenase genes (42) and is discussed in Section “3.d. New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds”.

Of the remaining articles, one was concerned with the aerobic degradation of TCE. The potential of non-aromatic substrates to support TCE cooxidation in five strains of toluene oxidizing bacteria was investigated (61). Fructose was found to support TCE cooxidation if TCE was used to induce oxidative enzymes. In particular, cells of *Pseudomonas mendocina* KR1

degraded significant amounts of TCE during cell growth on non- aromatic substrates. The other three research articles on higher chlorinated ethenes from this quarter covered different aspects of anaerobic degradation or bioremediation. The first anaerobic paper evaluated the feasibility of enhancing PCE biodegradation using cane molasses and sludge cakes as the electron-donating substrates under methanogenic and iron reducing conditions (27). Based on the chemical oxygen demand (COD) tests, approximately 28 and 248 mg of biodegradable COD can be released from 1 g of sludge cake and 1 g of cane molasses, which theoretically could convert 70 and 620 mg of PCE to ethylene (ETH), respectively. Microcosms established with aquifer sediments and PCE were incubated with sludge cake and molasses and reductive dechlorination of PCE was enhanced under methanogenic conditions. Rates of PCE degradation of 0.025 to 0.091 $\mu\text{M g}^{-1}$ sediment d^{-1} were observed with various sediment samples. The second anaerobic paper evaluated the potential of ethanol as an e-donor to bioremediate residual dense non-aqueous phase liquids (DNAPLs) after DNAPL flushing with an aqueous solution containing 95% ethanol (32). The ethanol flushing with 34,000 L removed 60% of the PCE at a field site. 2720 L of residual ethanol was left in the subsurface, which provided e-donor for enhancement of biological processes in the source zone and down-gradient areas. Groundwater monitoring for over 3 years showed decreasing concentrations of PCE in the source zone from initial values of 4-350 μM to less than 150 μM during the last sampling event. Initially there was little to no daughter product formation in the source zone, but after 3 years, measured concentrations were 242 μM for *cis*-dichloroethylene (*cis*-DCE), 13 μM for VC, and 0.43 μM for ethene. First-order rate constants based on the change in total mass estimated from contour plots of the groundwater concentration data were 0.75 y^{-1} for *cis*-DCE formation and -0.50 y^{-1} for PCE removal. Taken as a whole, the results show that combined solvent flushing and bioremediation is a promising technique for treating PCE source zones. The last anaerobic paper describes the field remediation of a petroleum hydro-carbon, TCE and metal contaminated site in London (16). Chemical analyses of groundwater samples show elevated aqueous concentrations of chloroethenes with a classical reduction pathway for TCE leading to an accumulation of VC. The possibility the petroleum hydro-carbons at the site can be used as e-donors to support TCE dechlorination is being investigated.

Carbon Tetrachloride (CT) and Chloroform (CF)

Two reports were found during the period of review on the microbial degradation of carbon tetrachloride (CT) (19) (60). The effect of zero-valent iron (ZVI, Fe^0) on the efficiency of a

combined microbial-ZVI treatment process was investigated in batch assays (19). Cathodic H₂ generated by ZVI was used as e-donor for reductive biotransformation of CT. Increasing ZVI dosage, and thus the available surface area, had a stimulatory effect possibly due to the increased production of cathodic H₂. However, high ZVI doses had an inhibitory effect due to a corrosion-induced increase in pH beyond the optimum range of the bacteria. The authors conclude that microbial activity might enhance the performance of permeable reactive iron barriers.

Shewanella oneidensis MR-1 was shown to produce two different factors capable of dehalogenating CT (60). A derivative of the menaquinone precursor, 1,4-dihydroxy-2-naphthoic acid, was found to be the factor involved in the reductive transformation of CT by aerobically grown *S. oneidensis*. The factor produced under anaerobic growth conditions was not identified, but it was shown to not be a product of the menaquinone biosynthetic pathway.

Chloromethane (CM) and Dichloromethane (DCM)

No reports concerning the microbial dechlorination of chloromethane and dichloromethane were found during the review period.

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

Three studies tested the microbial degradation of 1,2-DCA this quarter (11, 17, 52). In addition, one study examined the degradation of 2-chloroethanol and chloroacetate under aerobic and denitrifying conditions (14).

Eleven bacterial strains were screened for their ability to degrade 1,2-dichloroethane (1,2-DCA), 2,3-dichloropropionic acid, and 2-monochloroacetic acid by hydrolytic dechlorination under aerobic conditions (52). All of the isolated strains degraded MCA. Only two strains were able to degrade 1,2-DCA, and one strain degraded 2,3-dichloropropionic acid. Based on 16S rDNA sequence information, eight different species were identified, including *Pseudomonas plecoglossicida*, *Xanthobacter flavus*, *Ralstonia eutropha*.

Conversion of 1,2-DCA (40 mg l⁻¹) in groundwater to ethene was demonstrated in lab-scale experiments with *Desulfitobacterium dichloroeliminans* strain DCA1 (11). The DCA1 strain was shown to compete for nutrients in the presence of fast-growing *Enterococcus faecalis*, a microorganism detected in the enrichment culture from which strain DCA1 was isolated. The maximum 1,2-DCA dechlorination rate exceeded 350 nmol Cl⁻ released min⁻¹ mg⁻¹ bacterial protein.

The feasibility of treating 1,2-DCA in contaminated groundwater via reductive dechlorination by injection of an aqueous solution of methanol, ammonium chloride and sodium chloride was investigated in a field study (17). Nutrients were supplemented into the confined aquifer using an array of eight boreholes. Analyses of 1,2-DCA and degradation products provided evidence for the reductive dechlorination of the parent compound. Biodegradation of 1,2-DCA appeared to be localized within the lowest depth of the confined aquifer (31–32 m depth). The main degradation products detected were methane and ethene. For other sampling depths, in-situ biodegradation was either absent (i.e. 27–28 m depth) or unclear (i.e. 23–24 m depth). This was attributed to limited mixing of the carbon substrate within the test zone. In addition, clogging of recharge wells complicated groundwater circulation.

The bacterium tentatively identified as *Pseudomonas stutzeri* strain JJ was shown to utilize 2-chloroethanol and chloroacetate as sole carbon and energy source with nitrate (NO_3^-) or oxygen (O_2) as electron acceptor (14). This is the first report of oxidation of 2-chloroethanol under denitrifying conditions by a pure bacterial culture. With nitrate as e-acceptor, optimum growth occurred at 30°C with a μ_{max} of 0.14 h^{-1} and a yield of 4.4 g protein per mol 2-chloroethanol metabolized. Under aerobic conditions, the μ_{max} was 0.31 h^{-1} . Nitrite (NO_2^-) also served as e-acceptor, but not Fe(III), Mn(IV), sulfate (SO_4^{2-}), fumarate or ClO_3^- . Additional experiments with various bacterial strains, including some closely related *Pseudomonas stutzeri* strains, showed that *Pseudomonas stutzeri* strain LMD 76.42, *Pseudomonas putida* US2 and *Xanthobacter autotrophicus* GJ10, grew on 2-chloroethanol aerobically, but not under denitrifying conditions.

Chlorobenzenes (CB)

Three reports were found regarding the microbial degradation of several chlorobenzene compounds, namely chlorobenzene (59), 1,2,4-trichlorobenzene (47), pentachlorobenzene (25), and hexachlorobenzene (25).

Microbial degradation of chlorobenzene by five bacterial strains (*Acidovorax facilis* 13517, *Cellulomonas turbata* B529, *Pseudomonas veronii* 13547, *Pseudomonas veronii* B549, and *Paenibacillus polymyxa* B550) under oxygen-limited conditions was found to lead to accumulation of 3-chlorocatechol in the presence and absence of nitrate (1 mM) (59). The presence of nitrate did not influence the biological conversion pattern.

Biomineralisation of C^{14} -labelled 1,2,4-trichlorobenzene (1,2,4-TCB) in an agricultural soil and in a soil from a contaminated site was investigated (47). No significant degradation of the

trichlorobenzene was recorded in the agricultural soil (1% removed after 23 days). In contrast, 1,2,4-TCB was readily degraded by the indigenous microorganisms which had been previously exposed to chloroaromatic pollutants in the contaminated soil. Up to 62% of the initially applied amount of 1,2,4-TCB was mineralised by the adapted microbial population within 23 days. Formation of high amounts of non extractable C-14-residues was observed in the contaminated soil. Nutrient supplementation ((NH₄)₂HPO₄) lead to considerable enhancement of the mineralisation rates in the contaminated soil.

The anaerobe *Dehalococcoides* sp strain CBDB1 was shown to be capable of halo-respiration utilizing hexachlorobenzene (HCB) and pentachlorobenzene (PeCBz) as e-acceptors with hydrogen as e-donor (25). This is the first report describing reductive dechlorination of HCB and PCBz via dehalorespiration by a pure bacterial culture. The growth yield of strain CBDB1 by dehalorespiration with HCB and PCBz was 2.1 ±0.24 and 2.90±0.15 g mol⁻¹ Cl⁻, respectively. These growth yields are similar to those described previously for other bacteria growing by dehalorespiration. The proposed pathway of HCB and PCB reductive dechlorination by *Dehalococcoides* sp. strain CBDB1 is illustrated in Figure 1. HCB was reductively dechlorinated to PCB, which was converted to a mixture of 1,2,3,5- and 1,2,4,5-tetrachlorobenzene (TeCB). The final end-products of HCB and PCB dechlorination were 1,3,5-TCB, 1,3- and 1,4-dichlorobenzene (DiCB), which were formed in a ratio of about 3:2:5. The microorganism utilized two different pathways to dechlorinate highly chlorinated benzenes. Strain CBDB1 converted 1,2,3,5-TeCB to 1,3,5-TCB through the removal of doubly flanked chlorine substituents, and 1,2,4,5-TeCB to 1,2,4-TCB by removing singly flanked chlorine substituents, as reported previously (*Adrian et al. 2000. Bacterial dehalorespiration with chlorinated benzenes. Nature 408:580-583*). The specific reductive dehalogenase activity of strain CBDB1 towards HCB was highest in cultures grown with HCB, while the specific activity towards 1,2,3,4-TeCB was three to five times higher in cultures grown with PCBz or HCB than with 1,2,3-TCB. This suggests that different dehalogenase activities might be induced by different chlorobenzene congeners.

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

In this quarter, two studies report on the fungal degradation of chlorinated dibenzo-*p*-dioxins (CDDs) (53) (30). The white rot fungus *Bjerkandera* sp. strain MS325 was shown to be capable of degrading tetrachlorodibenzo-*p*-dioxin (30). Production of extracellular peroxidases, namely, lignin peroxidase (LiP), manganese peroxidase (MnP), and manganese-independent per-

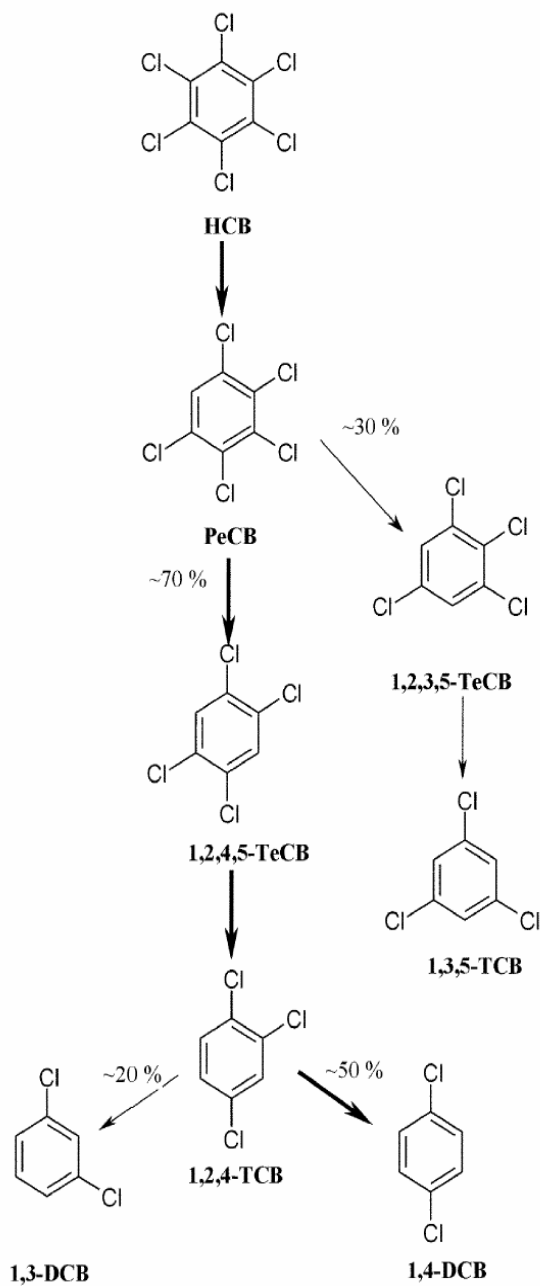


Figure 1. Proposed pathway of hexachlorobenzene and pentachlorobenzene reductive dechlorination by *Dehalococcoides* sp. strain CBDB1. The values indicate the relative amounts of product formation and are related to the total amount of products detected. Other reactions are not catalyzed by strain CBDB1, as determined using the different chlorobenzene congeners separately. **Bold arrows:** Major dechlorination pathway (ref. 25).

oxidase (MiP), under various conditions by the MS325 strain and 37 other MnP-producing white rot strains was compared with investigated. The *Bjerkandera* sp. strain was among the best producers of the three different peroxidases. High LiP and MnP activities were detected for this strain under various conditions, e.g., nutrient nitrogen-sufficient or -limited conditions, conditions with or without Mn(II), and changes in temperature (15-37°C). Based on these results, the authors conclude that *Bjerkandera* sp. strain MS325 is an interesting microorganism for application in the remediation of CDD-contaminated soils.

A PCR-based assay was developed to monitor the fungal strain, *Ceriporia* sp. MZ-340, at a CDD-bioremediation site (53). The white rot fungal strain, which was isolated from white rotten wood, was shown to have a high ability to degrade dioxin. The species-specific assay developed permitted detection of the fungus in contaminated soil at concentrations of only 2 mg g⁻¹ mycelium.

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, four publications reported on the microbial degradation of PCBs. Three of these publications address aerobic degradation of PCBs (1, 28, 45). Enzymatic attack of chlorobiphenyl metabolites formed from PCBs by plants (20) will be discussed in section “3.c. *In Vitro* Degradation of Chlorinated Compounds”.

A new aerobic gram-positive bacterium with a unique ability to degrade *ortho*- and *para*-chlorinated biphenyls was isolated from contaminated soil (45). The strain, identified as a *Microbacterium* sp. B51, could degrade *ortho*- and *para*-substituted mono-, di-, and trichlorinated biphenyls (MCBp, DCBp, and TCBp, respectively). Unlike the known PCB degraders, *Microbacterium* sp. B51 is able to oxidize the *ortho*-chlorinated ring of 2,2'-DCBp and 2,4'-DCBp and the *para*-chlorinated ring of 4,4'-DCBp. 2,4'-DCBp and 4,4'-DCBp were transformed to 4-chlorobenzoic acid (4-CBA). The strain was able to utilize 2-MCBp, 2,2'-DCBp, and their intermediate 2-CBA and to oxidize the mono(*ortho*)-chlorinated ring of 2,4,2'-TCBp and the di(*ortho-para*)-chlorinated ring of 2,4,4'-TCBp. A co-culture of *Microbacterium*

sp. B51 and the 4-CBA-degrading bacterium *Arthrobacter* sp. H5 utilized 2,4'-DCB (1 g l^{-1}) as the sole source of carbon and energy.

Ralstonia eutropha H850 was labeled chromosomally with a *gfp* marker gene encoding for the green fluorescent protein to facilitate monitoring of the bacterium in soil microcosm experiments spiked with 2,2',5,5'-tetrachlorobiphenyl (TeCBp) (1). The modified bacterium was detected by viable plate counts and most-probable-number/PCR after 102 days in TeCBp-contaminated soil microcosms, but did not degraded TeCBp over a 102 day period.

Plant terpenes were shown to induce expression of multiple aromatic ring hydroxylation oxygenase genes in *Rhodococcus* sp strain T104 (28). The three distinct genes for aromatic oxygenase were found to be putatively involved in the degradation of aromatic substrates including biphenyl, limonene, and phenol. The genes were differentially expressed and well induced by limonene, cymene, and a terpenic plant extract compared to biphenyl and/or glucose.

Miscellaneous Chlorinated Compounds

The search query used is specifically designed to review literature on the target compounds listed in the *Introduction* section. Interesting publications concerned with compounds outside of the range list which are found in the search process are briefly discussed below. This quarter our search retrieved 10 reports on the biodegradation of miscellaneous chlorinated pollutants, including, DDT (26), hexachlorocyclohexane (35, 44), endosulfan (49), tetrabromobisphenol-A (2), 3,5,6-trichloro-2-pyridinol (18), halosubstituted benzyl alcohols (22), and chlorophenols (12, 33).

A bacterial strain, identified as a *Pseudomonas* species, was isolated from insecticide-contaminated soil (26). The strain could transform 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) into 2,3-dihydroxy-DDT, which undergoes *meta*-ring cleavage, ultimately yielding 4-chlorobenzoic acid.

The fate of hexachlorocyclohexane (HCH, lindane) in heavily contaminated soil after 8 years of remediation by liming was investigated (35). Lime treatment promoted HCH dechlorination leading to the formation of metabolites of increased mobility. Nonetheless, HCH remained largely in the soil. Microcosm studies showed that the alkaline pH conditions resulting from liming caused inhibition of microbial respiration. In the absence of liming, addition of HCH led to a temporary increase in soil respiration, and stimulation occurred with oxygen and/or nutrient addition. The authors conclude that bioremediation rather than chemical treatments with lime should be the preferred option for the clean up of the contaminated soil. In a second study

concerning HCH, the stability of bacterial populations in tropical soil amended with 100 mg HCH kg⁻¹ was evaluated by monitoring bacterial cell concentrations, metabolic versatility, and genetic diversity (16S rDNA/denaturing gradient gel electrophoresis) (44). HCH persisted in the soil environment (30% elimination in 70 days). Overall, no effect of HCH was observed on the metabolic versatility and genetic diversity in these soils, although bacterial cell counts decreased by 50%.

Complete disappearance of both α - and β -endosulfan (100 mg l⁻¹) was observed during incubation with *Fusarium ventricosum* and a *Pandora* species (49). Both fungal and bacterial strains formed less toxic endosulfan diol and endosulfan ether as metabolites during metabolism of endosulfan. Biodegradation kinetics of the organochlorine pesticide by the both microorganisms were studied (49).

Enrichment of an anaerobic microbial culture capable of reductive debromination of the flame retardant tetrabromobisphenol-A, and identification of the intermediate metabolites produced, was reported (2). Three intermediate products found to accumulate in the assays were tentatively identified as tri-, di-, and mono-brominated bisphenol-A. Additional results suggest that the culture utilized requires some organic components in order to sustain debromination.

Aerobic biodegradation of 3,5,6-trichloro-2-pyridinol, a primary degradation product of the insecticide chlorpyrifos and the herbicide triclopyr, by a newly isolated bacterium, *Pseudomonas* sp. strain ATCC 700113, was reported (18). The strain was capable of using 3,5,6-trichloro-2-pyridinol as a sole source of carbon and energy, leading to the formation of CO₂, chloride, and unidentified polar metabolites.

The effect of the type and position of the halogen atom in the aromatic ring on the degradation rate of halosubstituted benzyl alcohols was examined (22). Biodegradation results of 2-halosubstituted benzyl alcohols correlate with the size of the halogen atom, while for 4-halosubstituted benzyl alcohols, a good correlation with the energy of C-X bond was observed.

Biodegradation and bioremediation of pentachlorophenol (PCP) were reviewed by Dercova *et al.* (2003) (12). Several chlorophenols including, PCP, 2,3,5,6-tetrachlorophenol and 2,4,6-trichlorophenol, were shown to be degraded by mixed and pure cultures (33).

Cloning, expression, and characterization of a *cis*-3-chloroacrylic acid dehalogenase from coryneform bacterium strain FG41 was accomplished (39). The dehalogenase was classified as member of a new family of enzymes within the 4-oxalocrotonate tautomerase family.

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

Two papers this quarter evaluate metal metalloporphyrin enzyme cofactors for the dehalogenation of chlorinated compounds (15, 51). The first of these evaluated several metalloporphyrins and found that their solubility was an important determinant in their ability to behave as a catalyst in reductive PCE dechlorination (15). The second paper evaluated isotopic fractionation during vitamin B12 catalyzed reduction of PCE and TCE (51). In laboratory experiments, 10 mg/L vitamin B12 degraded >90% of the initial 20 mg/L PCE with TCE, the primary product of PCE degradation, accounting for between 64% and 72% of the PCE degraded. In experiments with TCE, 147 mg/L vitamin B12 degraded >90% of the initial 20 mg/L TCE with cis-DCE, the primary product of degradation accounting for between 30% and 35% of the TCE degraded. Degradation of both PCE and TCE exhibited first-order kinetics. Strong isotopic fractionation of the reactant PCE and of the reactant TCE was observed over the course of degradation.

Biphenyl dioxygenases (BPDO) from *Burkholderia* sp LB400 and *Comamonas testosteroni* B-356 were shown to catalyze oxygenation of *ortho*-substituted chlorinated hydroxybiphenyls by biphenyl dioxygenase (BPDO) in vitro (20). *Ortho*-substituted chlorinated hydroxybiphenyls are degradation products of PCB metabolism. The chlorine substitutions in these compounds are on the hydroxyl-substituted ring. Oxygenation of the chloro-hydroxybiphenyls tested was observed exclusively on the non-substituted ring and proceeded via *ortho*-meta oxygenation.

Four additional publications were concerned with the characterization of dehalogenase enzymes, specifically, haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26 (8, 34, 40) and halohydrin dehalogenase from *Agrobacterium radiobacter* (56). Haloalkane dehalogenases are bacterial enzymes capable of carbon-halogen bond cleavage in halogenated compounds. The activity and specificity of haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26 was modified by site-directed mutagenesis of its entrance tunnel (8). Purified protein variants were kinetically characterized by determination of specific activities with 12 halogenated substrates and steady-state kinetic parameters with two substrates. In the second study, the crystal structure of the haloalkane dehalogenase LinB from *S. paucimobilis* UT26 was presented at 0.95 Å resolution (34). Structural data, molecular dynamics simulations and quantum mechanics calculations indicated that the positions of the catalytic residues and charge state of the catalytic base are important for determining reaction energetics in LinB. In the third study, the kinetics of the conversion of the substrates 1-chlorohexane,

chlorocyclohexane, and bromocyclohexane by LinB from strain UT26 were studied (40). The ultimate objective of the study was to study the catalytic mechanism of LinB. A mechanism was proposed that consist of three main steps: *i*) substrate binding; *ii*) cleavage of the carbon-halogen bond with simultaneous formation of an alkyl-enzyme intermediate; and *iii*) hydrolysis of the alkyl-enzyme intermediate. A similar mode of action was also observed for the haloalkane dehalogenase Dh1A from *Xantobacter autotrophicus* GJ10 and the haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous* NCIMB 13064. Different rate-limiting steps were detected for three enzymes, *i.e.*, hydrolysis of the alkyl-enzyme intermediate in LinB, halide release in Dh1A, and liberation of an alcohol in DhaA, which were attributed to specific features in the protein structures. In the last publication, halohydrin dehalogenase (HheC) from *Agrobacterium radiobacter* was investigated (56). The fluorescence properties of the enzyme are strongly influenced by halide binding. Tryptophan residues (W139, W192, W238, and W249) in the enzyme were individually mutated to a phenylalanine to examine their role in halide binding and catalysis. All mutations, except for W238F, influenced the enzymatic properties. A number of alterations in the fluorescence were noted and the significance was discussed in relation to the enzyme's properties.

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

Stable Isotope Fractionation

The application of stable isotopic analysis as a tool to investigate and monitor the sources and fates of contaminant compounds in the environment was reviewed in two recent papers (50) (46). These papers discuss reported results, including numerous studies concerned with chlorinated hydrocarbons such as CT, TCE, and PCBs. Additionally, isotopic fractionation during the vitamin B12 catalyzed reduction of PCE and TCE was discussed previously under heading 3.c. *In Vitro Degradation of Chlorinated Compounds* (51).

Age-dating techniques

The application of chlorofluorocarbon and tritium as marker for estimating the minimum age of a chlorinated solvent plume in groundwater was examined (36). Chlorofluorocarbon and tritium are anthropogenic atmospheric pollutants that recharge the groundwater through precipitation.

Analytical methods

Analytical methods for the isolation and identification of intermediates from the biodegradation of low chlorinated PCBs (Delor-103) were developed that permitted to obtain chlorobiphenyls and chlorobiphenyldiols as an almost pure fraction (58). Two new PCB metabolites, dihydrodihydroxytrichloro- and tetrahydrodihydroxytrichloro-biphenyl, were identified.

Models for bioremediation

Several studies this quarter propose new methods of modeling or mapping bioremediation of sites contaminated with chlorinated ethenes. The first publication provides an analytical solution to the network of reaction involved in PCE reductive dechlorination to ethane (54). The solution can be plugged into existing chlorinated solvent remediation model, Biochlor. A second paper proposes new graphical curve methods to estimate kinetic parameters for the reductive dechlorination of PCE and its daughter products (10). The estimated kinetic parameters were validated with published experimental data. A third paper describes an innovative radial diagram approach is applied to illustrate natural attenuation trends for BTEX and chlorinated ethenes at a former fire training area at Plattsburgh Air Force Base, New York (7). The radial diagram map suggests that there is a spatial correlation between decreasing TCE parent compound concentrations and increasing or stable daughter product concentrations. This provides secondary evidence of intrinsic biodegradation of TCE down-gradient from the source area. The last article describes a model for estimating PCE and TCE in indoor air above a contaminated chlorinated solvent plume (13).

4. MICROBIAL CHLORINATION

4.a. General Reviews

Various aspects of marine natural products, among which a wide array of halogenated metabolites are found, were reviewed in three different papers published in the 2004 issue of *Natural Products Reports* (4-6).

Blunt and coworkers (4) published a review of marine natural products that illustrates the wealth and diversity of halogenated metabolites produced by marine organisms. The review covers the literature published in 2002 for marine natural products, with 579 citations referring to 677 new compounds isolated from marine microorganisms and phytoplankton, green algae,

brown algae, red algae, sponges, coelenterates, bryozoans, molluscs, tunicates and echinoderms. Brominated secondary metabolites are especially frequently encountered; these include simple molecules such as bromoform, dibromomethane that are synthesized on a large scale by some aquatic species. In addition, a substantial diversity of halogenated marine natural products has been described, including some chlorinated derivatives (Figure 2). Novel organochlorine compounds have been identified in sponges (macrolides with chlorocyclopropyl side chain – cited refs. 253–255–), red alga (chloro-bromo-monoterpenes and sesquiterpenes – cited refs. 159-167, 170–), and cyanobacteria (hectochlorin and a dichlorinated thiazole macrolide – cited refs. 87 and 102–). A wide variety of new brominated compounds were also isolated from marine organisms, e.g., from red alga (brominated diterpenes – cited ref. 163–), brominated

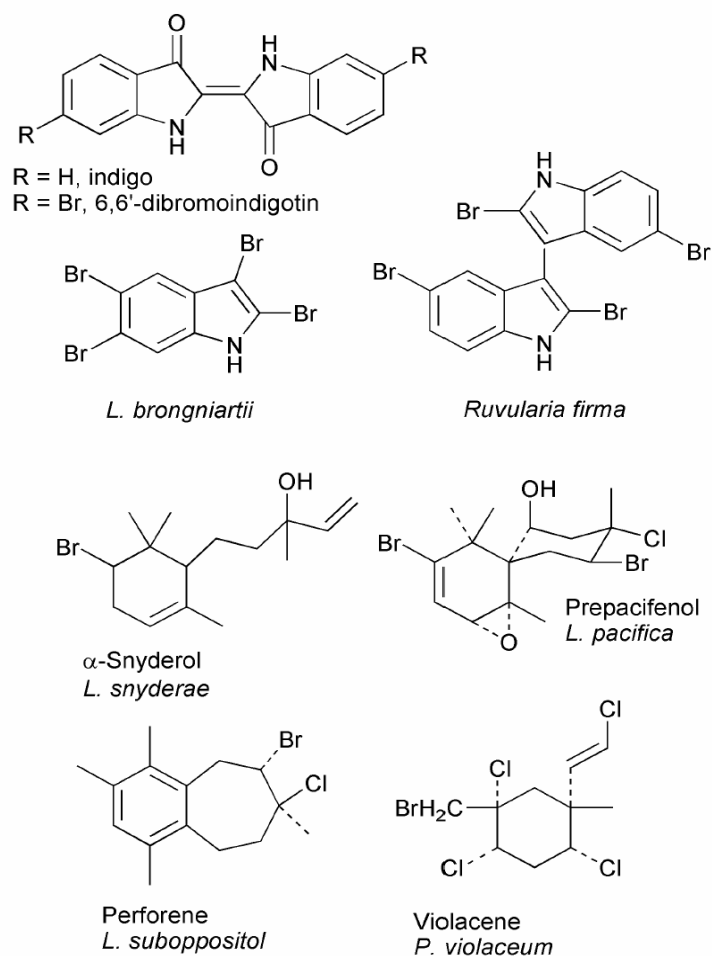


Figure 2. Examples of halogenated marine natural products (Ref. 6).

sesquiterpenes –cited refs. 168, 171–, a chlorinated C15 acetogenin –cited ref. 163–; C15 acetogenins containing a terminal bromoallene moiety –cited ref. 168–, and a bromoindol –cited ref. 182–); from green algae (prenylated bromohydroquinones –cited ref. 134–); from sponges (bromoindols –cited refs. 286, 335–; bromopyrroles –cited refs. 310-314, 326–); and from bryozoans (several bromoalkaloids –cited refs. 453, 457, 459–). Two review papers, one on bromo- and iodo-containing alkaloids from marine microorganisms and sponges (cited ref. 20), the other on is also cited in the natural halogenated fatty acids (cited ref. 17) are also listed in the paper.

The report by Bugni & Ireland (2004) (5) reviews the structures and biological activities of secondary from marine-derived fungi. Several new chlorinated organics were isolated from sponge-derived fungi (chlorinated sesquiterpenes –cited ref. 63–; chlorinated polyketides –cited ref. 64–) and other marine fungal isolates (chlorogentisylquinone –cited ref. 126–).

The role of vanadium bromoperoxidase in the biosynthesis of halogenated marine natural products was reviewed in a third paper covering relevant literature in the period 1998-2003 (6). Vanadium haloperoxidases catalyze the oxidation of halides (iodide, bromide, chloride) by hydrogen peroxide (Figure 3). Vanadium haloperoxidases are classified according to the most electronegative halogen oxidized. Thus vanadium chloroperoxidases (V-ClPOs) can oxidize chloride, bromide and iodide, while vanadium bromoperoxidases (V-BrPOs) can oxidize bromide and iodide. V-ClPOs have been isolated primarily from dematiaceous hyphomycete fungi, and have yet to be isolated from marine organisms, whereas V-BrPO has been isolated and characterized from all the different classes of marine algae. The paper reviews the structure and catalytic activity of vanadium haloperoxidases, including the reactivity of these enzymes with indole substrates and organic sulfides. In addition, the authors review bromination and cyclization reactions of terpene substrates that are catalyzed by V-BrPO and the role of these

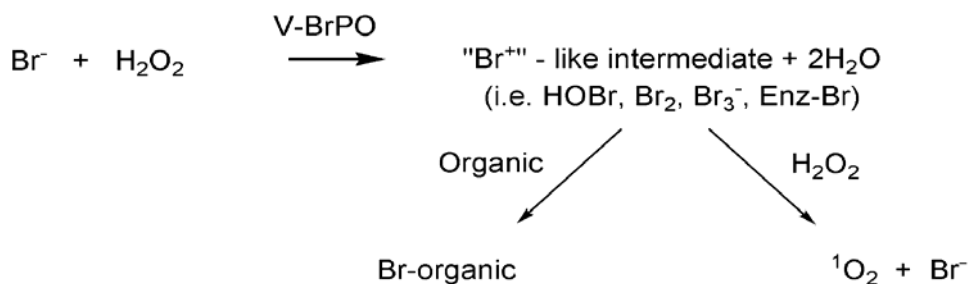


Figure 3. Overall reaction scheme of vanadium haloperoxidases (Ref. 6).

enzymes on the biogenesis of halogenated natural products by marine alga. Halogenated natural products are frequently reported metabolites in marine seaweeds. These compounds span a range from halogenated indoles, terpenes, acetogenins, phenols, *etc.*, to volatile halogenated hydrocarbons that are produced on a very large scale.

4.b. Microbial Chlorination in the Environment

Chloromethanes

No reports were found during the review period concerning the microbial formation of chloromethane. Physiological and biochemical aspects of methyl halide production in rice plants were examined by Redeker *et al.* (2004) (41).

Chlorinated Compounds

Only one publication was found during this quarter on the formation of organohalogen, other than methyl halides, in the terrestrial environment. The genes required for biosynthesis of the dichloroacetyl component of chloramphenicol in *Streptomyces venezuelae* ISP5230 were characterized (38).

An interesting study examined the biodegradation of chlorinated hydroquinone metabolites (CHMs) synthesized by basidiomycete fungi by anaerobic dehalogenating bacteria (31). CHMs were shown to be subject to anaerobic demethylation and dechlorination by indigenous microbial populations in sediments and by several dehalogenating desulfitobacteria. In sediment microcosms, the CHMs 2,3,5,6-tetrachloro-1,4-dimethoxybenzene and 2,3,5,6-tetrachloro-4-methoxyphenol (TeCMP) were anaerobically demethylated to tetrachlorohydroquinone (TCHQ). Subsequently, TCHQ was converted to trichlorohydroquinone and 2,5-dichlorohydroquinone (2,5-DCHQ) in freshwater and estuarine enrichment cultures. *Desulfitobacterium hafniense* strains DCB2 and PCP1, *D. chlororespirans* strain Co23, and *D. dehalogenans* JW/DU1 completely dechlorinated TeCMP to 1,4-dihydroquinone (HQ). Degradation pathways of CHMs by anaerobic enrichment cultures and axenic cultures of desulfitobacteria in relation to aerobic degradation of TCHQ and lindane are compared in Figure 4. This is the first report on the anaerobic degradation of fungal CHMs, The report establishes a fundamental role for microbial reductive degradation of natural organochlorine compounds in the global halogen cycle.

Chlorinated Natural Organic Matter

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

4.c. Chlorination by Marine and Freshwater Organisms

Chloromethanes

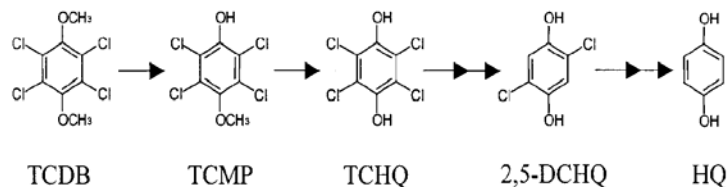
This quarter there was only one report on the natural formation of chloromethanes and related methylhalides by marine microorganisms. Two strains of iodine-producing bacteria, both closely related to *Roseovarius tolerans*, were isolated from marine samples (21). Both strains produced free iodine and organic iodine (chiefly CH_2I_2 , CHI_3 and CH_2CII) from iodide. Cells were necessary for production of CH_2I_2 and CH_2CII . In contrast, CHI_3 was produced by spent media with H_2O_2 or free iodine.

Other Chlorinated Compounds

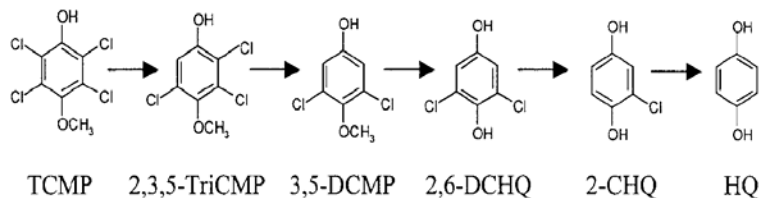
Several studies report on the identification and characterization of new halogenated metabolites from a freshwater alga (57), and from diverse marine organisms, including: fungi (24), bryozoans (37), sponges (43), and corals (55).

The unicellular soil-freshwater alga *Chlamydomonas reinhardtii* secretes compounds that mimic bacterial signals and interfere with quorum sensing regulation in bacteria (57). The compounds secreted by the alga were shown to be halogenated furanones. The known organohalogen dechlorogriseofulvin was isolated from the fungus *Penicillium* sp. derived from the Mediterranean sponge *Axinella verrucosa* (24). Ten brominated alkaloids were isolated from the North Sea bryozoan *Flustra foliacea* (37). Several of these compounds were shown to display antibacterial activity. Chagosensine, a new chlorinated macrolide, was isolated from the Red Sea sponge *Leucetta chagosensis* (43). Its structure was elucidated on the basis of spectroscopic data. Juncin N, a new chlorinated briarane-type diterpenoid, was isolated from the gorgonian coral *Junceella juncea* (55). The structure of the new compound was elucidated by spectral data analysis.

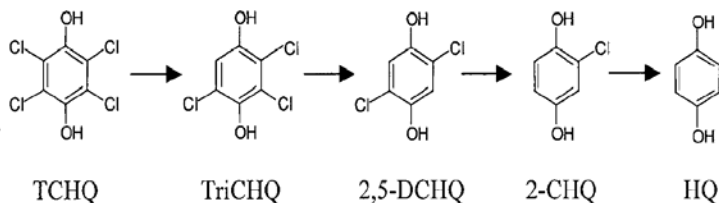
A: Biodegradation of CHMs by anaerobic enrichment cultures (this study).



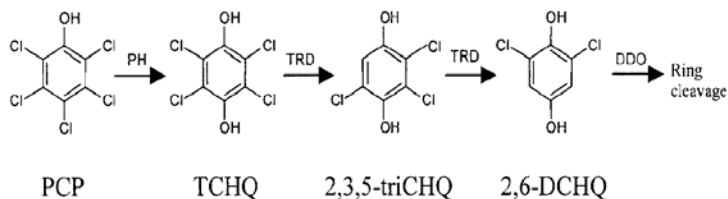
B: Anaerobic biodegradation of TCMP by desulfitobacteria (this study).



C: Anaerobic dechlorination of TCHQ by desulfitobacteria (this study).



D: Aerobic biodegradation of TCHQ (7).



E: Aerobic biodegradation of lindane (<http://umbbd.ahc.umn.edu>, 24).

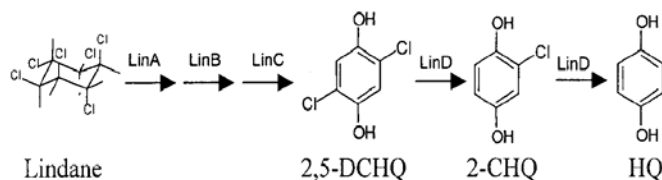


Figure 4. Degradation pathways of chlorinated hydroquinone metabolites (CHMs) by anaerobic enrichment cultures and axenic cultures of desulfitobacteria in relation to aerobic degradation of TCHQ and lindane. PCP is aerobically degraded by a series of enzymes including pentachlorophenol hydroxylase (PH), TRD, and dichlorohydroquinone dioxygenase. Lindane is degraded aerobically by a series of proteins (LinA, -B, -C, and -D), including the 2,5-DCHQ reductive dehalogenase (LinD). HQ, 1,4-dihydroquinone; 2,6-DCHQ, 2,6-dichlorohydroquinone; 2-CHQ, 2-chlorohydroquinone (Ref. 31).

4.d. Chlorinating Enzymes

Except for the aforementioned review article on vanadium bromoperoxidases (6), there were no other articles on the enzymatic formation of organohalogenes this quarter.

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6. ANNEX

Abbey, A. M. I., L. A. Beaudette, et al. (2003). "Polychlorinated biphenyl (PCB) degradation and persistence of a gfp-marked *Ralstonia eutropha* H850 in PCB-contaminated soil." *Applied Microbiology and Biotechnology* 63(2): 222-230.

Ralstonia eutropha H850 was labelled chromosomally with a gfp marker gene encoding for the green fluorescent protein, and designated *R. eutropha* H850g13. Visual observation of green fluorescent cells under an epifluorescence microscope, and PCR amplification products, confirmed that the bacterium was labelled with gfp. Southern blot hybridization products further confirmed the gfp was chromosomally labelled. Using resting cell assays, it was determined that insertion of the gfp gene decreased the microorganisms' ability to degrade biphenyl compared to the parent strain. However, this marker facilitated the identification and monitoring of *R. eutropha* H850g13 survival in soil microcosm experiments. Survival and polychlorinated biphenyl degradation by *R. eutropha* H850g13 was analysed in soil microcosms spiked with 2,2',5,5'-tetrachlorobiphenyl (TeCB). *R. eutropha* H850g13 was detected by viable plate counts and most-probable-number/PCR after 102 days in TeCB-contaminated soil microcosms, and was likely outcompeted by indigenous soil microorganisms in microcosms amended with oil and Daramend (an organic amendment, <http://www.adventusremediation.com>). *R. eutropha* H850g13 did not degrade TeCB in any of the soil microcosms. This research confirmed that gfp was useful as a marker to distinguish *R. eutropha* H850g13 from indigenous soil microorganisms over a 102 day period and that, under the experimental conditions used, *R. eutropha* H850g13 did not degrade TeCB.

Arbeli, Z. and Z. Ronen (2003). "Enrichment of a microbial culture capable of reductive debromination of the flame retardant tetrabromobisphenol-A, and identification of the intermediate metabolites produced in the process." *Biodegradation* 14(6): 385-395.

Tetrabromobisphenol-A is a reactive flame retardant used in the production of many plastic polymers. In previous research, it was demonstrated that anaerobic microorganisms from contaminated sediment debrominate tetrabromobisphenol-A to bisphenol-A, but an enrichment culture was not established. The current study was carried out to identify the intermediate metabolites in this process and to determine the factors facilitating enrichment of debrominating microorganisms. During the enrichment process in an anaerobic semi-continuous batch reactor, tetrabromobisphenol-A debromination gradually slowed down with concurrent accumulation of three intermediate products. These compounds were tentatively identified using GC-MS as tri-, di-, and mono-brominated bisphenol-A. GC-MS and HPLC analyses showed one dominant metabolite of dibromobisphenol-A, and NMR analysis identified it as 2,2'-dibromobisphenol-A. Addition of sterile sediment (15% wt/wt) to the reactor stimulated debromination of tetrabromobisphenol-A. Furthermore, different solid amendments such as surface soil and pulverized gray chalk from the site subsurface (100 m below ground) were also stimulating agents. We conclude that organic matter is involved in stimulation since the stimulation effect of the sediment, soil and gray chalk was abolished after it was heat-treated to 550degreesC. Our study suggests that the debrominating culture requires some organic components found in the sediment, soil, and chalk in order to sustain activity and perhaps to survive. The possible mechanisms of stimulation by these solids are discussed.

Aust, S. D., P. R. Swaner, et al. (2004). *Detoxification and metabolism of chemicals by white-rot fungi. Pesticide Decontamination and Detoxification*. 863: 3-14.

White-rot fungi can degrade a wide variety of environmental pollutants using a variety of extracellular enzymes and chemicals normally involved in lignin degradation. Examples of toxic chemicals shown to be degraded by white-rot fungi include pentachlorophenol, trinitrotoluene, trichloroethylene, cyanide and polyaromatic hydrocarbons. Pentachlorophenol is methylated by a transmembrane methyl transferase. Trinitrotoluene is reduced by a transmembrane redox potential associated with a proton pump that the fungus uses to establish a rather low (4.5) extracellular pH. Trichloroethylene is aerobically dechlorinated by peroxidases using the carboxylate anion radical. The peroxidases oxidize either veratryl alcohol or manganese which oxidize oxalic acid to form the carboxylate anion radical for reductive dechlorinations. Other chemicals can either be directly or indirectly oxidized

to radicals by the peroxidases. In all cases the chemicals are detoxified such that relatively high concentrations of these chemicals can be degraded. In addition, sites contaminated with multiple chemicals, including these toxic chemicals, can be remediated.

Blunt, J. W., B. R. Copp, et al. (2004). "Marine natural products." *Natural Products Reports* 21: 1-49.

Covering: 2002. Previous review: *Nat. Prod. Rep.*, 2003, 20, 1. This review covers the literature published in 2002 for marine natural products, with 579 citations (413 for the period January to December 2002) referring to compounds isolated from marine microorganisms and phytoplankton, green algae, brown algae, red algae, sponges, coelenterates, bryozoans, molluscs, tunicates and echinoderms. The emphasis is on new compounds (677 for 2002), together with their relevant biological activities, source organisms and country of origin. Syntheses that lead to the revision of structures or stereochemistries have been included (114), including any first total syntheses of a marine natural product.

Carey, G. R., P. J. Van Geel, et al. (2003). "A modified radial diagram approach for evaluating natural attenuation trends for chlorinated solvents and inorganic redox indicators." *Ground Water Monitoring and Remediation* 23(4): 75-84.

Selection of monitored natural attenuation as a ground water remedy requires that sound scientific documentation clearly illustrating the effectiveness of this remedial alternative be presented to regulatory agencies and concerned citizens. An innovative radial diagram approach is applied to illustrate natural attenuation trends for total benzene, toluene, ethylbenzene, and xylenes (BTEX) and chlorinated ethenes at a former fire training area at Plattsburgh Air Force Base, New York. A BTEX-CAH (chlorinated aliphatic hydrocarbons) radial diagram map shows that concentrations of site contaminants are generally decreasing along the primary flowpath downgradient from the source area. This radial diagram map also suggests that there is a spatial correlation between decreasing CAH parent compound concentrations and increasing or stable daughter product concentrations. This provides secondary evidence of intrinsic biodegradation of TCE downgradient from the source area. A SEQUENCE-Redox(TM) map suggests that there is a spatial correlation between trends in electron acceptor and metabolic byproduct concentrations, and the decline in total BTEX concentrations downgradient from the source area. This correlation provides secondary evidence for the intrinsic biodegradation of total BTEX in the aquifer. This study demonstrates that radial diagram visual aids can provide a clear and efficient approach for documenting natural attenuation lines of evidence, as an alternative or a complement to using multiple contour maps, tabulated data, or log-linear plots.

Chaloupkova, R., J. Sykorova, et al. (2003). "Modification of activity and specificity of haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26 by engineering of its entrance tunnel." *Journal of Biological Chemistry* 278(52): 52622-52628.

Structural comparison of three different haloalkane dehalogenases suggested that substrate specificity of these bacterial enzymes could be significantly influenced by the size and shape of their entrance tunnels. The surface residue leucine 177 positioned at the tunnel opening of the haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26 was selected for modification based on structural and phylogenetic analysis; the residue partially blocks the entrance tunnel, and it is the most variable pocket residue in haloalkane dehalogenase-like proteins with nine substitutions in 14 proteins. Mutant genes coding for proteins carrying all possible substitutions in position 177 were constructed by site-directed mutagenesis and heterologously expressed in *Escherichia coli*. In total, 15 active protein variants were obtained, suggesting a relatively high tolerance of the site for the introduction of mutations. Purified protein variants were kinetically characterized by determination of specific activities with 12 halogenated substrates and steady-state kinetic parameters with two substrates. The effect of mutation on the enzyme activities varied dramatically with the structure of the substrates, suggesting that extrapolation of one substrate to another may be misleading and that a systematic characterization of the protein variants with a number of substrates is essential. Multivariate analysis of activity data revealed that catalytic activity of mutant enzymes generally increased with the introduction of small and nonpolar amino acid in position 177. This result is consistent with the phylogenetic analysis showing that glycine and alanine are the most commonly occurring amino acids in this position among

haloalkane dehalogenases. The study demonstrates the advantages of using rational engineering to develop enzymes with modified catalytic properties and substrate specificities. The strategy of using site-directed mutagenesis to modify a specific entrance tunnel residue identified by structural and phylogenetic analyses, rather than combinatorial screening, generated a high percentage of viable mutants.

Chen, G. (2004). "Reductive dehalogenation of tetrachloroethylene by microorganisms: current knowledge and application strategies." *Applied Microbiology and Biotechnology* 63(4): 373-377.

Reductive anaerobic dehalogenation is a useful method for remediation of sites contaminated by chlorinated ethylenes, where hydrogen concentration plays the key role. Under anaerobic conditions, dehalogenating bacteria compete best against methanogenic consortia when the hydrogen level is low; and methanogenic consortia outplay dehalogenating bacteria when the hydrogen level is high. Thus, in an anaerobic mixed culture, efficient use of hydrogen for dehalogenation can be achieved by strategies that maintain hydrogen at a certain low concentration. However, due to the role of acetate, expected dehalogenating results cannot be obtained and unexpected methane formation can be encountered in practice.

Corapcioglu, M. Y., K. Sung, et al. (2004). "Parameter determination of sequential reductive dehalogenation reactions of chlorinated hydrocarbons." *Transport in Porous Media* 55(2): 169-182.

Chlorinated hydrocarbons such as tetrachloroethylene (PCE) and trichloroethylene (TCE) are not directly mineralized, but rather are transformed into one or more intermediate compounds before converting into a final compound. Since the biotransformation rate coefficients of each intermediate compound are different, the coefficients in each step of reductive dehalogenation must be determined to establish an effective treatment operation. These parameters can be estimated by fitting the experimental data to Michaelis-Menten equation. In this study, we introduce a methodology, using both the curve-fitting and graphical methods, to estimate the rate of maximum biotransformation and half-saturation coefficients of parent and intermediate compounds. First-order rate coefficients are also estimated by simplifying the Michaelis-Menten equation for both curve-fitting and graphical methods. The results show that both methods produce similar parameter values for each rate equation. Estimated first-order kinetic parameters are employed to predict the compound concentrations from the analytical solutions of governing equations for sequential dehalogenation. Comparisons of predicted and experimental values show favorable agreement.

De Wildeman, S., G. Linthout, et al. (2004). "Complete lab-scale detoxification of groundwater containing 1,2-dichloroethane." *Applied Microbiology and Biotechnology* 63(5): 609-612.

The suspected carcinogenic solvent 1,2-dichloroethane (1,2-DCA) is the most abundant chlorinated C-2 groundwater pollutant on earth. However, an efficient reductive in situ detoxification technology for this compound is not known. Detoxification results of 1,2-DCA with the recently isolated anaerobic bacterium *Desulfotobacterium dichloroeliminans* strain DCA1 are presented. First, it was verified that strain DCA1 could compete for nutrients in the presence of fast-growing *Enterococcus faecalis*; the latter was observed in the enrichment culture from which strain DCA1 was isolated. Subsequently, lab-scale bioaugmentation of the strain to groundwater containing 40 mg 1,2-DCA/l indicated that the bacterium has strong metabolic activity under prevailing environmental conditions, converting the pollutant into ethene. During exponential growth, the maximum 1,2-DCA dechlorination rate exceeded 350 nmol chloride released per min per mg total bacterial protein. Growth and dechlorination within the community with autochthonous bacteria indicated a high competitive strength of strain DCA1. Interestingly this dechlorination process does not produce any toxic byproducts, such as vinyl chloride. Furthermore, complete groundwater detoxification happens within a short time-frame (days) and is robust in terms of bacterial competition, oxygen tolerance, high ionic strength, and pH range.

Dercova, K., Z. Kyselova, et al. (2003). "Biodegradation and bioremediation of pentachlorophenol." *Chemicke Listy* 97(10): 991-1002.

Pentachlorophenol (PCP) has been widely used in a number of industrial applications. As a consequence and due to its stability, it has become a widespread contaminant in soil, sediments and landfills. Because classic remediation technologies (such as incineration) are generally non-ecological and uneconomical, alternative methods involving biodegradation by microbial populations have been developed. The two known pathways of biodegradation (oxidative and reductive) as well as factors affecting PCP degradation by microbial strains are reviewed here. The proposed bioremediation strategies and those recently developed are outlined.

DeVaull, G., R. Ettinger, et al. (2002). "Chemical vapor intrusion from soil or groundwater to indoor air: Significance of unsaturated zone biodegradation of aromatic hydrocarbons." *Soil & Sediment Contamination* 11(4): 625-641.

The soil vapor to indoor air exposure pathway is considered in a wide number of risk-based site management programs. In screening-level assessments of this exposure pathway, models are typically used to estimate the transport of vapors from either subsurface soils or groundwater to indoor air. Published studies indicate that the simple models used to evaluate this exposure pathway often over estimate the impact for aromatic hydrocarbons (e.g., benzene, toluene, ethylbenzene, and xylene or BTEX), while showing reasonable agreement for estimates of chlorinated hydrocarbon impacts (e.g., PCE, TCE, DCE). Aerobic biodegradation of the petroleum hydrocarbons is most often attributed as the source of this disparity in the model/data comparisons. This paper looks at the significance of aerobic biodegradation of aromatic hydrocarbons as part of the assessment of chemical vapor intrusion from soil or groundwater to indoor air. A review of relevant literature summarizing the available field data as well as various modeling approaches that include biodegradation is presented. This is followed by a simple modeling analysis that demonstrates the potential importance of biodegradation in the assessment of the soil vapor to indoor air exposure pathway. The paper concludes with brief discussions of other model considerations that are often not included in simple models but may have a significant impact on the intrusion of vapors into indoor air.

Dijk, J. A., A. J. M. Stams, et al. (2003). "Anaerobic oxidation of 2-chloroethanol under denitrifying conditions by *Pseudomonas stutzeri* strain JJ." *Applied Microbiology and Biotechnology* 63(1): 68-74.

A bacterium that uses 2-chloroethanol as sole energy and carbon source coupled to denitrification was isolated from 1,2-dichloroethane-contaminated soil. Its 16 S rDNA sequence showed 98% similarity with the type strain of *Pseudomonas stutzeri* (DSM 5190) and the isolate was tentatively identified as *Pseudomonas stutzeri* strain JJ. Strain JJ oxidized 2-chloroethanol completely to CO₂ with NO₃⁻ or O₂ as electron acceptor, with a preference for O₂ if supplied in combination. Optimum growth on 2-chloroethanol with nitrate occurred at 30 degreesC with a $\mu(\text{max})$ of 0.14 h⁻¹ and a yield of 4.4 g protein per mol 2-chloroethanol metabolized. Under aerobic conditions, the $\mu(\text{max})$ was 0.31 h⁻¹. NO₂⁻ also served as electron acceptor, but reduction of Fe(OH)₃, MnO₂, SO₄²⁻, fumarate or ClO₃⁻ was not observed. Another chlorinated compound used as sole energy and carbon source under aerobic and denitrifying conditions was chloroacetate. Various different bacterial strains, including some closely related *Pseudomonas stutzeri* strains, were tested for their ability to grow on 2-chloroethanol as sole energy and carbon source under aerobic and denitrifying conditions, respectively. Only three strains, *Pseudomonas stutzeri* strain LMD 76.42, *Pseudomonas putida* US2 and *Xanthobacter autotrophicus* GJ10, grew aerobically on 2-chloroethanol. This is the first report of oxidation of 2-chloroethanol under denitrifying conditions by a pure bacterial culture.

Dror, I. and M. A. Schlautman (2004). "Metalloporphyrin solubility: A trigger for catalyzing reductive dechlorination of tetrachloroethylene." *Environmental Toxicology and Chemistry* 23(2): 252-257.

Metalloporphyrins are well known for their electron-transfer roles in many natural redox systems. In addition, several metalloporphyrins and related tetrapyrrole macrocycles complexed with various core metals have been shown to catalyze the reductive dechlorination of certain organic compounds, thus demonstrating the potential for using naturally occurring metalloporphyrins to attenuate toxic and persistent chlorinated organic pollutants in the environment. However, despite the great interest in reductive dechlorination reactions and the wide variety of natural and synthetic porphyrins currently available, only soluble porphyrins, which comprise a small fraction of this particular family of organic macrocycles, have been used as electron-transfer shuttles in these reactions. Results from the present study clearly demonstrate that metalloporphyrin solubility is a key factor in their ability to catalyze

the reductive dechlorination of tetrachloroethylene and its daughter compounds. Additionally, we show that certain insoluble and nonreactive metalloporphyrins can be activated as catalysts merely by changing solution conditions to bring about their dissolution. Furthermore, once a metalloporphyrin is fully dissolved and activated, tetrachloroethylene transformation proceeds rapidly, giving nonchlorinated and less toxic alkenes as the major reaction products. Results from the present study suggest that if the right environmental conditions exist or can be created, specific metalloporphyrins may provide a solution for cleaning up sites that are contaminated with chlorinated organic pollutants.

Dyer, M. (2003). "Field investigation into the biodegradation of TCE and BTEX at a former metal plating works." *Engineering Geology* 70(3-4): 321-329.

The paper is based on a recent programme of groundwater monitoring at an industrial site in west London. Redevelopment of the site in 1997 revealed high levels of soil and groundwater pollution by hydrocarbon fuels, trichloroethylene (TCE) and soluble metal salts (e.g. free cyanide, chromium VI and nickel). The pollution originated from a previous metal plating and galvanising works at the site. As part of the redevelopment works, the owners undertook limited excavation works and groundwater extraction to remove the pollutant. However, groundwater sampling has continued to show high levels of pollution. Following discussion with the environment regulator in late 1998, a groundwater monitoring programme was agreed to investigate the potential for co-degradation of the petroleum fuel and TCE. Groundwater samples have been taken from six existing boreholes (1C to 6C). The location of the monitoring boreholes relates to past pollution spillages and the layout of the new factory building. Chemical analyses of groundwater samples show elevated aqueous concentrations of chloroethenes with a classical reduction pathway for trichloroethylene (TCE) leading to an accumulation of vinyl chloride. (C) 2003 Elsevier B.V. All rights reserved.

Dyer, M., E. van Heiningen, et al. (2003). "A field trial for in-situ bioremediation of 1,2-DCA." *Engineering Geology* 70(3-4): 315-320.

Historic spillages of chlorinated hydrocarbons at a vinyl chloride plant in the Rotterdam Botlek area in The Netherlands have led to contamination of the underlying aquifer. The principal contaminant is 1,2-dichloroethane (1,2-DCA). The contamination is temporarily contained by a pump-and-treat system. A field trial was carried out to investigate the feasibility of treating the dissolved phase of 1,2-DCA via reductive dechlorination by injection of an aqueous solution of methanol, ammonium chloride and sodium chloride into the confined aquifer using an array of eight boreholes. Biodegradation of 1,2-DCA was localised. This was attributed to limited mixing of the carbon substrate within the test zone. In addition, clogging of recharge wells complicated groundwater circulation. (C) 2003 Elsevier B.V. All rights reserved.

Feng, Y. C. (2004). *Microbial and photolytic degradation of 3,5,6-trichloro-2-pyridinol. Pesticide Decontamination and Detoxification*. 863: 15-24.

3,5,6-Trichloro-2-pyridinol (TCP) is a primary degradation product of the insecticide chlorpyrifos and the herbicide triclopyr. A bacterium, *Pseudomonas* sp. strain ATCC 700113, capable of using TCP as a sole source of carbon and energy, was isolated from a soil treated repeatedly with chlorpyrifos. TCP was metabolized to CO₂, chloride, and unidentified polar metabolites. *Pseudomonas* sp. ATCC 700113 immobilized on diatomaceous earth beads also mineralized [2,6-C-14]TCP rapidly; about 75% of the initial radioactivity was recovered as (CO₂)-C-14. Immobilized cells effectively removed TCP from wastewater generated from a chlorpyrifos-manufacturing plant; however, degradation of TCP was inhibited by high concentrations of NaCl. Photolysis of TCP occurred rapidly upon UV irradiation and released CO₂, chloride, dichlorodihydroxypyridine isomers, and reductive dechlorination products. Resting cell cultures of *Pseudomonas* sp. ATCC 700113 can only degrade the reductive dechlorination products in the mixture of photodegradation products, suggesting TCP degradation by this organism involves a reductive dechlorination pathway.

Fernandez-Sanchez, J. M., E. J. Sawvel, et al. (2004). "Effect of Fe-0 quantity on the efficiency of integrated microbial-Fe-0 treatment processes." *Chemosphere* 54(7): 823-829.

Batch experiments were conducted with different reaction systems to investigate how the treatment efficiency of integrated microbial-Fe-0 processes is affected by the amount of Fe-0 added. Abiotic experiments with

hexavalent chromium and carbon tetrachloride mixtures corroborated that different pollutants could compete for reactive sites on the iron surface, which would hinder specific degradation rates when the available Fe-0 surface area is relatively small (e.g., 11 m² l⁻¹). In such cases, reductive precipitation of chromium could occlude reactive sites and significantly inhibit removal efficiency. Microbial participation in the cleanup process was also influenced by the amount of Fe-0 added. Increasing the Fe-0 dose (and thus the available surface area) had a stimulatory effect possibly due to a higher production of cathodic H₂, which can be used as electron donor for reductive biotransformation of many pollutants. However, high Fe-0 doses had an inhibitory effect due to a corrosion-induced increase in pH beyond the optimum range of the bacteria. This suggests that there may be a system-specific, optimum quantity of Fe-0 that satisfies availability requirements to preclude contaminant competition for reactive sites and biological requirements for H₂ production while minimizing inhibitory increases in pH. Results also confirmed extensive RDX mineralization in bioaugmented (but not in abiotic) Fe-0 systems, and support the notion that permeable reactive iron barriers performance might be enhanced by the participation of some microorganisms. (C) 2003 Elsevier Ltd. All rights reserved.

Francova, K., M. Mackova, et al. (2004). "Ability of bacterial biphenyl dioxygenases from Burkholderia sp LB400 and Comamonas testosteroni B-356 to catalyze oxygenation of ortho-hydroxychlorobiphenyls formed from PCBs by plants." *Environmental Pollution* 127(1): 41-48.

Capacity of enzymes of the biphenyl/chlorobiphenyl pathway, especially biphenyl dioxygenase (BPDO) of two polychlorinated biphenyls (PCB) degrading bacteria, *Burkholderia* sp. LB400 and *Comamonas testosteroni* B-356, to metabolize ortho-substituted hydroxybiphenyls was tested. These compounds found among plant products of PCB metabolism, are carrying chlorine atoms on the hydroxyl-substituted ring. The abilities of His-tagged purified LB400 and B-356 BPDOs to catalyze the oxygenation of 2-hydroxy-3-chlorobiphenyl, 2-hydroxy-5-chlorobiphenyl and 2-hydroxy-3,5-dichlorobiphenyl were compared. Both enzyme preparations catalyzed the hydroxylation of the three chloro-hydroxybiphenyls on the non-substituted ring. Neither LB400 BPDO nor B-356 BPDO oxygenated the substituted ring of the ortho-hydroxylated biphenyl. The fact that metabolites generated by both enzymes were identical for all three hydroxychlorobiphenyls tested; exclude any other mode of attack of these compounds by LB400 BPDOs than the ortho-meta oxygenation.

Fuse, H., H. Inoue, et al. (2003). "Production of free and organic iodine by *Roseovarius* spp." *Fems Microbiology Letters* 229(2): 189-194.

Two strains of iodine-producing bacteria were isolated from marine samples. 16S rRNA gene sequences indicated the strains were most closely related to *Roseovarius tolerans*, and phylogenetic analysis indicated both belong to the same genus. 5 mM iodide inhibited the growth of strain 2S5-2 almost completely, and of strain S6V slightly. Both strains produced free iodine and organic iodine from iodide. CH₂I₂, CHI₃ and CH₂CII were the main organic iodines produced by strain 2S5-2, and CHI₃ and CH₂I₂ by strain S6V. Experiments using cells and spent media suggested that the organic iodines were produced from the compounds released or contained in the media and cells were necessary for the considerable production of CH₂I₂ and CH₂CII, though CHI₃ was produced by spent media with H₂O₂ or free iodine. (C) 2003 Federation of European Microbiological Societies.

Gong, W. L., K. J. Sears, et al. (2004). "Toxicity of model aliphatic amines and their chlorinated forms." *Environmental Toxicology and Chemistry* 23(2): 239-244.

Aliphatic amines can be found in many wastewater effluents from industry, agriculture, pharmacy, and food processing. Amines can induce toxicological responses that are relevant in biochemical treatment processes, as well as in natural waters. This research compared the toxicity and inhibition caused by three aliphatic amines (n-propylamine, ethylmethylamine, and trimethylamine) and their chlorinated derivatives. The chemistry of chlorine interactions with these compounds was characterized by using membrane introduction mass spectrometry (MIMS). Acute toxicity assays were conducted by using a Microtox(R) system with *Phosphobacterium phosphoreum* (also known as *Vibrio fischeri*) for the aliphatic amine compounds and their corresponding chlorinated derivatives, as identified by MIMS. Inhibition tests were conducted by using the oxygen utilization rate test with an enhanced nitrifier culture. The median effective concentration (EC₅₀) values for chloropropylamine, chloroethylmethylamine,

and chlorodimethylamine obtained by Microtox with a contact time of 15 min were 12.68, 19.72, and 15.92 μM , respectively. The EC50 values of these aliphatic chloramines from the Microtox test decreased by roughly one order of magnitude as a result of chlorination. Inhibition of nitrifiers also was observed in these amines. Trimethylamine and n-propylamine caused greater inhibition to nitrifiers than did ethylmethylamine under similar concentrations. Nitrifier inhibition from these amines increased after chlorination. The results of these tests indicated that aliphatic amines and their chlorinated derivatives could induce environmentally relevant toxicity responses in treatment settings and in receiving waters.

Grm, K. S. W., M. Vrtacnik, et al. (2003). "Biodegradation and photooxidation studies of model organic pollutants." *Critical Reviews in Analytical Chemistry* 33(4): 333-338.

In the paper we present the results of biodegradation and photooxidation studies of halosubstituted benzyl alcohols. The basic goal of our research was to examine the effect of the type and position of the halogen atom in the aromatic ring on the degradation rate and to use the experimental data for the development of HQSAR models for predicting degradation rates. Biodegradation results of 2-halosubstituted benzyl alcohols correlate with the size of the halogen atom, while for 4-halosubstituted benzyl alcohols, a good correlation with the energy of C-X bond was observed. In the photooxidation process, the impact of position of the halogen on the rate of reaction is smaller. The HQSAR model was derived only for photooxidation data sets, while for the biodegradation it was not possible to obtain a model with satisfactory statistical characteristics.

Hagblom, M. M., Y. B. Ahn, et al. (2003). Anaerobic dehalogenation of organohalide contaminants in the marine environment. *Advances in Applied Microbiology*, Vol 53. 53: 61-+.

Jadulco, R., R. A. Edrada, et al. (2004). "New communesin derivatives from the fungus *Penicillium* sp. derived from the Mediterranean sponge *Axinella verrucosa*." *Journal of Natural Products* 67(1): 78-81.

The ethyl acetate extract of *Penicillium* sp., derived from the Mediterranean sponge *Axinella verrucosa*, yielded the known compound communesin B (1) and its new congeners communesins C (2) and D (3), as well as the known compounds griseofulvin, dechlorogriseofulvin, and oxaline. All structures were unambiguously established by 1D and 2D NMR and MS data. In several bioassays performed on different leukemia cell lines, the communesins exhibited moderate antiproliferative activity.

Jayachandran, G., H. Gorisch, et al. (2003). "Dehalorespiration with hexachlorobenzene and pentachlorobenzene by *Dehalococcoides* sp strain CBDB1." *Archives of Microbiology* 180(6): 411-416.

The chlororespiring anaerobe *Dehalococcoides* sp. strain CBDB1 used hexachlorobenzene and pentachlorobenzene as electron acceptors in an energy-conserving process with hydrogen as electron donor. Previous attempts to grow *Dehalococcoides* sp. strain CBDB1 with hexachlorobenzene or pentachlorobenzene as electron acceptors failed if these compounds were provided as solutions in hexadecane. However, *Dehalococcoides* sp. strain CBDB1 was able to grow with hexachlorobenzene or pentachlorobenzene when added in crystalline form directly to cultures. Growth of *Dehalococcoides* sp. strain CBDB1 by dehalorespiration resulted in a growth yield (Y) of 2.1 ± 0.24 g protein/mol Cl⁻ released with hexachlorobenzene as electron acceptor; with pentachlorobenzene, the growth yield was 2.9 ± 0.15 g/mol Cl⁻. Hexachlorobenzene was reductively dechlorinated to pentachlorobenzene, which was converted to a mixture of 1,2,3,5- and 1,2,4,5-tetrachlorobenzene. Formation of 1,2,3,4-tetrachlorobenzene was not detected. The final end-products of hexachlorobenzene and pentachlorobenzene dechlorination were 1,3,5-trichlorobenzene, 1,3- and 1,4-dichlorobenzene, which were formed in a ratio of about 3:2:5. As reported previously, *Dehalococcoides* sp. strain CBDB1 converted 1,2,3,5-tetrachlorobenzene exclusively to 1,3,5-trichlorobenzene, and 1,2,4,5-tetrachlorobenzene exclusively to 1,2,4-trichlorobenzene. The organism therefore catalyzes two different pathways to dechlorinate highly chlorinated benzenes. In the route leading to 1,3,5-trichlorobenzene, only doubly flanked chlorine substituents were removed, while in the route leading to 1,3- and 1,4-dichlorobenzene via 1,2,4-trichlorobenzene singly flanked chlorine substituents were also removed. Reductive dehalogenase activity measurements using whole cells pregrown with different chlorobenzene congeners as electron

acceptors indicated that different reductive dehalogenases might be induced by the different electron acceptors. To our knowledge, this is the first report describing reductive dechlorination of hexachlorobenzene and pentachlorobenzene via dehalorespiration by a pure bacterial culture.

Kamanavalli, C. M. and H. Z. Ninnekar (2004). "Biodegradation of DDT by a Pseudomonas species." *Current Microbiology* 48(1): 10-13.

A bacterial strain capable of degrading 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) was isolated from insecticide-contaminated soil by biphenyl enrichment culture and identified as a *Pseudomonas* species. The organism degraded DDT through the intermediate formation of 2,3-dihydroxy-DDT, which undergoes meta-ring cleavage, ultimately yielding 4-chlorobenzoic acid as a stable metabolite.

Kao, C. M., Y. L. Chen, et al. (2003). "Enhanced PCE dechlorination by biobarrier systems under different redox conditions." *Water Research* 37(20): 4885-4894.

The industrial solvent tetrachloroethylene (PCE) is among the most ubiquitous chlorinated compounds found in groundwater contamination. The objective of this study was to evaluate the (1) feasibility of enhancing PCE biodegradation using cane molasses and sludge cakes as the primary substrates under methanogenic and iron reducing conditions, and (2) potential of installation a sludge cake/cane molasses biobarrier to clean up PCE-contaminated aquifers. The biodegradability of sludge cake (from secondary wastewater treatment system) and cane molasses was tested using bioavailability experiments. Results show that biodegradable materials were released from sludge cake/cane molasses and utilized by microbial consortia. Based on the chemical oxygen demand (COD) tests, approximately 28 and 248 mg of biodegradable COD can be released from 1 g of sludge cake and 1 g of cane molasses under anaerobic conditions, which have the potential to convert 70 and 620 mg of PCE to ethylene (ETH), respectively. Reductive dechlorination was evaluated using microcosms containing primary substrates (sludge cake/cane molasses) and inocula (aquifer sediments). Results indicate that sludge cake and cane molasses can serve as the diffusion sources of primary substrates, and enhance the reductive dechlorination of PCE under methanogenic processes. However, results from this study were not sufficient enough to show that reductive dechlorination of PCE would occur under iron-reducing conditions. This indicates that more studies need to be performed to further evaluate the role of iron reduction on the PCE dechlorination. Results reveal that it is feasible and applicable to install a sludge cake or cane molasses biobarrier to clean up PCE contaminated aquifers. From an engineering point of view, the sludge cake/cane molasses biobarrier has the potential to become an environmentally and economically acceptable technology for PCE bioremediation. (C) 2003 Elsevier Ltd. All rights reserved.

Kim, B. H., E. T. Oh, et al. (2003). "Plant terpene-induced expression of multiple aromatic ring hydroxylation oxygenase genes in *Rhodococcus* sp strain T104." *Journal of Microbiology* 41(4): 349-352.

Recent studies have shown that some of the PCB (polychlorinated biphenyl)-degraders are able to effectively degrade PCB in the presence of monoterpenes, which act as inducers for the degradation pathway. *Rhodococcus* sp. T104, an effective PCB degrader, has been shown to induce the degradation pathway by utilizing limonenes, cymenes, carvones, and pinenes as sole carbon sources which can be found in the natural environment. Moreover, the strain T104 proved to possess three separate oxidation pathways of limonene, biphenyl, and phenol. Of these three, the limonene can also induce the biphenyl degradation pathway. In this work, we report the presence of three distinct genes for aromatic oxygenase, which are putatively involved in the degradation of aromatic substrates including biphenyl, limonene, and phenol, through PCR amplification and denaturing gradient gel electrophoresis (DGGE). The genes were differentially expressed and well induced by limonene, cymene, and plant extract A compared to biphenyl and/or glucose. This indicates that substrate specificity must be taken into account when biodegradation of the target compounds are facilitated by the plant natural substrates.

Kuntz, R. L., L. R. Brown, et al. (2003). "Isopropanol and acetone induces vinyl chloride degradation in *Rhodococcus rhodochrous*." *Journal of Industrial Microbiology & Biotechnology* 30(11): 651-655.

In situ bioremediation of vinyl chloride (VC)-contaminated waste sites requires a microorganism capable of degrading VC. While propane will induce an oxygenase to accomplish this goal, its use as a primary substrate in

bioremediation is complicated by its flammability and low water solubility. This study demonstrates that two degradation products of propane, isopropanol and acetone, can induce the enzymes in *Rhodococcus rhodochrous* that degrade VC. Additionally, a reasonable number of cells for bioremediation can be grown on conventional solid bacteriological media (nutrient agar, tryptic soy agar, plate count agar) in an average microbiological laboratory and then induced to produce the necessary enzymes by incubation of a resting cell suspension with isopropanol or acetone. Since acetone is more volatile than isopropanol and has other undesirable characteristics, isopropanol is the inducer of choice. It offers a nontoxic, water-soluble, relatively inexpensive alternative to propane for in situ bioremediation of waste sites contaminated with VC.

Manji, S. and A. Ishihara (2004). "Screening of tetrachlorodibenzo-p-dioxin-degrading fungi capable of producing extracellular peroxidases under various conditions." *Applied Microbiology and Biotechnology* 63(4): 438-444.

Forty-six pulp-bleaching fungi were screened for production of key enzymes for conversion of polychlorinated dibenzo-p-dioxins-lignin peroxidase (LiP), manganese peroxidase (MnP), and manganese-independent peroxidase (MiP)-under various conditions that would allow their utilization in the environment. Of 38 MnP-producing strains with MiP activity, 22 produced LiP. Three of the new isolates, *Bjerkandera* sp. strains MS191, MS325, and MS1167, were the best producers of the three different peroxidases, and had reasonable growth rates. The most promising *Bjerkandera* sp. strain, MS325, exhibited significant levels of LiP and MnP activities under various conditions, e.g., nutrient nitrogen-sufficient or -limited conditions, conditions with or without Mn(II), and changes in temperature (15-37degreesC). Furthermore, the ability of this strain to degrade 1,3,6,8-tetrachlorodibenzo-p-dioxin was confirmed. The results presented here indicate that utilization of *Bjerkandera* sp. strain MS325 on a practical scale in the environment has several advantages over many white rot fungi, which produce extracellular peroxidases only under specific conditions such as nutrient limitation.

Milliken, C. E., G. P. Meier, et al. (2004). "Microbial anaerobic demethylation and dechlorination of chlorinated hydroquinone metabolites synthesized by basidiomycete fungi." *Applied and Environmental Microbiology* 70(1): 385-392.

The synthesis and degradation of anthropogenic and natural organohalides are the basis of a global halogen cycle. Chlorinated hydroquinone metabolites (CHMs) synthesized by basidiomycete fungi and present in wetland and forest soil are constituents of that cycle. Anaerobic dehalogenating bacteria coexist with basidiomycete fungi in soils and sediments, but little is known about the fate of these halogenated fungal compounds. In sediment microcosms, the CHMs 2,3,5,6-tetrachloro-1,4-dimethoxybenzene and 2,3,5,6-tetrachloro-4-methoxyphenol (TCMP) were anaerobically demethylated to tetrachlorohydroquinone (TCHQ). Subsequently, TCHQ was converted to trichlorohydroquinone and 2,5-dichlorohydroquinone (2,5-DCHQ) in freshwater and estuarine enrichment cultures. Screening of several dehalogenating bacteria revealed that *Desulfitobacterium hafniense* strains DCB2 and PCP1, *Desulfitobacterium chlororespirans* strain Co23, and *Desulfitobacterium dehalogenans* JW/DU1 sequentially dechlorinate TCMP to 2,3,5-trichloro-4-methoxyphenol and 3,5-dichloro-4-methoxyphenol (3,5-DCMP). After a lag, these strains demethylate 3,5-DCMP to 2,6-DCHQ, which is then completely dechlorinated to 1,4-dihydroquinone (HQ). 2,5-DCHQ accumulated as an intermediate during the dechlorination of TCHQ to HQ by the TCMP-degrading desulfitobacteria. HQ accumulation following TCMP or TCHQ dechlorination was transient and became undetectable after 14 days, which suggests mineralization of the fungal compounds. This is the first report on the anaerobic degradation of fungal CHMs, and it establishes a fundamental role for microbial reductive degradation of natural organochlorides in the global halogen cycle.

Mravik, S. C., R. K. Sillan, et al. (2003). "Field evaluation of the solvent extraction residual biotreatment technology." *Environmental Science & Technology* 37(21): 5040-5049.

The Solvent Extraction Residual Biotreatment (SERB) technology was evaluated at a former dry cleaner site in Jacksonville, FL, where an area of tetra chloroethylene (PCE) contamination was identified. The SERB technology is a treatment train approach for complete site restoration, which combines an active in situ dense nonaqueous-phase liquid (DNAPL) removal technology, cosolvent extraction, with a passive enhanced in situ bioremediation technology, reductive dechlorination. During the in situ cosolvent extraction test, approximately 34 kL of 95% ethanol/5% water (v:v) was flushed through the contaminated zone, which removed approximately 60% of the estimated PCE mass. Approximately 2.72 kL of ethanol was left in the subsurface, which provided electron

donor for enhancement of biological processes in the source zone and downgradient areas. Quarterly groundwater monitoring for over 3 yr showed decreasing concentrations of PCE in the source zone from initial values of 4-350 μM to less than 150 μM during the last sampling event. Initially there was little to no daughter product formation in the source zone, but after 3 yr, measured concentrations were 242 μM for cis-dichloroethylene (cis-DICE), 13 μM for vinyl chloride, and 0.43 μM for ethene. In conjunction with the production of dissolved methane and hydrogen and the removal of sulfate, these measurements indicate that in situ biotransformations were enhanced in areas exposed to the residual ethanol. First-order rate constants calculated from concentration data for individual wells ranged from -0.63 to -2.14 yr^{-1} for PCE removal and from 0.88 to 2.39 yr^{-1} for cis-DCE formation. First-order rate constants based on the change in total mass estimated from contour plots of the groundwater concentration data were 0.75 yr^{-1} for cis-DICE, -0.50 yr^{-1} for PCE, and -0.33 yr^{-1} for ethanol. Although these attenuation rate constants include additional processes, such as sorption, dispersion, and advection, they provide an indication of the overall system dynamics. Evaluation of the groundwater data from the former dry cleaner site showed that cosolvent flushing systems can be designed and utilized to aid in the enhancement of biodegradation processes at DNAPL sites.

Murialdo, S. E., R. Fenoglio, et al. (2003). "Degradation of phenol and chlorophenols by mixed and pure cultures." *Water Sa* 29(4): 457-463.

The enrichment of mixed cultures for species capable of degrading phenol and chlorophenols, as well as the isolation of pure cultures are investigated. The cultures obtained are capable of degrading phenol and chlorophenols (pentachlorophenol 2,3,5,6 tetrachlorophenol and 2,4,6 trichlorophenol) but not 2,4,5 trichlorophenol. The results suggest the feasibility of the use of toxic chemicals as phenols, hexadecane and other chlorophenols as co-substrates in field decontamination processes. The inhibitory effect of PCP is shown, and the influence of a readily degradable ancillary carbon source on the performance of pure cultures is reported, as well as the preliminary identification of the bacteria that showed higher PCP degrading activity.

Oakley, A. J., M. Klvana, et al. (2004). "Crystal structure of haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26 at 0.95 angstrom resolution: Dynamics of catalytic residues." *Biochemistry* 43(4): 870-878.

We present the structure of LinB, a 33-kDa haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26, at 0.95 Angstrom resolution. The data have allowed us to directly observe the anisotropic motions of the catalytic residues. In particular, the side-chain of the catalytic nucleophile, Asp 108, displays a high degree of disorder. It has been modeled in two conformations, one similar to that observed previously (conformation A) and one strained (conformation 13) that approached the catalytic base (His272). The strain in conformation B was mainly in the C-alpha-C-beta-C-gamma angle (126degrees) that deviated by 13.4degrees from the "ideal" bond angle of 112.6degrees. On the basis of these observations, we propose a role for the charge state of the catalytic histidine in determining the geometry of the catalytic residues. We hypothesized that double-protonation of the catalytic base (His272) reduces the distance between the side-chain of this residue and that of the Asp108. The results of molecular dynamics simulations were consistent with the structural data showing that protonation of the His272 side-chain nitrogen atoms does indeed reduce the distance between the side-chains of the residues in question, although the simulations failed to demonstrate the same degree of strain in the Asp 108 C-alpha-C-beta-C-gamma angle. Instead, the changes in the molecular dynamics structures were distributed over several bond and dihedral angles. Quantum mechanics calculations on Lin3 with 1-chloro-2,2-dimethylpropane as a substrate were performed to determine which active site conformations and protonation states were most likely to result in catalysis. It was shown that His272 singly protonated at N-delta1 and Asp108 in conformation A gave the most exothermic reaction ($\Delta\text{H} = -22 \text{ kcal/mol}$). With His272 doubly protonated at N-delta1 and N-epsilon2, the reactions were only slightly exothermic or were endothermic. In all calculations starting with Asp108 in conformation B, the Asp108 C-alpha-C-beta-C-gamma angle changed during the reaction and the Asp108 moved to conformation A. The results presented here indicate that the positions of the catalytic residues and charge state of the catalytic base are important for determining reaction energetics in LinB.

Osterreicher-Cunha, P., T. Langenbach, et al. (2003). "HCH distribution and microbial parameters after liming of a heavily contaminated soil in Rio de Janeiro." *Environmental Research* 93(3): 316-327.

The closing down of a lindane factory near Rio de Janeiro, over 45 years ago, left an area heavily contaminated with hexachlorocyclohexane (HCH). Remediation by soil liming was applied by government authorities in 1995. This study aims to evaluate the HCH distribution and impact on soil microbiota due to contamination and liming. Microcosm experiments with uncontaminated soil mixed with HCH and lime indicated that lime-promoted dechlorination of HCH molecules led to leaching and volatilization of metabolites. The treatment applied transformed but did not solve the problem as most of the HCH remains in the soil. Reduced microbial respiratory activity was measured in contaminated field samples. Higher respiration rates in uncontaminated soil were reduced by HCH and lime addition; the sole addition of HCH caused a temporary increase in soil respiration, and stimulation occurred with oxygen and/or nutrient addition. A heterotrophic bacterial population around 10⁹ CFU/g was found in polluted field soil, some well-known degraders having been isolated. Native soil microbiota showed resistance to high amounts of HCH and alkaline pH. The results allow considering bioremediation rather than chemical treatments to clean up the area. (C) 2003 Elsevier Inc. All rights reserved.

Oudijk, G. (2003). "Estimating the minimum age of a chlorinated solvent plume in groundwater with chlorofluorocarbon (CFC) and tritium methodologies: A case study." *Environmental Forensics* 4(1): 81-88.

Numerous age-dating techniques are available to estimate the time frame of contaminant releases to the environment. One method, historically used in the hydrology field to determine recharge ages of groundwater, can be applied to estimating the minimum age of contaminant discharges. Chlorofluorocarbons and tritium are anthropogenic substances present in the atmosphere over the past half century. These constituents recharge the groundwater through precipitation and can be used as an age marker. In cases where the recharge water is in contact with contaminants, the CFCs and tritium may be used to estimate the minimum age of the contaminant release.

Peters, L., G. M. König, et al. (2003). "Secondary metabolites of *Flustra foliacea* and their influence on bacteria." *Applied and Environmental Microbiology* 69(6): 3469-3475.

The North Sea bryozoan *Flustra foliacea* was investigated to determine its secondary metabolite content. Gas chromatography-mass spectrometry analysis of a dichloromethane extract of the bryozoan enabled 11 compounds to be identified. Preparative high-performance liquid chromatography of the extract resulted in the isolation of 10 brominated alkaloids (compounds 1 to 10) and one diterpene (compound 11). All of these compounds were tested to determine their activities in agar diffusion assays against bacteria derived from marine and terrestrial environments. Compounds 1, 3 to 7, 10, and 11 exhibited significant activities against one or more marine bacterial strains originally isolated from *F. foliacea* but only weak activities against all of the terrestrial bacteria. By using the biosensors *Pseudomonas putida* (pKR-02), *P. putida* (pAS-C8), and *Escherichia coli* (pSB403) the antagonistic effect on N-acyl-homoserine lactone-dependent quorum-sensing systems was investigated. Compounds 8 and 10 caused reductions in the signal intensities in these bioassays ranging from 50 to 20% at a concentration of 20 µg/ml.

Pirae, M., R. L. White, et al. (2004). "Biosynthesis of the dichloroacetyl component of chloramphenicol in *Streptomyces venezuelae* ISP5230: genes required for halogenation." *Microbiology-Sgm* 150: 85-94.

Five ORFs were detected in a fragment from the *Streptomyces venezuelae* ISP5230 genomic DNA library by hybridization with a IPCIR product amplified from primers representing a consensus of known halogenase sequences. Sequencing and functional analyses demonstrated that ORFs 11 and 12 (but not ORFs 13-15) extended the partially characterized gene cluster for chloramphenicol (Cm) biosynthesis in the chromosome. Disruption of ORF11 (cmlK) or ORF12 (cmkS) and conjugal transfer of the insertionally inactivated genes to *S. venezuelae* gave mutant strains VS1111 and VS1112, each producing a similar series of Cm analogues in which unhalogenated acyl groups replaced the dichloroacetyl substituent of Cm. H-1-NMR established that the principal metabolite in the disrupted strains was the alpha-N-propionyl analogue. The sequence of CmlK implicated the protein in adenylation, and involvement in halogenation was inferred from biosynthesis of analogues by the cmlK-disrupted mutant. A role in generating the dichloroacetyl substituent was supported by partial restoration of Cm biosynthesis when a cloned

copy of *cmlK* was introduced in trans into VS1111. Complementation of the mutant also indicated that inactivation of *cmlK* rather than a polar effect of the disruption on *cmlS* expression had interfered with dichloroacetyl biosynthesis. The deduced *CmlS* sequence resembled sequences of FADH(2)-dependent halogenases. Conjugal transfer of *cmlK* or *cmlS* into *S. venezuelae cml-2*, a chlorination-deficient strain with a mutation mapped genetically to the *Cm* biosynthesis gene cluster, did not complement the *cml-2* lesion, suggesting that one or more genes in addition to *cmlK* and *cmlS* is needed to assemble the dichloroacetyl substituent. Insertional inactivation of ORF13 did not affect *Cm* production, and the products of ORF14 and ORF15 matched *Streptomyces coelicolor* A3(2) proteins lacking plausible functions in *Cm* biosynthesis. Thus *cmlS* appears to mark the downstream end of the gene cluster.

Poelarends, G. J., H. Serrano, et al. (2004). "Cloning, expression, and characterization of a cis-3-chloroacrylic acid dehalogenase: Insights into the mechanistic, structural, and evolutionary relationship between isomer-specific 3-chloroacrylic acid dehalogenases." *Biochemistry* 43(3): 759-772.

The gene encoding the cis-3-chloroacrylic acid dehalogenase (*cis-CaaD*) from coryneform bacterium strain FG41 has been cloned and overexpressed, and the enzyme has been purified to homogeneity and subjected to kinetic and mechanistic characterization. Kinetic studies show that *cis-CaaD* processes cis-3-haloacrylates, but not trans-3-haloacrylates, with a turnover number of similar to 10^4 s^{-1} . The product of the reaction is malonate semialdehyde, which was confirmed by its characteristic H-1 NMR spectrum. The enzyme shares low but significant sequence similarity with the previously studied trans-3-chloroacrylic acid dehalogenase (*CaaD*) and with other members of the 4-oxalocrotonate tautomerase (4-OT) family. While 4-OT and *CaaD* function as homo- and heterohexamers, respectively, *cis-CaaD* appears to be a homotrimeric protein as assessed by gel filtration chromatography. On the basis of the known three-dimensional structures and reaction mechanisms of *CaaD* and 4-OT, a sequence alignment implicated Pro-1, Arg-70, Arg-73, and Glu-114 as important active-site residues in *cis-CaaD*. Subsequent site-directed mutagenesis experiments confirmed these predictions. The acetylene compounds, 2-oxo-3-pentynoate and 3-bromo- and 3-chloropropiolate, were processed by *cis-CaaD* to products consistent with an enzyme-catalyzed hydration reaction previously established for *CaaD*. Hydration of 2-oxo-3-pentynoate afforded acetopyruvate, while the 3-halopropiolates became irreversible inhibitors that modified Pro-1. The results of this work revealed that *cis-CaaD* and *CaaD* have different primary and quaternary structures, and display different substrate specificity and catalytic efficiencies, but likely share a highly conserved catalytic mechanism. The mechanism may have evolved independently because sequence analysis indicates that *cis-CaaD* is not a 4-OT family member, but represents the first characterized member of a new family in the tautomerase superfamily that probably resulted from an independent duplication of a 4-OT-like sequence. The discovery of a fifth family of enzymes within this superfamily further demonstrates the diversity of activities and structures that can be created from 4-OT-like sequences.

Prokop, Z., M. Monincova, et al. (2003). "Catalytic mechanism of the haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26." *Journal of Biological Chemistry* 278(46): 45094-45100.

Haloalkane dehalogenases are bacterial enzymes capable of carbon-halogen bond cleavage in halogenated compounds. To obtain insights into the mechanism of the haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26 (*LinB*), we studied the steady-state and presteady-state kinetics of the conversion of the substrates 1-chlorohexane, chlorocyclohexane, and bromocyclohexane. The results lead to a proposal of a minimal kinetic mechanism consisting of three main steps: (i) substrate binding, (ii) cleavage of the carbon-halogen bond with simultaneous formation of an alkyl-enzyme intermediate, and (iii) hydrolysis of the alkyl-enzyme intermediate. Release of both products, halide and alcohol, is a fast process that was not included in the reaction mechanism as a distinct step. Comparison of the kinetic mechanism of *LinB* with that of haloalkane dehalogenase *DhlA* from *Xantobacter autotrophicus* GJ10 and the haloalkane dehalogenase *DhaA* from *Rhodococcus rhodochrous* NCIMB 13064 shows that the overall mechanisms are similar. The main difference is in the rate-limiting step, which is hydrolysis of the alkyl-enzyme intermediate in *LinB*, halide release in *DhlA*, and liberation of an alcohol in *DhaA*. The occurrence of different rate-limiting steps for three enzymes that belong to the same protein family indicates that extrapolation of this important catalytic property from one enzyme to another can be misleading even for evolutionary closely related proteins. The differences in the rate-limiting step were related to: (i) number and size of

the entrance tunnels, (ii) protein flexibility, and (iii) composition of the halide-stabilizing active site residues based on comparison of protein structures.

Redeker, K. R., S. L. Manley, et al. (2004). "Physiological and biochemical controls over methyl halide emissions from rice plants." *Global Biogeochemical Cycles* 18(1).

This paper investigates physiological and biochemical aspects of methyl halide production in rice plants over two growing seasons. Multiple separate mechanisms appear to be responsible for production of methyl halides in rice plant tissues. Evidence for multiple mechanisms is found in timing of peak emissions of methyl halides from rice, inconsistent effects of competitive inhibitors on methyl halide emissions, and large differences in methyl halide emission rates when compared to plant tissue halide concentrations. Other results show that chloride, bromide, and iodide ion concentrations in plant tissue appear to be regulated throughout the season, and observed changes in leaf tissue concentration cannot explain observed methyl halide emissions. The K_m for methyl iodide formation in leaf tissue cell-free extract is 0.018 mM, suggesting a very efficient mechanism. Of the seven competitive inhibitors used, only thiol had a consistently strong effect on both methyl iodide and methyl bromide.

Regeard, C., J. Maillard, et al. (2004). "Development of degenerate and specific PCR primers for the detection and isolation of known and putative chloroethene reductive dehalogenase genes." *Journal of Microbiological Methods* 56(1): 107-118.

Degenerate and specific PCR primers were designed for the detection of chloroethene reductive dehalogenases (CE-RDase), the key enzymes of chloroethene dehalorespiration, based on sequence information of three CE-RDases and three chlorophenol (CP) RDases. For the design of the degenerate primers, seven conserved amino-acid blocks identified with different bioinformatic tools were used. For one block degenerate, primers containing a 5'-consensus clamp region specific for CE-RDases and a 3'-end degenerate core region specific for RDases in general were designed using the Consensus-Degenerate Hybrid Oligonucleotide Primer (CDHOP) design method. Applying the degenerate primers to genomic DNA of *Sulfurospirillum multivorans* strain K, *Dehalobacter restrictus* strain PER-K23, and *Desulfitobacterium* sp. strain PCE1 led to the isolation of the known CE-RDase genes and three new genes encoding putative reductive dehalogenases that cluster with CE-RDases and not with CP-RDases. In addition, primers designed to be specific for the three known CE-RDase genes, namely *pceA* of *S. multivorans*, *pceA* of *D. restrictus*, and *tceA* of *Dehalococcoides ethenogenes* were successfully tested on genomic DNA of different chloroethene-dehalorespiring bacteria. Nested PCR using degenerate primers followed by a PCR with specific primers allowed a sensitive detection of only 102 copies per reaction. (

Rezanka, T., L. Hanus, et al. (2003). "Chagosensine, a new chlorinated macrolide from the Red Sea sponge *Leucetta chagosensis*." *European Journal of Organic Chemistry*(20): 4073-4079.

Chagosensine, a sixteen-membered chlorinated macrolide, was isolated from the Red Sea calcareous sponge *Leucetta chagosensis*. Its structure was elucidated mainly on the basis of NMR spectroscopic data. The relative and absolute configurations were determined by analysis of H-1 and C-13 NMR, NOESY, and CD data, by the modified Mosher's method, and by using degradation products. ((C) Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2003).

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.

Rybkina, D. O., E. G. Plotnikova, et al. (2003). "A new aerobic gram-positive bacterium with a unique ability to degrade ortho- and para-chlorinated biphenyls." *Microbiology* 72(6): 672-677.

Strain B51 capable of degrading polychlorinated biphenyls (PCB) was isolated from soil contaminated with wastes from the chemical industry. Based on its morphological and chemotaxonomic characteristics, the strain was identified as a *Microbacterium* sp. Experiments with washed cells showed that strain B51 is able to degrade ortho- and para-substituted mono-, di-, and trichlorinated biphenyls (MCB, DCB, and TCB, respectively). Unlike the known PCB degraders, *Microbacterium* sp. B51 is able to oxidize the ortho-chlorinated ring of 2,2'-DCB and 2,4'-DCB and the para-chlorinated ring of 4,4'-DCB. The degradation of 2,4'-DCB and 4,4'-DCB was associated with the accumulation of 4-chlorobenzoic acid (4-CBA) in the medium in amounts comprising 80-90% of the theoretical yield. The strain was able to utilize 2-MCB, 2,2'-DCB, and their intermediate 2-CBA and to oxidize the mono(ortho)-chlorinated ring of 2,4,2'-TCB and the di(ortho-para)-chlorinated ring of 2,4,4'-TCB. A mixed culture of *Microbacterium* sp. B51 and the 4-CBA-degrading bacterium *Arthrobacter* sp. H5 was found to grow well on 1 g/l 2,4'-DCB as the sole source of carbon and energy.

Schmidt, T. C., L. Zwank, et al. (2004). "Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges." *Analytical and Bioanalytical Chemistry* 378(2): 283-300.

Compound-specific stable isotope analysis (CSIA) using gas chromatography-isotope ratio mass spectrometry (GC/IRMS) has developed into a mature analytical method in many application areas over the last decade. This is in particular true for carbon isotope analysis, whereas measurements of the other elements amenable to CSIA (hydrogen, nitrogen, oxygen) are much less routine. In environmental sciences, successful applications to date include (i) the allocation of contaminant sources on a local, regional, and global scale, (ii) the identification and quantification of (bio)transformation reactions on scales ranging from batch experiments to contaminated field sites, and (iii) the characterization of elementary reaction mechanisms that govern product formation. These three application areas are discussed in detail. The investigated spectrum of compounds comprises mainly n-alkanes, monoaromatics such as benzene and toluene, methyl tert-butyl ether (MTBE), polycyclic aromatic hydrocarbons (PAHs), and chlorinated hydrocarbons such as tetrachloromethane, trichloroethylene, and polychlorinated biphenyls (PCBs). Future research directions are primarily set by the state of the art in analytical instrumentation and method development. Approaches to utilize HPLC separation in CSIA, the enhancement of sensitivity of CSIA to allow field investigations in the $\mu\text{g L}^{-1}$ range, and the development of methods for CSIA of other elements are reviewed. Furthermore, an alternative scheme to evaluate isotope data is outlined that would enable estimates of position-specific kinetic isotope effects and, thus, allow one to extract mechanistic chemical and biochemical information.

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.

Sergejevova, M. and J. Ruzicka (2003). "Potentials of aerobic microbial degradation of trichloroethene." *Chemicke Listy* 97(10): 986-990.

The article reviews contemporary basic knowledge of microbial degradation of trichloroethene. It gives a number of literature data on key enzymes, appropriate microorganisms, degradation products and also suggests unclarified problems of the process. Perspectives of microbiological decontamination of underground waters by possibly complete mineralization of the pollutant are discussed. The key role is attributed to the choice of an appropriate microorganism.

Siddique, T., B. C. Okeke, et al. (2003). "Biodegradation kinetics of endosulfan by *Fusarium ventricosum* and a *Pandoraea* species." *Journal of Agricultural and Food Chemistry* 51(27): 8015-8019.

Endosulfan, classified as an organochlorine pesticide, is rated by the U.S. EPA as a Category 1 pesticide with extremely high acute toxicity. This study describes the biodegradation kinetics of endosulfan and the metabolic pathway utilized by *Fusarium ventricosum* and a *Pandoraea* sp. Complete disappearance of both alpha- and beta-endosulfan was observed during 12 days of incubation with *F. ventricosum* in flasks containing 100 mg L⁻¹ of endosulfan. The rate constants (k) for biodegradation of alpha- and beta-endosulfan by *F. ventricosum* using zero-order kinetics were 14.22 and 6.60 mg L⁻¹ day⁻¹, respectively. The *Pandoraea* sp. degraded about 95 and 100% of alpha- and beta-endosulfan, respectively, in 18 days of incubation in flasks spiked with 100 mg L⁻¹ of endosulfan. The rate constants (k) for biodegradation of alpha- and beta-endosulfan by the *Pandoraea* sp. were 8.19 and 3.78 mg L⁻¹ day⁻¹, respectively. Both fungal and bacterial strains formed less toxic endosulfan diol and endosulfan ether as metabolites during metabolism of endosulfan. The results of this study suggest that these novel strains may be used for the bioremediation of endosulfan-contaminated sites.

Slater, G. E. (2003). "Stable isotope forensics - When isotopes work." *Environmental Forensics* 4(1): 13-23.

Stable isotopic analysis-particularly compound specific stable carbon isotopic analysis-is being increasingly investigated and applied as a tool to investigate and monitor the sources and fates of contaminant compounds in the environment. Results of an increasing number of studies indicate that stable isotopic analysis is a promising tool in environmental chemistry. This paper discusses reported results and presents a case study of stable carbon isotopic analysis of volatile organic groundwater contaminants to illustrate the present abilities and limitations of the application of stable isotopic analysis to environmental contaminants. Though this paper focuses on stable carbon isotopic analysis of volatile organic compounds, the principles discussed herein are relevant to all applications of isotopic analysis as an environmental forensic tool.

Slater, G. F., B. S. Lollar, et al. (2003). "Carbon isotope fractionation of PCE and TCE during dechlorination by vitamin B12." *Ground Water Monitoring and Remediation* 23(4): 59-67.

Reductive dechlorination of perchloroethylene (PCE) and trichloroethylene (TCE) by vitamin B12 is an analogue of the microbial reductive dechlorination reaction and is presently being applied as a remediation technique. Stable carbon isotopic analysis, an effective and powerful tool for the investigation and monitoring of contaminant remediation, was used to characterize the isotopic effects of reductive dechlorination of PCE and TCE by vitamin B12 in laboratory microcosms. In laboratory experiments, 10 mg/L vitamin B12 degraded >90% of the initial 20 mg/L PCE with TCE, the primary product of PCE degradation, accounting for between 64% and 72% of the PCE degraded. In experiments with TCE, 147 mg/L vitamin B12 degraded >90% of the initial 20 mg/L TCE with cis-dichloroethene (cDCE), the primary product of degradation accounting for between 30% and 35% of the TCE degraded. Degradation of both PCE and TCE exhibited first-order kinetics. Strong isotopic fractionation of the reactant PCE and of the reactant TCE was observed over the course of degradation. This fractionation could be described with a Rayleigh model using enrichment factors of -16.5parts per thousand and -15.8parts per thousand for PCE, and -17.2parts per thousand and -16.6parts per thousand for TCE. Fractionation was similar in all experiments, with a mean enrichment factor of -16.5parts per thousand +/-0.6parts per thousand. The occurrence of such large enrichment factors indicates that isotopic analysis can be used to monitor the dechlorination of PCE and

TCE by vitamin B 12 and remediation of ground water plumes. Evidence indicates that isotopic fractionation is taking place during complexation of the chlorinated ethenes to vitamin B12, as has been suggested for reductive dechlorination by zero valent iron. The differences between epsilon values for this reaction and those observed for anaerobic biodegradation of the chlorinated ethenes suggest that there may be differences in the rate-determining step for these two processes.

Song, J. S., D. H. Lee, et al. (2003). "Characteristics of several bacterial isolates capable of degrading chloroaliphatic compounds via hydrolytic dechlorination." *Journal of Microbiology* 41(4): 277-283.

Haloaliphatic hydrocarbons have been widely used as solvents and ingredients of pesticides and herbicides. However, when these compounds contaminate the environment, they can be very hazardous to animals and humans because of their potential toxicity and carcinogenicity. Therefore, lots of studies have been made for microbial degradation of those pollutant chemicals. In this study, 11 bacterial strains capable of degrading 1,2-dichloroethane (1,2-DCA), 2-chloropropionic acid (2-CPA), 2,3-dichloropropionic acid (2,3-DCPA), and 2-monochloroacetic acid (2-MCA) by hydrolytic dechlorination under aerobic conditions were isolated from wastewaters and rice paddy soil samples. Their morphological and biochemical characteristics and their degradation capabilities of haloaliphatic hydrocarbons were examined. On the basis of the 16S rDNA sequences, 8 different kinds of microbial species, including *Pseudomonas plecoglossicida*, *Xanthobacter flavus*, *Ralstonia eutropha*, were identified. All of the isolated strains can degrade MCA. In particular, strains UE-2 and UE-15 degraded 1,2-DCA, and strain CA-11 degraded 2,3-DCPA, which are hardly degraded by other strains.

Suhara, H., C. Daikoku, et al. (2003). "Monitoring of white-rot fungus during bioremediation of polychlorinated dioxin-contaminated fly ash." *Applied Microbiology and Biotechnology* 62(5-6): 601-607.

Bioremediation is a low-cost treatment alternative for the cleanup of polychlorinated-dioxin-contaminated soils and fly ash when pollution spread is wide-ranging. An interesting fungus, *Ceriporia* sp. MZ-340, with a high ability to degrade dioxin, was isolated from white rotten wood of a broadleaf tree from Kyushu Island in Japan. We have attempted to use the fungus for bioremediation of polychlorinated-dioxin-contaminated soil on site. However, we have to consider that this trial has the potential problem of introducing a biohazard to a natural ecosystem if this organism is naturalized. We have therefore developed a monitoring system for the introduced fungus as a part of the examination and evaluation of bioremediation in our laboratory. We have also developed a PCR-based assay to reliably detect the fungus at the bioremediation site. DNA isolated from the site was amplified by PCR using a specific primer derived from internal transcribed spacer region (ITS: ITS1, 5.8S rDNA and ITS2) sequences of *Ceriporia* sp. MZ-340. We successfully monitored *Ceriporia* sp. MZ-340 down to 100 fg/mul DNA and down to 2 mg/g mycelium. We also successfully monitored the fungus specifically at the bioremediation site. The polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran content was observed to decrease in response to treatment with the fungus. The species-specific PCR technique developed in the present work is useful in evaluating the possibility of on-site bioremediation using the fungus *Ceriporia* sp. MZ-340.

Sun, Y. W., X. J. Lu, et al. (2004). "An analytical solution of tetrachloroethylene transport and biodegradation." *Transport in Porous Media* 55(3): 301-308.

In this manuscript, we consider a transport system with a dechlorination reaction network, in which tetrachloroethylene (PCE) reacts to produce trichloroethylene (TCE), TCE reacts to form three daughter products, cis-1,2-dichloroethylene (cis-1,2-DCE), trans-1,2-dichloroethylene (trans-1,2-DCE), and 1,1-dichloroethylene (1,1-DCE), three DCEs further react to produce vinyl chloride (VC), finally VC reacts to produce ethylene (ETH). Because the partial differential equation describing the reactive transport of VC, is coupled by three reactant concentrations, currently the problem must be solved numerically. Following Lu et al. (2003), we extend the analytical solution from five species to the entire PCE reaction network. Using the singular value decomposition (SVD) the system of transport equations with convergent reactions is decoupled into seven orthogonal subsystems. Previously published analytical solutions of single species transport become the basic solutions in the transformed domain for each independent subsystem. The solutions in real concentration domain are obtained using the inverse

transform. The solution derived in this study can then be used instead of Sun et al. (1999) in BIOCHLOR for simulating more realistic systems of biodegradation and reactive transport.

Sung, P. J., T. Y. Fan, et al. (2003). "Juncin N, a new briarane-type diterpenoid from the gorgonian coral *Junceella juncea*." *Heterocycles* 61: 587-+.

A new chlorinated briarane-type diterpenoid, juncin N (1), has been isolated from the gorgonian coral *Junceella juncea*. The structure, including the relative configuration of the new compound (1), was elucidated by the combination of extensive spectral data analysis, especially in 1D and 2D NMR.

Tang, L. X., A. E. J. van Merode, et al. (2003). "Steady-state kinetics and tryptophan fluorescence properties of halohydrin dehalogenase from *Agrobacterium radiobacter*. Roles of W139 and W249 in the active site and halide-induced conformational change." *Biochemistry* 42(47): 14057-14065.

Halohydrin dehalogenase (HheC) from *Agrobacterium radiobacter* AD1 is a homotetrameric protein containing four tryptophan residues per subunit. The fluorescence properties of the enzyme are strongly influenced by halide binding. To examine the role of the tryptophans (W139, W192, W238, and W249) in halide binding and catalysis, they were individually mutated to a phenylalanine. All mutations, except for W238F, influenced the enzymatic properties. Mutating W192 to phenylalanine inactivated the enzyme and led to dissociation into dimers and monomers. In the structure of HheC, residue W139 and residue W249 from the opposite subunit are close to the active site of the enzyme. Substitution of W139 mainly affected K_m values with all tested substrates and reduced the enantioselectivity for *p*-nitro-2-bromo-1-phenylethanol. Replacing W249 increased both k_{cat} and K_m values with all tested substrates except for the (S)-enantiomer of *p*-nitro-2-bromo-1-phenylethanol, for which k_{cat} was 3-fold decreased, resulting in a 6-fold increase of the enantioselectivity. Fluorescence measurements revealed that in the ligand-free state the intrinsic protein fluorescence of mutant W139F is higher than that of the wild-type enzyme, while the fluorescence intensity of mutants W238F and W249F was lower. The fluorescence intensities of the W238F and W249F enzymes were increased when they were unfolded or when bromide was added, whereas the fluorescence of mutant W139F was not increased by unfolding or addition of bromide. These results demonstrate that the fluorescence of residues W238 and W249 is partially quenched in the folded ligand-free state, and that W139 is completely quenched and acts as an energy acceptor for the other tryptophan residues as well. Changes of the maximum fluorescence emission wavelength of the HheC variants and the results of acrylamide quenching experiments confirmed that bromide binding induces a local conformational change around the active site, resulting in residue W139 and the quencher group being separated.

Teplitski, M., H. C. Chen, et al. (2004). "Chlamydomonas reinhardtii secretes compounds that mimic bacterial signals and interfere with quorum sensing regulation in bacteria." *Plant Physiol.* 134(1): 137-146.

Chlamydomonas reinhardtii was found to secrete substances that mimic the activity of the N-acyl-L-homoserine lactone (AHL) signal molecules used by many bacteria for quorum sensing regulation of gene expression. More than a dozen chemically separable but unidentified substances capable of specifically stimulating the LasR or CepR but not the LuxR, AhyR, or CviR AHL bacterial quorum sensing reporter strains were detected in ethyl acetate extracts of *C. reinhardtii* culture filtrates. Colonies of *C. reinhardtii* and *Chlorella* spp. stimulated quorum sensing-dependent luminescence in *Vibrio harveyi*, indicating that these algae may produce compounds that affect the AI-2 furanosyl borate diester-mediated quorum sensing system of *Vibrio* spp. Treatment of the soil bacterium *Sinorhizobium meliloti* with a partially purified LasR mimic from *C. reinhardtii* affected the accumulation of 16 of the 25 proteins that were altered in response to the bacterium's own AHL signals, providing evidence that the algal mimic affected quorum sensing-regulated functions in this wild-type bacterium. Peptide mass fingerprinting identified 32 proteins affected by the bacterium's AHLs or the purified algal mimic, including GroEL chaperonins, the nitrogen regulatory protein PII, and a GTP-binding protein. The algal mimic was able to cancel the stimulatory effects of bacterial AHLs on the accumulation of seven of these proteins, providing evidence that the secretion of AHL mimics by the alga could be effective in disruption of quorum sensing in naturally encountered bacteria.

Triska, J., G. Kuncova, et al. (2004). "Isolation and identification of intermediates from biodegradation of low chlorinated biphenyls (Delor-103)." *Chemosphere* 54(6): 725-733.

Microorganism *Pseudomonas* species P2 metabolizes polychlorinated biphenyls (PCBs) and biphenyl, producing the whole spectrum of intermediates, among them coloured intermediates, which are suitable for the monitoring of PCBs degradation by optical sensors. Knowledge of chemical structures and conditions of development of colour metabolites is necessary for application of optical analytical methods. The main goal of this work was the isolation and identification of intermediates from the biodegradation of the mixture of low chlorinated biphenyls (Delor-103), which is based on the solid phase extraction (SPE) of the whole mixture using LiChrolut EN cartridges, then silylation of the extract as one way to the identification of one part of intermediates by GC-MS, and acetylation of the extract as a way for the further concentration and analysis of more polar chlorobiphenylols and chlorobiphenyldiols. The combination of SPE and following acetylation allows to obtain chlorobiphenylols and chlorobiphenyldiols as an almost pure fraction. The acetylation method could be also used instead SPE procedure with the same final concentration effect. Using the simulation mass spectrometry program, two new compounds, dihydrodihydroxytrichloro- and tetrahydrodihydroxytrichlorobiphenyl, as silyl derivatives, were identified. (C) 2003 Elsevier Ltd. All rights reserved.

Vogt, C., D. Simon, et al. (2004). "Microbial degradation of chlorobenzene under oxygen-limited conditions leads to accumulation of 3-chlorocatechol." *Environmental Toxicology and Chemistry* 23(2): 265-270.

Five bacterial strains (*Acidovorax facilis* 13517, *Cellulomonas turbata* B529, *Pseudomonas veronii* 13547, *Pseudomonas veronii* B549, and *Paenibacillus polymyxa* B550) isolated on chlorobenzene as the sole source of carbon and energy were screened for the accumulation of the putative metabolic intermediate 3-chlorocatechol during growth on chlorobenzene under oxygen-limited conditions in the presence and absence of nitrate (1 mM). 3-Chlorocatechol accumulated in the growth media of all five strains, but accumulation was significantly less in cultures of *A. facilis* B517 compared to the other four strains. The presence of nitrate did not influence the biological conversion pattern. However, biologically produced nitrite reacted with 3-chlorocatechol chemically, a reaction that masked the accumulation of 3-chlorocatechol. For *P. veronii* B549, a clear relationship between the presence of 3-chlorocatechol in the medium and low oxygen concentrations was demonstrated. The assumption is made that accumulation of 3-chlorocatechol is due to the low enzymatic turnover of the 3-chlorocatechol cleaving enzyme, catechol-1,2-dioxygenase, at low oxygen concentrations.

Ward, M. J., Q. S. Fu, et al. (2004). "A derivative of the menaquinone precursor 1,4-dihydroxy-2-naphthoate is involved in the reductive transformation of carbon tetrachloride by aerobically grown *Shewanella oneidensis* MR-1." *Applied Microbiology and Biotechnology* 63(5): 571-577.

Transformation of carbon tetrachloride (CT) by *Shewanella oneidensis* MR-1 has been proposed to involve the anaerobic respiratory-chain component menaquinone. To investigate this hypothesis a series of menaquinone mutants were constructed. The *menF* mutant is blocked at the start of the menaquinone biosynthetic pathway. The *menB*, *menA* and *menG* mutants are all blocked towards the end of the pathway, being unable to produce 1,4-dihydroxy-2-naphthoic acid (DHNA), demethyl-menaquinone and menaquinone, respectively. Aerobically grown mutants unable to produce the menaquinone precursor DHNA (*menF* and *menB* mutants) showed a distinctly different CT transformation profile than mutants able to produce DHNA but unable to produce menaquinone (*menA* and *menG* mutants). While DHNA did not reduce CT in an abiotic assay, the addition of DHNA to the *menF* and *menB* mutants restored normal CT transformation activity. We conclude that a derivative of DHNA, that is distinct from menaquinone, is involved in the reduction of CT by aerobically grown *S. oneidensis* MR-1. When cells were grown anaerobically with trimethylamine-N-oxide as the terminal electron acceptor, all the menaquinone mutants showed wild-type levels of CT reduction. We conclude that *S. oneidensis* MR-1 produces two different factors capable of dehalogenating CT. The factor produced under anaerobic growth conditions is not a product of the menaquinone biosynthetic pathway.

Yeager, C. M., K. M. Arthur, et al. (2004). "Trichloroethylene degradation by toluene-oxidizing bacteria grown on non-aromatic substrates." *Biodegradation* 15(1): 19-28.

The potential of trichloroethylene (TCE) to induce and non-aromatic growth substrates to support TCE degradation in five strains (*Pseudomonas mendocina* KR1, *Ralstonia pickettii* PKO1, *Pseudomonas putida* F1, *Burkholderia cepacia* G4, *B. cepacia* PR1) of toluene-oxidizing bacteria was examined. LB broth and acetate did not support TCE degradation in any of the wild-type strains. In contrast, fructose supported the highest specific levels of TCE oxidation observed in each of the strains tested, except *B. cepacia* G4. We discuss the potential mechanisms and implications of this observation. In particular, cells of *P. mendocina* KR1 degraded significant amounts of TCE during cell growth on non-aromatic substrates. Apparently, TCE degradation was not completely constrained by any given factor in this microorganism, as was observed with *P. putida* F1 (TCE was an extremely poor substrate) or *B. cepacia* G4 (lack of oxygenase induction by TCE). Our results indicate that multiple physiological traits are required to enable useful TCE degradation by toluene-oxidizing bacteria in the absence of aromatic cosubstrates. These traits include oxygenase induction, effective TCE turnover, and some level of resistance to TCE-mediated toxicity.