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

6th Quarterly Report
2nd Quarter Year 2002

Report prepared for EUROCHLOR

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ACRONYMS

CAR	Carbazole
CB	Chorobenzene
CDDs	Chlorinated dibenzo- <i>p</i> -dioxins
CDFs	Chlorinated dibenzo- <i>p</i> -furans
CF	Chloroform
CT	Carbon tetrachloride
CPY	Cytochrome P450
2,4-D	2,4-dichlorophenoxyacetate
1,2-DCA	1,2-dichloroethane
<i>Cis</i>-DCE	<i>Cis</i> -1,2-dichloroethene
<i>Trans</i>-DCE	<i>Trans</i> -1,2-dichloroethene
DCM	Dichloromethane
DDD	1,1-dichloro-2,2- bis(<i>p</i> -chlorophenyl)-ethane
DDE	1,1-dichloro-2,2-bis(<i>p</i> - chlorophenyl)-ethylene
DhIA	Haloalkane Dehalogenase A
DiCDD	Di-chlorinated dibenzo- <i>p</i> -dioxins
DDT	1,1,1-trichloro-2,2-bis(<i>p</i> -chlorophenyl)-ethane
DF	Dibenzofuran
DGGE	Denaturing Gradient Gel Electrophoresis
DNAPL	Dense Non-Aqueous Phase Liquid
E-acceptor	Electron Acceptor
E-donor	Electron Donor
ETH	Ethene
FISH	Fluorescent <i>In Situ</i> Hybridization
HCB	Hexachlorobenzene
HCH	Hexachlorohexane
PCBs	Polychlorinated Biphenyls
PCE	Tetrachloroethylene
PCR	Polymerase Chain Reaction
TCE	Trichlorethylene
TeCDD	Tetra-chlorinated dibenzo- <i>p</i> -dioxins
VC	Vinyl Chloride

ACRONYMS (Continued)

VFA	Volatile Fatty Acids
ZVI	Zero Valent Iron
VOC	Volatile Organic Carbon

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1. INTRODUCTION

This report presents a review of scientific literature published during the second quarter of 2002 (covering June to August, 2002) on the microbial halogenation and dehalogenation of the following compounds: vinyl chloride, dichloroethane, chloroform, dichloromethane, hexachlorobenzene, chlorobenzene, 1,2,4-1,2,3-1,3,5-trichlorobenzene, hexachlorobutadiene, octachlorostyrene, dioxins and chlorinated furans. In addition, reports regarding the microbial chlorination of compounds structurally related to those listed above were also reviewed.

2. SUMMARY OF MOST IMPORTANT DEVELOPMENTS

2.a. Microbial Dechlorination

The most important findings on microbial dechlorination in this quarter are several reports on the aerobic degradation of lower chlorinated ethenes (8, 48), 1,2-dichloroethane (8), as well as several reports on the aerobic degradation of chlorinated dioxins (19, 20, 21, 38). An enrichment culture was reported that could aerobically degrade vinyl chloride (VC) as a sole source of carbon and energy (48). Although similar findings are known from the past, this new report also demonstrates how *cis*-dichloroethene (*cis*-DCE) could be cometabolized with VC as the primary substrate, which is a new finding. Another study reports for the first time the isolation of a bacterium (*Polaromonas vacuolata*) which is able to degrade *cis*-DCE as a sole

carbon and energy source (8). Previously, only aerobic cometabolism of *cis*-DCE was known. The study goes on to demonstrate that other chlorinated solvents could be cometabolized by *P. vacuolata* utilizing *cis*-DCE as the primary substrate (growth substrate). One of these compounds was 1,2-dichloroethane.

This quarter is also characterized by numerous reports of chlorinated dibenzo-*p*-dioxin (CDDs) degradation by aerobic microorganisms. Among the most noteworthy of these is the report of the bacterium *Sphingomonas wittichi* strain KA1 capable of oxidizing 2,7-dichloro- and 1,2,3,4-tetrachloro-dibenzo-*p*-dioxin to metabolites which were identified as chlorocatechols (and chloroguaiacols) (21). Also noteworthy is a report of a genetically modified yeast (*Saccharomyces cerevisiae*) which received cytochrome P450 genes from a mammal (rat) and was shown to be capable of oxidizing polychlorinated dibenzo-*p*-dioxins (2,7-dichloro-CDD and 2,3,7-trichloro-CDD) to a variety of metabolites, including metabolites formed by loss of chlorine and ring-opening (38).

2.b. Microbial Chlorination

The most important highlight from this quarter is the discovery of the natural formation of vinyl chloride (VC) in the terrestrial environment (24). The article reports on significant natural formation of VC in soils. VC formation is demonstrated upon wetting dried soils. A mechanism is proposed by which VC is formed during the oxidation of *o*-quinone or catechol groups in humus by Fe(III) in the presence of chloride. The authors provide evidence to support the proposed mechanism, and they demonstrate VC formation from the oxidation of catechol as well as demonstrate enhanced formation of VC in soils by addition of oxidants.

3. MICROBIAL DECHLORINATION

3.a. General Reviews

In this quarter there were four review articles on microbial dechlorination. These include: one review on polychlorinated biphenyl (PCB) degradation (1), one review on chemotaxis of microorganisms to chlorinated compounds and priority pollutants (34), and two reviews on phytoremediation, including reference to chlorinated compounds (17, 42).

The review on PCB provides an overview of the current knowledge of aerobic and anaerobic degradation of PCBs in microbial consortia and in the environment, including novel approaches to determine *in situ* PCB degradation (1). The article observes that PCB-degrading microbial communities are quite diverse. The recent advances under anaerobic conditions are observations of isotope fractionation and preferred enantiomer degradation providing new information on degradation of PCBs. Also the first defined community capable of halo-respiration of PCBs (organisms able to grow using PCB chlorine as electron acceptor (e-acceptor)) has been described, and important community members have been identified. The recent advances in aerobic PCB degradation is the discovery of an antibiotic compound, protoanemonin, as a dead-end intermediate or in other cases as a temporal intermediate.

Chemotaxis, the ability of motile bacteria to detect and respond to specific chemicals in the environment, can increase an organism's chances of locating useful sources of carbon, nitrogen and energy, and could thus play an important role in the biodegradation process. A review article from this quarter compiles information on evidence for chemotaxis towards environmental pollutants (34). Evidence is given for 3- and 4-chlorobenzoate, 2,4-dichlorophenoxyacetate (2,4-D), *cis*-dichloroethene (*cis*-DCE), trichloroethene (TCE) and perchloroethylene (PCE).

Phytoremediation is a bioremediation technology that uses plants to degrade, assimilate, metabolize, or detoxify metals, hydrocarbons, pesticides, and chlorinated solvents. This quarter one review article covers *in situ*, *in vivo* and *in vitro* methods of application for describing remediation of these compounds (42). The role of enzymes in transforming organic chemicals in plants is also presented. Data is reviewed for degradation of chlorinated compounds in the rhizosphere (zone of influence in soil by roots where microorganisms probably have an important impact). The chlorinated compounds reviewed are chlorobenzoates, PCE, TCE, PCB's, trichloro- and hexachlorobenzene. Another review article proposes a model to describe the transformation of environmentally widespread persistent organic pollutants (including DDT, HCH, PCBs) in the air-plant-soil system (17).

3.b. Microbial Dechlorination

Vinyl chloride and Other Chlorinated Ethenes

As indicated in previous reports, a large number of studies involve research evaluating the degradation of the higher chlorinated ethenes, perchloroethylene (PCE) and trichloroethene (TCE) because these are major groundwater contaminants. Thus information regarding the

degradation of lower chlorinated ethenes, vinyl chloride and dichloroethenes, is found in studies evaluating the higher chlorinated ethenes.

Vinyl Chloride (VC) and Dichloroethenes (DCE). An aerobic bacterial enrichment culture, capable of growing on VC as the sole source of carbon and energy is described. The enrichment culture is able to cometabolize *cis*-DCE while growing on VC (48). Also *trans*-DCE is cometabolized but to a lesser extent. *Pseudomonas aeruginosa* strain MF1 (previously isolated from the enrichment culture) also readily cometabolizes *cis*-DCE. The initial intermediates of the oxidation of both DCE isomers were the corresponding epoxides of DCE isomers. The presence of *cis*-DCE was shown to reduce the rate at which VC is oxidized. The article provides kinetic information. The rate of VC degradation and of *cis*-DCE degradation by VC-grown cells of *P. aeruginosa* strain MF1 was 0.41 and 0.44 $\mu\text{mol}/\text{mg}$ total suspended solids/day, respectively. The corresponding half-velocity constants were 0.26 and 22 μM , respectively. The results taken as a whole can explain observations at contaminated sites, that the removal of *cis*-DCE is associated with the removal of VC. It is also noted that *cis*-DCE is the most common isomer of DCE during anaerobic bioremediation of PCE/TCE and thus it is also fortunate that it is the easiest isomer to be cometabolized by aerobic bacteria.

Another aerobic bacterial isolate was found that could utilize *cis*-DCE as a sole carbon and energy source, which is the first time *cis*-DCE has been observed without cometabolism (8). Based on 16S-ribosomal DNA analysis, the microorganism was found to be the β -proteobacterium, *Polaromonas vacuolata*. Based on the high level of recovery of inorganic chloride, the complete mineralization of *cis*-DCE chlorine was demonstrated. The bacterium had a doubling time of 73 h. The half-velocity constant for *cis*-DCE transformation was 1.6 μM , and the maximum specific substrate utilization rate ranged from 12.6 to 16.8 $\text{nmol}/\text{min}/\text{mg}$ of protein. Cells cultivated on *cis*-DCE could oxidize VC as well as a number of other chlorinated compounds: *trans*-DCE, TCE, ethene and 1,2-dichloroethane (1,2-DCA). Epoxide was formed from ethene, suggesting an epoxidation reaction as the first step in degradation of *cis*-DCE.

Laboratory microcosms were established from groundwater and sediments at Dover Air Force Base (Dover, DE, USA), which is a large contaminated PCE site. The microcosms were used to obtain direct evidence for the microbial degradation of PCE and its intermediates under a variety of redox conditions (8). Evidence was established that VC and *cis*-DCE could be mineralized under aerobic conditions throughout the Dover site. Also evidence was found for the anaerobic reductive dehalogenation for all steps between PCE to ethene. Enrichment cultures capable of the oxidative degradation of *cis*-DCE and VC were obtained from

groundwater across the aquifer demonstrating the possible importance of direct (meaning use as sole carbon and sole energy source), non-cometabolic oxidation of *cis*-DCE and VC in natural attenuation. Using 16S-ribosomal DNA probes, recovery of sequences affiliated with phylogenetic groups containing known anaerobic-halorespiring organisms such as *Desulfitobacterium* and *Dehalobacter* provided qualitative support for a role of reductive dechlorination processes in the aquifer. The presence and distribution of microorganisms was found to be consistent with a microbially-driven attenuation of chlorinated ethenes within the aquifer, suggesting that both reductive and oxidative mechanisms are involved which is in accordance with the geochemistry of the site (having a heterogenous distribution of anaerobic and oxic zones).

In a companion article, the hydrogeochemistry of the Dover Air Force Base site was surveyed in relation to the natural attenuation of PCE (49). Chlorinated hydrocarbon and biogeochemical data were used to develop a site-specific conceptual model where both anaerobic and aerobic biological processes are responsible for the destruction of PCE, TCE, and daughter metabolites. Regions of depleted dissolved oxygen with elevated dissolved methane and hydrogen concentrations were observed. In these anaerobic regions, reductive dechlorination most likely dominated as evidenced by declines in PCE and TCE levels associated with a simultaneous increase in *cis*-DCE, VC, ethene, and dissolved chloride. Near the anaerobic/aerobic interface, concentrations of *cis*-DCE and VC decreased to below detection limits, presumably due to aerobic biotransformation processes. In conclusion, the contaminant and daughter product plumes appear to have been naturally attenuated by a combination of active anaerobic and aerobic biotransformation processes.

A pilot study was conducted at Idaho National Engineering and Environmental Laboratory's field site to study carbon isotopic effects during the anaerobic bioremediation of TCE utilizing lactate as an electron donor (e-donor) (39). The isotope ratios of TCE and its biodegradation by products, *cis*-DCE, *trans*-DCE, VC, and ethene, in groundwater samples collected during the pilot study were preconcentrated with a combination of purge-and-trap and cryogenic techniques in order to allow for reproducible isotopic measurements of the compounds at low concentrations. Isotope monitoring allowed for the distinction between groundwater transport, DNAPL dissolution, and enhanced bioremediation. In the bioremediation zone (where lactate was added) kinetic isotope effects were found to be the largest during the reduction of *cis*-DCE to VC, and VC to ethene. Eventually, despite initial large isotopic variations, ethene in all these wells reached the carbon isotope ratios of the initial dissolved TCE, confirming the complete conversion of dissolved TCE to ethene. The isotope

ratios of *trans*-DCE were never affected indicating that this intermediate was not biodegraded at the site.

Perchloroethylene (PCE) and Trichloroethene (TCE). Anaerobic reductive dechlorination of chlorinated ethenes was studied in batch and in continuous columns utilizing a mixture of six volatile fatty acids (VFA: acetic, propionic, butyric, valeric and caproic acids) as e-donor (28). The fates of PCE, *cis*-DCE and VC were evaluated in the presence and absence of the VFA mixture. The results showed that VFAs stimulated complete reductive dechlorination of chlorinated ethenes, either as direct substrates for the dechlorinating bacteria or via H₂ formed during VFA-degradation. There was sequential utilization of different VFAs by fermenting bacteria. In the batch microcosm, propionic acid was the first to be used, followed by acetic, butyric, isobutyric, valeric, and isovaleric acids.

A microcosm study was established to study the combined anaerobic-aerobic degradation of PCE in closed serum bottles (29). Using anaerobic sludge as an inoculum, either molasses, gelatine and a polylactate polymer (known as HRC) were used as a slow release e-donor to promote the reductive dechlorination of PCE. Oxygen was periodically added to the headspace to promote aerobic degradation of reduced products from PCE. Both TCE and DCE were detected from the reductive dechlorination of PCE, but not VC nor ethene. ¹⁴C-PCE was mineralized extensively (60%) in 7 days to ¹⁴CO₂, demonstrating the extensive degradation of PCE in a system with both anaerobic and aerobic microenvironments.

The bioremediation of chloroethene- and nickel-contaminated sediment in a single anaerobic step under sulfate-reducing conditions was evaluated in a sediment column study (14). By stimulating the activity of sulfate-reducing bacteria through the addition of sulfate as supplementary e-acceptor and lactate as e-donor, complete dehalogenation of PCE and TCE to ethane and ethane was achieved. Chloride production was detected in the effluent of the columns, suggesting mineralization of organochlorines. Aside from ethene and ethane, other intermediates (eg. DCE) were only detected sporadically. The results of this study demonstrated that microbial activity stimulated under sulfate-reducing conditions can have a beneficial effect on both the precipitation of heavy metals and the complete dechlorination of organochlorines. Sulfide formed during sulfate reduction precipitated Ni in the sediment. The strongly negative redox potential created by the activity of sulfate-reducing bacteria may be one factor responsible for stimulating the activity of the dehalogenating bacteria in the test columns.

Natural attenuation of PCE in a mixture with toluene and carbon tetrachloride (1-10 mg/L) was investigated in a controlled experiment in which the test compounds were introduced to the aquifer using diffusive emitters (12). PCE transformation products identified included trichloroethene and *cis*-DCE, albeit only at trace levels. The authors estimated the half-life

values for toluene (58-62 days) and carbon tetrachloride (11-13 days). The half-life value for PCE could not be estimated reliably because the compound degraded too slowly.

The combined effect of zero valent iron (ZVI) and microorganisms on the reduction of TCE was evaluated in a column packed with granular ZVI (18). Bioaugmenting the column with the iron-reducing bacterium, *Shewanella algae* strain BRY, improved the reductive dechlorination of TCE from 50-70% to 80%. Microbial colonization of the ZVI-granules was confirmed with scanning electron microscopy.

Another hydrogeochemical analysis was conducted at a Superfund site containing TCE and an unusual copollutant, tetra kis(2-ethylbutoxy)silane (27). Geochemical analysis of contaminated groundwater indicated subsurface anaerobic zones, where reductive dechlorination of TCE to predominantly *cis*-DCE occurred. 16S rDNA analysis of the DNA extracted from the site indicated the occurrence of bacteria closely related to the TCE-dechlorinating, DCE-accumulating genus *Dehalobacter*, whereas the TCE-dechlorinating, ethene-producing species *Dehalococcoides ethenogenes* was not detectable. The geochemical and microbiological evidence was thus in agreement.

The effect of TCE bioavailability on TCE cooxidation by an aerobic bacterium, *Burkholderia cepacia*, was evaluated (15). This investigation was undertaken to evaluate the effectiveness of *B. cepacia* to regenerate used sorbents by degrading TCE from the sorbent directly or indirectly. The results of this investigation showed that *B. cepacia* was capable of reducing TCE attached to PM-100 clay but at significantly reduced rate due to the slow desorption rate. Conversely, it was shown that *B. cepacia* was capable of degrading TCE dissolved in n-hexadecane at the same rate as systems without n-hexadecane present. The reduction in TCE degradation when the TCE is attached to the PM-100 clay could be overcome by solvent rinsing the TCE from the clay with subsequent removal of the TCE from the n-hexadecane by *B. cepacia*.

Three additional reports on the microbial dechlorination of TCE are discussed elsewhere (In 3.d. *New Tools to Assess the Biodegradation of Chlorinated Compounds*) (5, 9, 36).

Carbon Tetrachloride (CT) and Chloroform (CF)

Anaerobic biotransformation of CT under various e-acceptor conditions was investigated using enrichment cultures (2). CT was biotransformed under sulfate-reducing, methanogenic, nitrate-reducing, iron-reducing, fermenting, and mixed e-acceptor conditions. The fastest removal of CT was observed under mixed e-acceptor conditions (degradation rate 0.039 $\mu\text{mol CT/day/mg}$ protein) followed in order by sulfate-reducing, methanogenic, fermenting, iron-reducing and nitrate-reducing conditions (CT degradation rate from 0.0013-0.0018 $\mu\text{mol CT/day/mg}$ protein).

Under mixed e-acceptor conditions, the CT was converted to methyl chloride, which was further metabolized. Under sulfate, iron, denitrifying, and methanogenic conditions, the major metabolite produced from CT metabolism was chloroform (CF). Under fermenting conditions, dichloromethane was produced from CT metabolism.

In situ natural attenuation of CT in groundwater contaminated with a mixture of CT, PCE and toluene (conc. 1-10 mg/L) was investigated in a controlled field experiment (12). The test compounds were introduced to the aquifer using diffusive emitters. CT degraded with a half-life of 11-13 days. By comparison, toluene degraded with a half-life of 58-62 days and PCE degraded too slowly for a reliable estimate of rate to be made. Chloroform (CF), an intermediate of CT degradation, was assessed using the flux estimates and was found to degrade with a half-life in the range of 10-34 days. CF was the only chlorinated methane detected, suggesting that the CT was completely dechlorinated by natural processes.

Chloromethane (CM) and Dichloromethane (DCM)

The methylotroph, *Methylobacterium chloromethanicum* CM4, was shown to require a specific set of tetrahydrofolate-dependent enzymes for growth with chloromethane (41). Experimental evidence for the existence of a C₁ oxidation pathway specific for CM in *M. chloromethanicum* CM4 was obtained by transcriptional analysis, gene inactivation studies, and enzyme measurements. The tetrahydrofolate-dependent enzymes (CmuA and CmuB) were previously purified and shown to catalyze the dehalogenation of CM *in vitro*. Dehalogenation occurs by the vitamin B₁₂-mediated transfer of the methyl group of CM to tetrahydrofolate.

The ability of methylotrophic α -proteobacteria to grow with DCM as source of C and energy is generally thought to depend solely on a single enzyme, DCM dehalogenase. This enzyme converts DCM to formaldehyde, a central intermediate of methylotrophic growth. A recent study provides evidence that additional genes and proteins may be required for growth of *Methylobacterium* strains with DCM (23).

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

Only one publication was found in this quarter that is concerned with the dechlorination of 1,2-DCA (8). Resting cells of an aerobic bacterium grown on *cis*-DCE were shown to be able to dechlorinate 1,2-DCA. The microorganism, a β -proteobacterium resembling *Polaromonas vacuolata* (97.9% identity of 16S rRNA sequence), could also transform ethene, VC, *trans*-DCE, TCE, and 1,2-DCA. An additional publication reports on the dechlorination of various chlorinated ethanes such as hexachloroethane, pentachloroethane, 1,1,1,2-tetrachloroethane,

and 1,1,2,2- tetrachloroethane by a purified reductive dehalogenase (PceA) obtained from *Desulfitobacterium* sp. strain Y51 (43).

Chlorobenzenes

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

Hexachlorobenzene (HCB):

An anaerobic enrichment culture was obtained from river sediments with the ability to dechlorinate hexachlorobenzene (HCB). The impact of an e-donor (lactate) and three e-acceptors (bicarbonate, sulfate and nitrate) on HCB dechlorination by the culture was investigated (6). The culture was acclimated to HCB in a yeast extract amended culture for 300 days. The acclimated culture was able to dechlorinate HCB (8 mM) to the major product 1,3,5-trichlorobenzene in about 4 days. Addition of lactate, or replacement of yeast extract with lactate, did not affect the dechlorination rate. Addition of sulfate to the HCB dechlorinating consortium generally had little or no effect on the dechlorination rate. Complete inhibition of HCB dechlorination in the mixed culture was observed under denitrifying conditions (Nitrate was added). Dechlorination was inhibited by 2-bromoethanesulfonate (methanogen inhibitor) but not by vancomycin (eubacteria inhibitor), suggesting that methanogens may play an important role in HCB dechlorination.

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

In this quarter, three studies report on the degradation of chlorinated dibenzo-*p*-dioxins (CDDs) by different strains of various aerobic bacteria: *Sphingomonas* sp. (16, 19, 21), *Terrabacter* sp. (20), and *Klebsiella* sp. (16). An additional study investigated the biodegradation of CDDs by a recombinant yeast (38).

Sphingomonas sp. strain KA1, an aerobic strain carrying a carbazole (CAR) dioxygenase gene homologue, was shown to have the ability of degrading CDDs in soil (19). Inoculation of strain KA1 into dioxin-contaminated model soil resulted in 96% and 70% degradation of 2-mono- and 2,3-di-CDD, respectively, after 7 days of incubation. Dibenzofuran-grown resting cells of *Sphingomonas wittichii* RW1 were shown to degrade the diaryl ethers, 2,7-dichloro-CDD (diCDD) and 1,2,3,4-tetra-CDD (TeCDD) (21). Biotransformation of 2,7-diCDD resulted in the formation of 4-chlorocatechol, while microbial degradation of 1,2,3,4-TeCDD

led to formation of the metabolites, 3,4,5,6-tetrachlorocatechol and 2-methoxy-3,4,5,6-tetrachlorophenol (tetrachloroguaiacol). In addition, the authors showed that strain RW1 transformed 3,4,5,6-tetrachlorocatechol to the corresponding tetrachloroguaiacol, an activity not previously described for this organism. The pathways proposed for the biotransformation of 2,7-diCDD and 1,2,3,4-TeCDD by *S. wittichii* RW1 are shown in Figure 1. The turnover rates determined for these two CDDs were very low, only 1-2% of the turnover rate previously reported for the non-chlorinated dibenzo-*p*-dioxin by *S. wittichii* RW. This is the first report describing aerobic biotransformation of 2,7-diCDD and 1,2,3,4-TeCDD by a bacterial strain together with identification of the corresponding metabolites.

Isolation and characterization of two novel aerobic bacteria capable of utilizing dibenzofuran (DF) as a sole carbon source, *Sphingomonas* sp. strain HL7 and *Klebsiella* sp. strain HL1, was reported (16). The *Sphingomonas* sp. strain degraded non-, mono- and also dichlorinated DF and dibenzo-*p*-dioxins. *Klebsiella* sp. strain HL1 was able to degrade non- and monochlorinated DFs and DDs, but not dichlorinated ones. The metabolites formed from DF by strains HL1 and HL7 were similar to those by the well-characterized dioxin-degrading bacteria *Sphingomonas* sp. strain RW1 except for salicylic acid and catechol. The two strains were shown to have dioxygenase genes encoding a DF degradation activity. The dioxygenase genes of strains HL1 and HL7 were different from each other, while those of *Sphingomonas* sp. strain HL7 and RW1 were alike.

Biotransformation of CDDs or CDFs in soil by the dibenzofuran-degrader *Terrabacter* sp. strain DBF63 was investigated (20). The aerobic strain degraded 90% of 2,8-diCDF (dichlorinated dibenzofuran, conc. 1 mg/L), but only 40% of 2,3-diCDD (conc. 1 mg/L), during a 7-day incubation period in a soil slurry system. In the same system, the biodegradation of 2-diCDF, 2-diCDD, 2,8-diCDF and 2,3-diCDF were approximately 89%, 65%, 78% and 32%, respectively. *Terrabacter* sp. strain degraded mono- to diCDFs more effectively than mono- to diCDDs. Microbial metabolites of the CDD/Fs were not characterized. Bioremediation experiments with dioxin-contaminated soil from an incineration site (total conc. of CDD/Fs 725 ng/g soil) showed that approximately 10% of tetra- to hexa-chlorinated congeners were decreased by a single inoculation with DBF63 cells after a 7-day incubation. These results suggest a potential for applying strain DBF63 to bioremediate soil polluted with dioxins.

Biodegradation of polychlorinated-*p*-dioxins by recombinant yeast expressing rat cytochrome P450 (CPY) 1A enzyme subfamily (CPY1A) was demonstrated (38). Either one of two genes of this subfamily, CPY1A1 and CPY1A2, were cloned into the yeast, *S. cerevisiae*. When each of the dibenzo-*p*-dioxins, mono-, di-, and tri-CDDs, were added to the cell culture of the recombinant yeast, metabolites were detected. The metabolites were formed by either

hydroxylation at an unsubstituted position, hydroxylation with migration of a chloride substituent, hydroxylation with elimination of a chloride substituent, and/or by dioxin ring opening. In most cases, the yeast expressing CPY1A1 showed much higher activity than CPY1A2. Kinetic studies revealed that 2,7-diCDD and 2,3,7-triCDD were good substrates for both CYP1A1 and CYP1A2. Yeast cells expressing each of rat CYP1A1 and CYP1A2 transformed 2,3,7-triCDD to 8-hydroxy-2,3,7-triCDD, an intermediate that was further metabolized to more hydrophilic compounds. Based on these results, the authors suggest the possibility of applying microorganisms expressing mammalian cytochrome P450 to bioremediation of contaminated soils with dioxins.

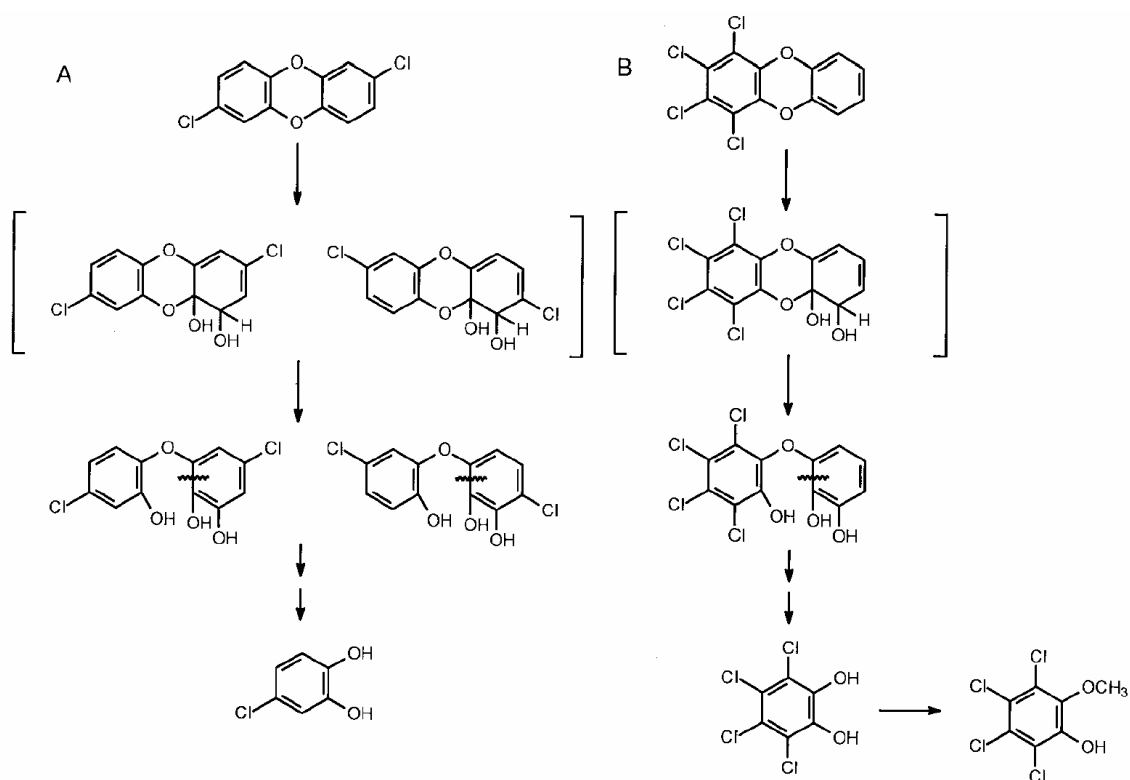


Fig. 1. Proposed pathways for the biotransformation of 2,7-dichlorodibenzo-p-dioxin (A) and 1,2,3,4-tetrachlorodibenzo-p-dioxin (B) by *Sphingomonas wittichii* RW1 (21).

Hexachlorobutadiene and Octachlorostyrene

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

Polychlorinated Biphenyls (PCBs)

Five publications concerned with the microbial dechlorination of PCBs were found this quarter. Aerobic degradation of commercial PCB mixtures (Delor 103 and Delor 106) by twelve different bacterial isolates obtained from PCB-contaminated sediments was investigated (46). Two out of the 12 strains, *Pseudomonas alcaligenes* KP2 and *P. fluorescens* KP12, were shown to significantly cometabolize Delor 103. Several 2,3-dihydroxybiphenyl dioxygenase genes involved in aerobic degradation of PCB by the aerobic bacterium, *Rhodococcus* sp strain RHA1, were characterized in another study (37). Studies with another aerobic microorganism, *Ralstonia eutropha* H850, showed that alterations in fatty acid composition and fluidity of cell membrane affected the accumulation of the PCB congener 2,2',5,5'-tetrachlorobiphenyl by the strain (25). Furthermore, two additional studies focusing on a new approach to assess the potential for *in situ* bioremediation of PCBs (22), and the susceptibility of PCBs to biodegradation (26) are discussed elsewhere (in 3.d. *New Tools to Assess the Biodegradation of Chlorinated Compounds*). Finally, a study was published on the enzymes involved in plant metabolism of PCBs (7). Compound transformation by peroxidases and oxidases were studied using various inhibitors and inducers of peroxidases and cytochrome P450. It was shown that both enzymatic systems are partially involved in the detoxification mechanism of PCBs in plants.

Miscellaneous

Several reports on the microbial degradation of miscellaneous chlorinated compounds are listed here. Two studies were published on the distribution and fate of DDE (13, 50), HCH (50), and other organochlorine pesticide residues (13) in river sediments. Substantial biodegradation of gamma-hexachlorocyclohexane (lindane, γ -HCH) and alpha-hexachlorocyclohexane (α -HCH) in water and a soil slurry by a species of the aerobic bacterium, *Pandoraea* sp. was reported (32). Rapid dehalogenation of trichloroacetate by the PCE reductive dehalogenase of *Dehalospirillum multivorans* and its corrinoid cofactor was demonstrated (30). Finally, a microorganism was constructed that is capable of growth on 1,2,3-trichloropropane (3). The microorganism was engineered using a combined strategy of random mutagenesis of haloalkane dehalogenase and genetic engineering of a chloropropanol- utilizing bacterium.

3.c. In Vitro Degradation of Chlorinated Compounds

In this quarter, six articles reported on the *in vitro* degradation of chlorinated compounds by enzymes. Four articles are concerned with haloalkane or haloacid dehalogenases. Two articles report on PCE dehalogenases.

Haloalkane dehalogenases catalyze the hydrolysis of haloalkanes to their corresponding alcohols and inorganic halides. The first article reports on the use of haloalkane dehalogenase (DhlA) as a model protein to explore the possibility of using molecular dynamics simulations as a tool to identify flexible regions in proteins that can serve as a target for stability enhancement by introduction of a disulfide bond (35). DhlA consists of two domains: an α/β -hydrolase fold main domain and a cap domain composed of five α -helices. Molecular dynamics simulations of DhlA showed high mobility in a helix-loop-helix region in the cap domain, involving residues 184-211. A disulfide cross-link was engineered between residue 201 of this flexible region and residue 16 of the main domain. The mutant enzyme showed substantial improvements in thermal stability as well as resistance to urea denaturation.

Molecular dynamics trajectories of three haloalkane dehalogenases (DhlA, LinB, and DhaA) were compared in the second article (33). The main domain was rigid in all three dehalogenases, whereas the substrate specificity-modulating cap domains showed considerably higher mobility. The functionally relevant motions were spread over the entire cap domain in DhlA, whereas they were more localized in LinB and DhaA. The exchange of water molecules between the enzyme active site and bulk solvent was very different among the three dehalogenases. The differences could be related to the flexibility of the cap domains and to the number of entrance tunnels.

Haloacid dehalogenases are hydrolytic enzymes that cleave the halogen-carbon bond(s) in haloalkanoic acids, yielding hydroxy- or oxyalkanoic acids. One article reports on a cryptic haloacid dehalogenase gene (*chd1*) from the bacterium, *Burkholderia cepacia*, which was expressed in *Escherichia coli* (47). The recombinant protein (CHD1) is unusual in having a long leader sequence, a property of periplasmic enzymes. In this paper, the functional role of this leader sequence is reported.

Two haloacetate dehalogenase genes, *dehH1* and *dehH2*, on the 65-kb plasmid pUO1 from the bacterium *Delftia acidovorans* strain B were reported to be located on transposable elements (40). A transposable element is a section of DNA that can jump at random to other locations in the genome, creating natural mutations. One of the transposons was functional and the other was defective.

PCE dehalogenases catalyze the reductive dechlorination of PCE. In the first article the substrate specificity of the tetrachloroethene reductive dehalogenase of *Dehalospirillum multivorans* and its corrinoid cofactor were studied (30). The dehalogenase could utilize methylviologen and titanium(III) as e-donors and PCE and TCE as substrates yielding *cis*-DCE as a product. In addition to chlorinated ethenes, chlorinated propenes were reductively dechlorinated solely by the native enzyme. These included: *trans*-1,3- dichloropropene, 1,1,3-trichloropropene and 2,3-dichloropropene, which were reduced to a mixture of monochloropropenes, 1,1- dichloropropene, and 2-chloropropene, respectively. Other halogenated compounds that were rapidly reduced by the enzyme were also dehalogenated abiotically by the heat-inactivated enzyme and by commercially available cyanocobalamin (vitamin B₁₂). The corrinoid cofactor purified from the PCE dehalogenase had 50- fold higher activity than that of vitamin B-12 with trichloro acetate as e-acceptor.

The second article, reports on the purification and characterization of PCE dehalogenase (PceA) of *Desulfitobacterium* sp. strain Y51 (43). The expression of the enzyme was induced in the presence of PCE and TCE. The purified enzyme catalyzed the reductive dehalogenation of PCE via TCE to *cis*-DCE. The specific activity was 113.6 nmol/min/mg protein. The apparent Km values for PCE and TCE were 105.7 and 535.3 μ M, respectively. No other chloroethenes were substrates, but the enzyme could reductively dechlorinate several chloroethanes such as hexachloroethane, pentachloroethane, 1,1,1,2-tetrachloroethane, and 1,1,2,2- tetrachloroethane. Immunoblot analyses located PceA in the periplasm (space between cell membrane and outer envelope) of the cell.

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

Estimation of Susceptibility to Biodegradation

Photochemical oxidation of PCBs in soils was proposed as a tool to assess the degradability of these halogenated compounds (26). Analysis of photooxidation (UV irradiation with TiO₂ as a catalyst) results for 12 PCBs in various soil types showed that the method considered proved to be unsuitable to predict PCB biodegradability.

New Methods for Compound Detection

A flow type microbial biosensor for direct measurement of TCE was developed [Han, 2002 #321]. The microbial sensor is based on the use of the TCE degrading bacterium *Pseudomonas*

aeruginosa J1104 and the electrical detection of the chloride ion released by microbial degradation in a closed, glass flow cell. The sensor showed a linear response in the range from 0.03 to 2 mg TCE/L.

Characterization of Microbial Populations

Three publications report on the application of molecular methods to characterize the structure of different microbial communities capable of dechlorinating PCE (9) and TCE (27, 36). The publication by Lowe and coworkers (27) was already discussed in heading “3.b. Microbial dechlorination: Vinyl chloride (VC) and other chlorinated ethenes”.

Microbial characterization of an aquifer contaminated with chlorinated ethenes (Area 6, Dover Air Force Base, Dover, IL) was undertaken by cultivation and direct recovery of 16S rRNA gene sequences (9). Results from these studies demonstrated that the aquifer contained relatively low biomass ($< 10^7$ bacteria/g of sediment) including physiologically diverse microorganisms (*i.e.*, iron reducers, acetogens, sulfate reducers, denitrifiers, aerobic and anaerobic heterotrophs). 16S rRNA probing suggested the presence of anaerobic microorganisms distributed primarily between the delta subdivision of the Proteobacteria and low- G + C gram positive. Recovery of sequences affiliated with phylogenetic groups containing known anaerobic-halorespiring organisms (eg. *Desulfitobacterium*, *Dehalobacter*) and certain groups of iron reducers indicated a role of reductive dechlorination processes in the aquifer. These results suggest that both reductive and oxidative microbial mechanisms are involved in the natural attenuation of chlorinated ethenes observed in the aquifer.

An anaerobic enrichment culture isolated from a TCE-contaminated site was characterized using three molecular methods concurrently: clone library construction/clone sequencing, terminal restriction fragment length polymorphism (T-RFLP) analysis, and fluorescent *in situ* hybridization (FISH) with rRNA probes (36). The culture was able to reductively dechlorinate TCE to ethene with transient accumulation of *cis*-DCE and VC using lactate as e-donor. The first two molecular approaches indicated that the dominant microbes in the enrichment culture belonged to three phylogenetic groups: *Dehalococcoides* species, *Desulfovibrio* species, and members of the *Clostridiaceae*. In addition, FISH results suggested that members of the *Cytophagav* / *Flavobacterium* / *Bacteroides* (CFB) cluster and high G + C Gram-positives were also important in the culture. *Dehalococcoides* is the only genus of bacteria known to completely reduce TCE to ethene.

Potential for *In Situ* Remediation

A polymerase chain reaction (PCR)-based assay was developed that could prove useful to determine the potential for *in situ* PCB bioremediation by the indigenous microorganisms in contaminated sediments (22). The authors used PCR of DNA and RNA extracted from aquatic sediments and amplified the biphenyl dioxygenase gene, *bphA1*. Various studies have shown that the BphA1 gene product determines PCB congener specificity, and therefore this assay could be a rapid method to screen for *in situ* aerobic catabolic activities for PCB mixtures in aquatic sediments.

Modeling of Phytoremediation

A model is presented which attempts to describe phytoremediation of TCE (5). The model takes into account root interactions with soil, water, and VOCs in time and space, as well as advective and dispersive transport in unsaturated soil. The developed model considers liquid-gas mass exchanges and permits root growth in the soil. VOC absorption and subsequent uptake into the roots with water were simulated using empirical equations. In addition, microbial activity in the rhizosphere, a zone of unique interactions between the roots and the soil microorganisms, was included in the model. Simulation results showed a greater degradation of TCE in the presence of cotton compared to the degradation in the absence of cotton.

4. MICROBIAL CHLORINATION

4.a. General Reviews

In this quarter there was one review article on the natural chlorine cycle (31). This report debates the previous contention that organochlorines are only xenobiotic. Evidence is presented that chlorine is involved in complex biogeochemical cycles, that chlorine is one of the major elements of soil organic matter, and that the amount of naturally-formed organic chlorine present in the environment can be counted in tons per km². The review article pieces together scattered information from different disciplines to gain a better insight in the natural chlorine cycle question. The present review enlightens four paradoxes based on preconceived ideas: 1) chlorinated organic compounds are xenobiotic even though more than 1,000 naturally produced chlorinated compounds have been identified; 2) only a few, rather specialized, organisms are able to convert chloride to organic chlorine even though it appears as if the ability among organisms to transform chloride to organic chlorine is more the rule than the exception; 3) all

chlorinated organic compounds are persistent and toxic even though the vast majority of naturally produced organic chlorine is neither persistent nor toxic; 4) chlorine is mainly found in its ionic form in the environment even though organic chlorine is as abundant or even more abundant than chloride in soil. Finally the article provides an outline of the terrestrial chlorine cycle by constructing a rough chlorine budget over a small forested catchment.

Furthermore, Dembitsky and coworkers published two reviews of naturally-occurring halogenated fatty acids (11) and halogenated alkaloids (10). Sources of fatty acids containing chlorine and/or other halogen atoms covalently bonded to carbon halogenated fatty acids include microorganisms, algae, marine invertebrates, higher plants, and some animals. The review identifies over 300 different halogenated fatty acids produced by different types of microorganisms and discusses their possible biological significance. In the second publication, the taxonomic distribution, structure, and biological activity of bromo- and iodo-containing alkaloids isolated from marine microorganisms and sponges were reviewed (10). The structures of nearly 140 natural bromo- and iodo-containing alkaloids are shown in the review paper. Bromine atoms are most commonly found in the halogenated alkaloids, whereas iodine atoms are very rare. Bromoalkaloids are predominantly found in marine eukaryotes but are practically absent from terrestrial plants and animals. Like the brominated alkaloids, chloro-containing alkaloids are widely distributed in nature and have been isolated until now from microorganisms, plants, terrestrial animals, and marine invertebrates. Chloroalkaloids are now considered in this literature review.

4.b. Microbial Chlorination in Soils

Chloromethanes

Reports on the formation of chloromethanes by soil microorganisms were not found in the second quarter of 2002.

Other Chlorinated Compounds

Keppler *et al.* (24) demonstrated for the first time that vinyl chloride can be formed naturally in the terrestrial environment. In this study, volatile chlorinated hydrocarbons were measured in ambient air and soil air from coastal salt marsh, peatland, and deciduous forest. Wetting of dried soils resulted in significant enhanced VC production beyond background in soil air. Although the microbial dechlorination of CH_3CCl_3 , TCE, and PCE could potentially account for the measured VC, the tropospheric concentration of these three known precursors of VC

were too low to account for the VC production observed. The mean concentration of VC in the topsoil air was in the range of 0.6-7.7 ng/L, which was up to 850 times greater than ambient air. Laboratory experiments using different soils and model compounds proved that VC could be produced during soil processes (Figure 2). VC formation is proposed to occur during the oxidative degradation of organic matter in soil, eg., in a reaction between humic substances, chloride ions and an oxidant (ferric ions or hydroxyl radicals). The redox-sensitive aromatic compounds in humus such as catechols and *o*-quinones are hypothesized to be the precursors of VC. During the reaction of catechol and Fe(III) in the presence of chloride, CO₂ production was shown to be accompanied by additional volatile breakdown products such as VC and other monochlorinated alkanes. In additional experiments, the influence of Fe(III) on VC formation was confirmed with natural soils (Figure 3). The production of VC was also accompanied by other chlorinated alkanes (chloromethane, chloroethane, chloropropane). A scheme depicting the proposed mechanism is shown in Figure 4. Possible VC formation by biotic soil processes was not investigated in this study.

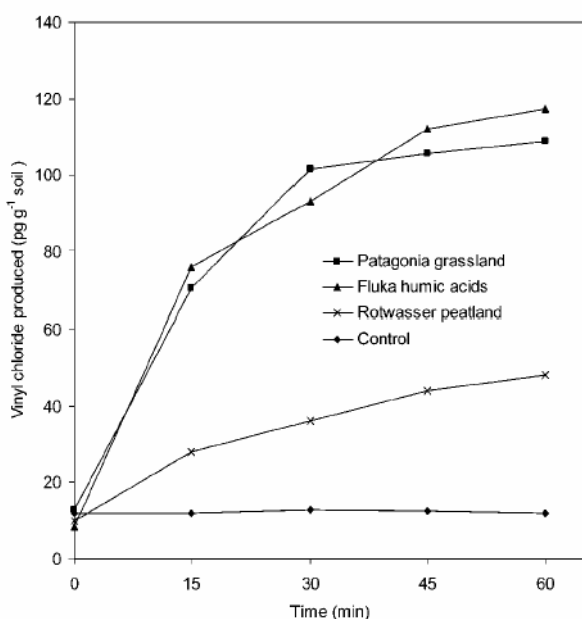


Fig. 2. Formation of VC by two organic-rich soils and humic acid. A total of 1000 mg of grassland, peatland, or humic acid was added to 10 mL of water in a 20-mL glass vial (n=3; RSD 12-33%). The pH in the medium was 4.1, 4.2, and 5.4 for grassland, peatland, and humic acid, respectively. (from ref. 24)

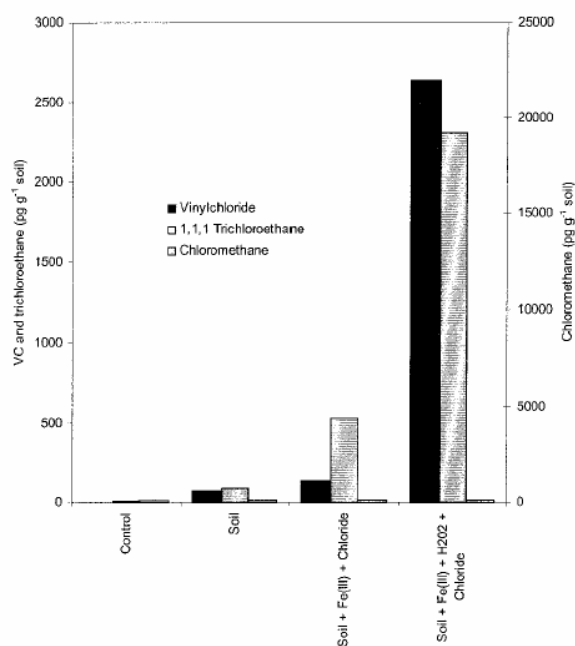


Fig. 3. Influence of Fe(III) and H₂O₂ on the formation of VC and chloromethane in soil. A total of 1000 mg of grassland soil from Patagonia was suspended in 10 mL of water containing 100 μmol of KCl. Before the flask was sealed, 100 μmol oxidant was added to the medium (n=3; RSD 4-48%). The pH in the medium was 2.5-3.2. (from ref. 24)

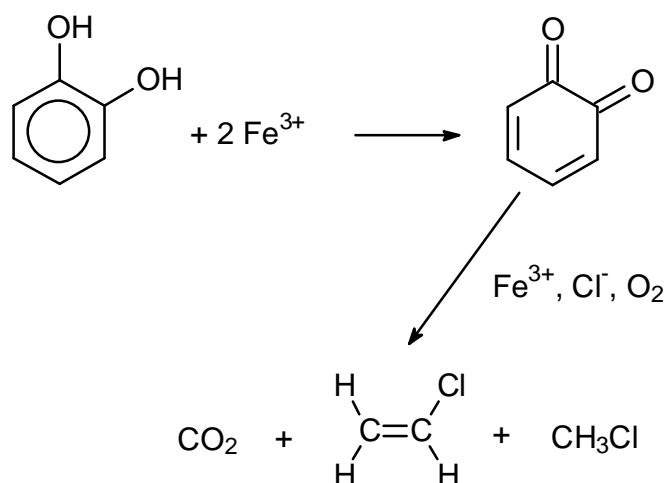


Fig. 4. Scheme depicting the proposed mechanism of natural vinyl chloride formation in soils via oxidation of catechol moieties in soil organic matter by ferric iron. (from ref. 24)

4.c. Chlorination by Marine and Freshwater Organisms

Chloromethanes

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

Other Chlorinated Compounds

Two different studies report on the production of halogenated terpenoids by red algae. Two new halogenated sesquiterpenes, oxachamigrene and 5-acetoxyoxachamigrene, have been isolated from the red alga *Laurencia obtusa* (4). Both compounds contained a chlorine and a bromine atom and they had empirical formulas of $\text{C}_{15}\text{H}_{24}\text{OBrCl}$ and $\text{C}_{17}\text{H}_{26}\text{O}_3\text{BrCl}$, respectively. The structures of the compounds were determined on the basis of spectroscopic evidence. A biogenetic route for these metabolites has been proposed. Two novel brominated labdane-type diterpenoids, along with known halogenated compounds, 2,10-dibromo-3-chloro-alpha-chamigrene, and microcladallene A, were isolated and identified from an Okinawan *Laurencia* sp. (44). The structures of these new compounds were established as ent-labdane-type bromoditerpenes. Both compounds were found to be inactive against several pathogenic bacteria. Species of the red algal genus *Laurencia* are well known to be prolific sources of diverse halogenated secondary metabolites, particularly terpenoids and C_{15} -acetogenins.

4.d. Chlorinating Enzymes

In this quarter the only article on a halogenating enzyme is a acid phosphatase from *Shigella flexneri* and *Salmonella enterica*. Acid phosphatases, vanadium haloperoxidases, and the bacterial class A nonspecific acid phosphatases have a conserved active site. It is shown that vanadate-substituted recombinant acid phosphatase from *S. flexneri* and *S. enterica* in the presence of H₂O₂ are able to oxidize bromide to hypobromous acid (45). Vanadate is essential for activity. The kinetic parameters for the artificial bromoperoxidases were determined. The half velocity coefficient (K_m) value for H₂O₂ is about the same as that for the vanadium bromoperoxidases from the seaweed *Ascophyllum nodosum*. However, the K_m value for Br⁻ is about 10-20 times higher, and the turnover values are much slower than those of the native bromoperoxidase. Thus despite the striking molecular similarities of the active sites, acid phosphatases is not optimized for haloperoxidase activity.

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50. **Zhang, Z. L., H. S. Hong, J. L. Zhou, G. Yu, W. Q. Chen, and X. H. Wang.** 2002. Transport and fate of organochlorine pesticides in the River Wuchuan, Southeast China. *Journal of Environmental Monitoring* **4**:435-441.

7. ANNEX

Abraham, W. R., B. Nogales, et al. (2002). "Polychlorinated biphenyl-degrading microbial communities in soils and sediments." *Current Opinion in Microbiology* 5(3): 246-253.

Recent advances in the degradation of polychlorinated biphenyls (PCBs) have focussed on the use of experimental enrichment cultures to obtain PCB-degrading communities, and the use of culture-independent approaches to characterize natural and experimental PCB-degrading communities and to identify the key members in this process. PCB-degrading communities can be surprisingly diverse. Novel types of composite bacteria-mineral biofilm communities have been described. Community metabolism of PCBs may lead to the formation of protoanemonin, a dead-end product in some instances but, in others, a seemingly productive intermediate. Analysis of isotope fractionation and preferred enantiomer degradation has provided new information on degradation of PCBs in anaerobic settings. The first defined community capable of dehalorespiration of PCBs has been described, and important community members identified. Here, we provide an overview of the current knowledge of aerobic and anaerobic degradation of PCBs in microbial consortia and in the environment, including novel approaches to determine in situ PCB degradation.

Boopathy, R. (2002). "Anaerobic biotransformation of carbon tetrachloride under various electron acceptor conditions." *Bioresource Technology* 84(1): 69-73.

The biotransformation of carbon tetrachloride (CT) under various electron acceptor conditions was investigated using enrichment cultures developed from the anaerobic digester sludge of Thibodaux sewage treatment plant. The results indicated that CT was biotransformed under sulfate-reducing, methanogenic, nitrate-reducing, iron-reducing, fermenting, and mixed electron acceptor conditions. However, the rates of CT removal varied among the conditions studied. The fastest removal of CT (100% removal in 12 days) was observed under mixed electron acceptor conditions followed in order by sulfate-reducing, methanogenic, fermenting, iron-reducing, and nitrate-reducing conditions. Under mixed electron acceptor conditions, the CT was converted to methyl chlorides, which was further metabolized. Under sulfate, iron, nitrate-reducing, and methanogenic conditions, the major metabolite produced from CT metabolism was chloroform (CF). Under fermenting conditions, methylene chloride was produced from CT metabolism. This study showed evidence for CT metabolism in a mixed microbial population system similar to many contaminated field sites where a heterogeneous microbial population exists. (C) 2002 Published by Elsevier Science

Bosma, T., J. Damborsky, et al. (2002). "Biodegradation of 1,2,3-trichloropropane through directed evolution and heterologous expression of a haloalkane dehalogenase gene." *Applied and Environmental Microbiology* 68(7): 3582-3587.

Using a combined strategy of random mutagenesis of haloalkane dehalogenase and genetic engineering of a chloropropanol- utilizing bacterium, we constructed an organism that is capable of growth on 1,2,3-trichloropropane (TCP). This highly toxic and recalcitrant compound is a waste product generated from the manufacture of the industrial chemical epichlorohydrin. Attempts to select and enrich bacterial cultures that can degrade TCP from environmental samples have repeatedly been unsuccessful, prohibiting the development of a biological process for groundwater treatment. The critical step in the aerobic degradation of TCP is the initial dehalogenation to 2,3-dichloro-1-propanol. We used random mutagenesis and screening on eosin-methylene blue agar plates to improve the activity on TCP of the haloalkane dehalogenase from *Rhodococcus* sp. m15-3 (DhaA). A second-generation mutant containing two amino acid substitutions, Cys176Tyr and Tyr273Phe, was nearly eight times more efficient in dehalogenating TCP than wild-type dehalogenase. Molecular modeling of the mutant dehalogenase indicated that the Cys176Tyr mutation has a global effect on the active-site structure, allowing a more productive binding of TCP within the active site, which was further fine tuned by Tyr273Phe. The evolved haloalkane dehalogenase was expressed under control of a constitutive promoter in the 2,3-dichloro-1- propanol-utilizing bacterium *Agrobacterium radiobacter* AD1, and the resulting strain was able to utilize TCP as the sole carbon and energy source. These results demonstrated that directed evolution of a key catabolic enzyme and its subsequent recruitment by a suitable host organism can be used for the construction of bacteria for the degradation of a toxic and environmentally recalcitrant chemical.

Brito, I., M. Cueto, et al. (2002). "Oxachamigrenes, new halogenated sesquiterpenes from *Laurencia obtusa*." *Journal of Natural Products* 65(6): 946-948.

Two new sesquiterpenes belonging to a novel oxacyclic structural type of chamigrene skeleton, oxachamigrene (1) and 5-acetoxyoachamigrene (2), have been isolated from the red alga *Laurencia obtusa*. The structures of the compounds were determined on the basis of spectroscopic evidence. A biogenetic route for these metabolites has been proposed.

Chang, Y. Y., B. Bae, et al. (2002). "Modelling plant-aided in-situ bioremediation of soils contaminated with a volatile organic compound (VOC)." *Acta Biotechnologica* 22(1-2): 21-34.

In this study, bioremediation of volatile organic compounds (VOCs) in soil, associated with plant root systems, was simulated by developing a mathematical model based on the assumption of homogeneous, isothermal and isotropic soil conditions, with mobile water but without density-driven gas flow. The proposed model includes root interactions with soil and water and VOCs in time and space, as well as advective and dispersive transport in unsaturated soil. The developed model considers liquid-gas mass exchanges and permits root growth in the soil. For the simulation of temporal and spatial changes of the root behaviour in soil and water and with VOCs, the time-specific distribution of root quantity through the soil was incorporated into the simulation model. VOC absorption and subsequent uptake into the roots with water were simulated using empirical equations. In addition, microbial activity in the rhizosphere, a zone of unique interactions between the roots and the soil microorganisms, was included in the model. Cotton was seeded on a hypothetical site contaminated with trichloroethylene (TCE), one of the ubiquitous volatile contaminants at many hazardous waste sites. The soil was assumed to be a sandy loam. Model parameters were independently estimated from the simulation of real data and a range of literature values to obtain a realistic simulation. The simulation results showed a greater degradation of TCE in the presence of cotton compared to the degradation in the absence of cotton. Significant sensitivity of the model to the diffusion in the gas phase shows that the degree of change of volatile organic distribution in the soil may be influenced by the transport of the gas phase as well as the plant-enhanced biodegradation. This mathematical model for understanding and predicting the fate and the transport of volatile compounds in plant-aided remediation will assist the effective application of plant-aided remediation to field contamination.

Chen, I. M., B. V. Chang, et al. (2002). "Reductive dechlorination of hexachlorobenzene under various additions." *Water Air and Soil Pollution* 139(1-4): 61-74.

Microorganisms collected from sediments of Ho-Tsin River in southern Taiwan were used in this study. The ability to dechlorinate hexachlorobenzene (HCB) was induced by enrichment incubation in yeast extract amended culture and acclimation with HCB. Addition of lactate or replacement of yeast extract by lactate did not enhance the dechlorination ability. With strong electron capturing capability, denitrifying bacteria resulted in complete inhibition of dechlorination in the mixed culture contained nitrate. In the culture amended with sulfate, sulfate reducing bacteria shared electrons and nutrient with HCB-dechlorinating consortium but grabbed more electrons when treated with vancomycin. Results from multi-factors tests indicate that the influences of factors on dechlorination were complicated. Dechlorinating microbes, electron suppliers, sulfate reducing bacteria and denitrifying bacteria, all possibly caused a great effect on dechlorination.

Chroma, L., M. Mackova, et al. (2002). "Enzymes in plant metabolism of PCBs and PAHs." *Acta Biotechnologica* 22(1-2): 35-41.

Recently it has been shown that plants are able to transform polychlorinated biphenyls (PCBs) as well as polycyclic aromatic hydrocarbons (PAHs), but the knowledge of enzymes involved in these metabolic processes is limited. Plant peroxidases generally play an important role in plant metabolism. On the other hand, cytochrome P450 is involved in the detoxification of various xenobiotics in the cells of higher organisms. In this work, several in vitro cultures of different plant species were screened for their ability to transform PCBs or PAHs, and compared regarding their total extra- and intracellular peroxidase activity. Cultures with good transformation ability exhibited in the presence of xenobiotics the same or higher levels of peroxidases as the controls incubated without contaminants. Cultures with markedly lower peroxidase activity exhibited also lower PCB/PAH conversion in the presence of PCBs/PAHs. It was attempted to identify lignin peroxidase and Mn-peroxidase in plants, originally described in white rot fungi to be responsible for the degradation of PCBs and other environmental pollutants. In addition to different types of peroxidases, RBBR oxidase was also detected in plants. The decolourisation of RBBR during the growth on agar plates was used as a rough screening method for plant

cells able to metabolise PCBs/PAHs efficiently. The exact type of transformation reaction (peroxidative or oxidative) was studied using various inhibitors and inducers of peroxidases and cytochrome P450. It was shown that both enzymatic systems are partially involved in the detoxification mechanism of chosen xenobiotics in plants.

Coleman, N. V., T. E. Mattes, et al. (2002). "Biodegradation of cis-dichloroethene as the sole carbon source by a beta-proteobacterium." *Applied and Environmental Microbiology* 68(6): 2726-2730.

An aerobic bacterium capable of growth on cis-dichloroethene (cDCE) as a sole carbon and energy source was isolated by enrichment culture. The 16S ribosomal DNA sequence of the isolate (strain JS666) had 97.9% identity to the sequence from *Polaromonas vacuolata*, indicating that the isolate was a beta- proteobacterium. At 20degreesC, strain JS666 grew on cDCE with a minimum doubling time of 73 7 h and a growth yield of 6.1 g of protein/mol of cDCE. Chloride analysis indicated that complete dechlorination of cDCE occurred during growth. The half-velocity constant for cDCE transformation was 1.6 +/- 0.2 muM, and the maximum specific substrate utilization rate ranged from 12.6 to 16.8 nmol/min/mg of protein. Resting cells grown on cDCE could transform cDCE, ethene, vinyl chloride, trans- dichloroethene, trichloroethene, and 1,2-dichloroethane. Epoxyethane was produced from ethene by cDCE-grown cells, suggesting that an epoxidation reaction is the first step in cDCE degradation.

Cortese, M. S., A. Paszczynski, et al. (2002). "Metal chelating properties of pyridine-2,6-bis(thiocarboxylic acid) produced by *Pseudomonas* spp. and the biological activities of the formed complexes." *Biometals* 15(2): 103-120.

We evaluated the ability of pyridine-2,6-bis(thiocarboxylic acid) (pdtc) to form complexes with 19 metals and 3 metalloids. Pdtc formed complexes with 14 of the metals. Two of these metal:pdtc complexes, Co:(pdtc)(2) and Cu:pdtc, showed the ability to cycle between redox states, bringing to 4 the number of known redox-active pdtc complexes. A precipitant formed when pdtc was added to solutions of As, Cd, Hg, Mn, Pb, and Se. Additionally, 14 of 16 microbial strains tested were protected from Hg toxicity when pdtc was present. Pdtc also mediated protection from the toxic effects of Cd and Te, but for fewer strains. Pdtc by itself does not facilitate iron uptake, but increases the overall level of iron uptake of *Pseudomonas stutzeri* strain KC and *P. putida* DSM301. Both these pseudomonads could reduce amorphous Fe(III) oxyhydroxide in culture. In vitro reactions showed that copper and pdtc were required for this activity. This reaction may derive its reducing power from the hydrolysis of the thiocarboxyl groups of pdtc.

Davis, J. W., J. M. Odom, et al. (2002). "Natural attenuation of chlorinated solvents at Area 6, Dover Air Force Base: characterization of microbial community structure." *Journal of Contaminant Hydrology* 57(1-2): 41-59.

A polyphasic approach based on cultivation and direct recovery of 16S rRNA gene sequences was utilized for microbial characterization of an aquifer contaminated with chlorinated ethenes. This work was conducted in order to support the evaluation of natural attenuation of chlorinated ethenes in groundwater at Area 6 at Dover Air Force Base (Dover, DE). Results from these studies demonstrated the aquifer contained relatively low biomass (e.g. direct microscopic counts of < 10(7) bacteria/g of sediment) comprised of a physiologically diverse group of microorganisms including iron reducers, acetogens, sulfate reducers, denitrifiers, aerobic and anaerobic heterotrophs. Laboratory microcosms prepared with authentic sediment and groundwater provided direct microbiological evidence that the mineralization of vinyl chloride and cis-dichloroethene as well as each step in the complete reductive dechlorination of tetrachloroethene to ethene can occur in the Area 6 aquifer. Enrichment cultures capable of the oxidative degradation of cis-1,2-dichloroethene (cis-DCE) and vinyl chloride (VC) were obtained from groundwater across the aquifer demonstrating the possible importance of direct, non-cometabolic oxidation of cis-DCE and VC in natural attenuation. Culture-independent analyses based upon recovery of 16S rRNA gene sequences revealed the presence of anaerobic organisms distributed primarily between two major bacterial divisions: the delta subdivision of the Proteobacteria and low- G + C gram positive. Recovery of sequences affiliated with phylogenetic groups containing known anaerobic-halorespiring organisms such as *Desulfitobacterium*, *Dehalobacter*, and certain groups of iron reducers provided qualitative support for a role of reductive dechlorination processes in the aquifer. This molecular data is suggestive of a functional linkage between the microbiology of the site and the apparent natural attenuation process. The presence and distribution of microorganisms were found to be consistent with a microbially driven attenuation of chlorinated ethenes within the aquifer and in accord with a conceptual model of aquifer geochemistry which suggest that both reductive and

oxidative mechanisms are involved in heterogeneous, spatially distributed processes across the aquifer. (C) 2002 Elsevier Science B.V All rights reserved.

Dembitsky, V. M. (2002). "Bromo- and iodo-containing alkaloids from marine microorganisms and sponges." *Russian Journal of Bioorganic Chemistry* 28(3): 170-182.

The taxonomic distribution, structure, and biological activity of halogenated alkaloids isolated from marine microorganisms and sponges are reviewed. The structures of nearly 140 natural bromo- and iodo-containing alkaloids are shown.

Dembitsky, V. M. and M. Srebnik (2002). "Natural halogenated fatty acids: their analogues and derivatives." *Progress in Lipid Research* 41(4): 315-367.

A comprehensive survey has been made of all fatty acids containing halogen atoms covalently bonded to carbon and which are deemed as naturally occurring. Generally thought to be minor components produced by many different organisms, these interesting compounds now number more than 300. Recent research, especially in the marine area, indicates this number will increase in the future. Sources of halogenated fatty acids include microorganisms, algae, marine invertebrates, and higher plants and some animals. Their possible biological significance has also been discussed (C) 2002 Elsevier Science Ltd. All rights reserved.

Devlin, J. F., M. McMaster, et al. (2002). "Hydrogeologic assessment of in situ natural attenuation in a controlled field experiment." *Water Resources Research* 38(1): U31-U41.

An experiment to investigate the natural attenuation of three volatile organic compounds, toluene, carbon tetrachloride, and tetrachloroethene (similar to 1-10 mg L⁻¹) was performed in a 3 in deep, sandy aquifer isolated within a 24 in long, 2 in wide, three-sided sheet pile alleyway (hereafter referred to as the gate). A constant flow was maintained in the test volume by pumping a well at the closed end of the gate at 130 mL min⁻¹. The test compounds were introduced to the aquifer using diffusive emitters installed inside 25 cm diameter wells located at the open end of the gate. Monitoring was performed by sampling along six multilevel fences (consisting of 12 sampling points each) ranging in distance from 1 to 22 in from the source wells. A bromide tracer experiment established that there were no significant hydraulic leaks, nor was there any continuous channeling through the gate. Degradation of the test compounds was assessed by mass balance calculations between fences located 1 and 7 m from the source, and the results were compared with degradation rate estimates from snapshot analyses and the analysis of fluxes. There was reasonably good agreement between rates estimated by these different methods. Toluene degraded with a half-life of 58-62 days, carbon tetrachloride degraded with a half-life of similar to 11-13 days, and tetrachloroethene degraded too slowly for a reliable estimate of rate to be made. Transformation products identified in the gate included acetate, possibly from toluene degradation, chloroform, trichloroethene, and cis-1,2, dichloroethene. The latter two compounds only appeared in trace quantities and could not be assessed for continuing degradation. However, chloroform degradation was assessed with the snapshot data and using the flux estimates and was found to degrade with a half-life in the range of 10-34 days. No additional chlorinated methanes were detected in the gate, suggesting that the carbon tetrachloride was completely dechlorinated by natural processes within 10 m of the source wells. This experiment demonstrated that degradation of chlorinated solvents occurs naturally at the Borden site but that the ethenes are more resistant to biodegradation than the methanes. In addition, the flux calculations were found to be the most robust in terms of estimating degradation rates.

Doong, R. A., Y. C. Sun, et al. (2002). "Distribution and fate of organochlorine pesticide residues in sediments from the selected rivers in Taiwan." *Chemosphere* 48(2): 237-246.

The contamination of organochlorine pesticides (OCPs) in sediments from selected rivers in Taiwan was investigated to evaluate the pollution potentials and hazard in river sediments. Da-han River and Erh-jen River were selected as the target rivers due to their serious pollution. A total of 40 surface sediment samples were collected at five sampling stations along the rivers. Results showed that the concentrations of various pesticides in sediments were in the range of 0.57-14.1 ng/g for SigmaHCH, 0.05-0.15 ng/g for aldrin, 0.12-5.8 ng/g for dieldrin, 0.22-0.64 for endrin, 0.24- 6.37 ng/g for endosulfan and 0.21-8.81 ng/g for SigmaDDT (p,p'- DDD, p,p'-DDE, p,p'-DDT). Among the OCPs, SigmaHCH, endosulfan and SigmaDDT were the most dominant compounds in the river sediments. Endosulfan sulfate was the most frequent detected compound in the sediments from the selected rivers. Also, SigmaDDT, dieldrin and beta-HCH were in abundance. Different contamination patterns between the selected river sediments were also observed. Da-han River was mainly contaminated with endosulfan sulfate and

SigmaDDT. Whereas the main pesticides in Erh-jen River were beta-HCH and SigmaDDT. Among the cyclodiene compounds, dieldrin was in abundance in most of the sediments. Moreover, the frequencies of detection of the metabolites were higher than those of parent compounds, depicting that the sediments have contaminated for a long time. The results obtained in this study showed that there still exist a variety of OCP residues in the river sediments in Taiwan. (C) 2002 Elsevier Science Ltd. All rights reserved.

Drzyzga, O., R. El Mamouni, et al. (2002). "Dehalogenation of chlorinated ethenes and immobilization of nickel in anaerobic sediment columns under sulfidogenic conditions." *Environmental Science & Technology* 36(12): 2630-2635.

A sediment column study was carried out to demonstrate the bioremediation of chloroethene- and nickel-contaminated sediment in a single anaerobic step under sulfate-reducing conditions. Four columns (one untreated control column and three experimental columns) with sediment from a chloroethene- and nickel-contaminated site were investigated for 1 year applying different treatments. By stimulating the activity of sulfate-reducing bacteria by the addition of sulfate as supplementary electron acceptor, complex anaerobic communities were maintained with lactate as electron donor (with or without methanol), which achieved complete dehalogenation of tetra- and trichloroethenes (PCE and TCE) to ethane and ethane. A few weeks after sulfate addition, production of sulfide increased, indicating an increasing activity of sulfate-reducing bacteria. The nickel concentration in the effluent of one nickel-spiked column was greatly reduced, likely due to the enhanced sulfide production, causing precipitation of nickel sulfide. At the end of the study, 94% of the initial amount of nickel added to that column was recovered in the sediment. As compared to the untreated (nonspiked) control column, all chloroethene-spiked columns (additions of PCE and TCE) showed a permanent release of small chloride ion quantities (similar to 0.5-0.7 mM chloride), which were detected in the effluents a few weeks after sulfide production was observed for the first time. The formation of ethane and ethane as final products after dechlorination of PCE and TCE was detected in some effluents and in some gas phases of the columns. Other metabolites or intermediates (such as DCE isomers) were only detected sporadically in negligible quantities. The results of this study demonstrated that microbial activity stimulated under sulfate-reducing conditions can have a beneficial effect on both the precipitation of heavy metals and the complete dechlorination of organochlorines. The strongly negative redox potential created by the activity of sulfate-reducing bacteria may be one factor responsible for stimulating the activity of the dehalogenating bacteria in the test columns.

French, W. T., L. R. Brown, et al. (2002). "Effects of n-hexadecane and PM-100 clay on trichloroethylene degradation by *Burkholderia cepacia*." *Journal of Hazardous Materials* 92(1): 89-102.

Trichloroethylene (TCE) is a non-flammable, volatile organochlorine compound which was a widely used degreasing agent, anesthetic, and coolant prior to 1960, but has since been placed on the Environmental Protection Agency's (EPA) list of priority pollutants. The inadequate disposal practices for TCE have created numerous TCE-contaminated superfund sites. The most commonly employed practice for remediating TCE-contaminated sites is to purge the contaminant from the source and trap it onto an adsorbent which is disposed of in a landfill or by incineration. This investigation was undertaken to evaluate the effectiveness of *Burkholderia cepacia* strain G4 (G4) to regenerate used sorbents by degrading TCE from the sorbent directly or indirectly. The results of this investigation showed that G4 was capable of reducing TCE attached to PM-100 clay but at significantly reduced rate due to the slow desorption rate. Conversely, it was shown that G4 was capable of degrading TCE dissolved in n-hexadecane at the same rate as systems without n-hexadecane present. The reduction in TCE degradation when the TCE is attached to the PM-100 clay could be overcome by solvent rinsing the TCE from the clay with subsequent removal of the TCE from the n-hexadecane by G4. (C) 2002 Elsevier Science B.V All rights reserved.

Fukuda, K., S. Nagata, et al. (2002). "Isolation and characterization of dibenzofuran-degrading bacteria." *Fems Microbiology Letters* 208(2): 179-185.

Two bacterial strains capable of utilizing dibenzofuran (DF) as a sole carbon source were isolated from soil samples of reclaimed land. The strains designated HL1 and HL7 were identified as *Klebsiella* sp. and *Sphingomonas* sp., respectively, on the basis of biochemical characteristics and the sequences of the 16S ribosomal DNA. *Sphingomonas* sp. strain HL7 degraded non-, mono- and also dichlorinated DF and dibenzo-p-dioxin (DD), *Klebsiella* sp. strain HL1 was able to degrade non- and monochlorinated DFs and DDs, but not dichlorinated ones. The metabolites formed from DF by strains HL1 and HL7 were similar to those by dioxin-degrading bacteria *Sphingomonas* sp. strain RW1 except for salicylic acid and catechol. Strain HL7 had a gene homologous to that

encoding the dioxin dioxygenase alpha-subunit (dxnA1) gene of *Sphingomonas* sp. strain RW1. However, Southern hybridization analysis showed that the size of an EcoRV-digested genomic fragment involving the dioxin dioxygenase gene of strain HL7 was smaller than that of strain RW1, and that strain HL1 did not have the homologous gene. Strains HL1 and HL7 provided useful information regarding the dioxygenase genes. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Galiulin, R. V., V. N. Bashkin, et al. (2002). "Review: Behavior of persistent organic pollutants in the air-plant-soil system." *Water Air and Soil Pollution* 137(1-4): 179-191.

A conceptual model for transformation of environmentally widespread persistent organic pollutants (DDT, HCH, PCBs and benz(a)pyrene) in the air-plant-soil system was developed. Based on this model, analyses and systematization were made in order to assess the empirical generalizations, models, and hypotheses from different literature sources related to studying the peculiarities of the above-mentioned phenomenon. They are illustrated by the examples of pollutant behavior on the agricultural areas of the former Soviet Union.

Gandhi, S., B. T. Oh, et al. (2002). "Degradation of TCE, Cr(VI), sulfate, and nitrate mixtures by granular iron in flow-through columns under different microbial conditions." *Water Research* 36(8): 1973-1982.

Flow-through aquifer columns packed with a middle layer of granular iron (Fe-0) were used to study the applicability and limitations of bio-enhanced Fe-0 barriers for the treatment of contaminant mixtures in groundwater. Concentration profiles along the columns showed extensive degradation of hexavalent chromium Cr(VI), nitrate, sulfate, and trichloroethene (TCE), mainly in the Fe-0 layer. One column was bioaugmented with *Shewanella* algae BRY, an iron-reducing bacterium that could enhance Fe-0 reactivity by reductive dissolution of passivating iron oxides. This strain did not enhance Cr(VI), which was rapidly reduced by iron, leaving little room for improvement by microbial participation. Nevertheless, BRY-enhanced nitrate removal (from 15% to 80%), partly because this strain has a wide range of electron acceptors, including nitrate. Sulfate was removed (55%) only in a column that was bioaugmented with a mixed culture containing sulfate-reducing bacteria. Apparently, these bacteria used H₂ (produced by Fe-0 corrosion) as electron donor to respire sulfate. Most of the TCE was degraded in the zone containing Fe-0 (50-70%), and bioaugmentation with BRY slightly increased the removal efficiency to about 80%. Microbial colonization of the Fe-0 surface was confirmed by scanning electron microscopy. (C) 2002 Elsevier Science Ltd. All rights reserved.

Habe, H., Y. Ashikawa, et al. (2002). "Sphingomonas sp strain KA1, carrying a carbazole dioxygenase gene homologue, degrades chlorinated dibenzo-p-dioxins in soil." *Fems Microbiology Letters* 211(1): 43-49.

Hybridization analysis showed that a newly isolated carbazole (CAR)-degrading bacterium *Sphingomonas* sp. strain KA1 did not possess the gene encoding the terminal oxygenase component (carAa) of CAR 1,9a-dioxygenase at high homology (more than 90% identity) to that of another CAR-degrader, *Pseudomonas resinovorans* strain CA10. However, PCR experiments using the primers for amplifying the internal fragment of the carAa gene (810 bp for strain CA10) showed that a PCR product of unexpected size (1100 bp) was amplified. Sequence analysis revealed that this DNA region contained the portion of two possible ORFs, which showed moderate homology to CarAa and CarBa from strain CA10 (61% and 40% identities at the amino acid level, respectively). Inoculation of strain KA1 into dioxin-contaminated model soil resulted in 96% and 70% degradation of 2-mono- and 2,3-dichlorinated dibenzo-p-dioxin, respectively, after 7-day incubation. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Habe, H., K. Ide, et al. (2002). "Degradation characteristics of a dibenzofuran-degrader *Terrabacter* sp strain DBF63 toward chlorinated dioxins in soil." *Chemosphere* 48(2): 201-207.

To obtain basic information towards applying a dibenzofuran (DF)-degrader *Terrabacter* sp. strain DBF63 to bioremediate dioxin-contaminated soil, we investigated the degradative potential of strain DBF63 for either chlorinated or polychlorinated dibenzo-p-dioxins and dibenzofurans (Cl_xDD/Cl_xDF) in soil. In the soil slurry system with a soil to water ratio of 1:5 (w/v), the DF-grown DBF63 cells degraded 90% of 1 ppm 2,8-Cl₂DF, whereas only 40% of 1 ppm 2,3-Cl₂DD during the 7-day incubation. The degradation rates of 2-Cl₁DF, 2-Cl₁DD, 2,8-Cl₂DF and 2,3-Cl₂DF by strain DBF63 in the soil slurry system (5-day incubation) were approximately 89%, 65%, 78% and 32%, respectively. These results suggest that strain DBF63 was able to degrade mono- to dichlorinated dibenzofurans more effectively than mono- to dichlorinated dibenzo-p-dioxins. Using the same soil slurry system, we performed a preliminary bioremediation experiment using the actual dioxin-contaminated soil at

an incineration site. We found that approximately 10% of tetra- to hexa-chlorinated congeners was decreased by a single inoculation with DBF63 cells within a 7-day incubation. (C) 2002 Elsevier Science Ltd. All rights reserved.

Han, T. S., S. Sasaki, et al. (2002). "Flow injection microbial trichloroethylene sensor." *Talanta* 57(2): 271-276.

A flow type microbial biosensor for direct measurement of trichloroethylene (TCE) was developed. The unique features of this novel microbial sensor were the use of the TCE degrading bacterium *Pseudomonas aeruginosa* J1104, the electrical detection of the chloride ion released by microbial degradation, and flow cell made of glass. Glass cell was used in order to suppress adsorption of TCE and made a closed reaction cell. Vaporization of TCE during the measurement was prevented using closed flow cell. The performance of the sensor was evaluated from following aspects; such as pH of the carrier solution, amount of the immobilized microbe, flow rate and injection volume. The sensor signals were linearly proportional to the concentration of TCE in the range from 0.03 to 2 mg l⁻¹, which is suitable for the determination of suspected samples to be drinkable water or not. The sensor performance was checked on the real sample, and this system showed good response in ground water, indicating its applicability for the on line monitoring at TCE contaminated areas or hazardous sites. (C) 2002 Published by Elsevier Science B.V.

Hong, H. B., Y. S. Chang, et al. (2002). "Biotransformation of 2,7-dichloro- and 1,2,3,4-tetrachlorodibenzo-p-dioxin by *Sphingomonas wittichii* RW1." *Applied and Environmental Microbiology* 68(5): 2584-2588.

Aerobic biotransformation of the diaryl ethers 2,7-dichlorodibenzo-p-dioxin and 1,2,3,4-tetrachlorodibenzo-p-dioxin by the dibenzo-p-dioxin-utilizing strain *Sphingomonas wittichii* RW1, producing corresponding metabolites, was demonstrated for the first time. Our strain transformed 2,7-dichlorodibenzo-p-dioxin, yielding 4-chlorocatechol, and 1,2,3,4-tetrachlorodibenzo-p-dioxin, producing 3,4,5,6-tetrachlorocatechol and 2-methoxy-3,4,5,6-tetrachlorophenol; all of these compounds were unequivocally identified by mass spectrometry both before and after N,O-bis(trimethylsilyl)-trifluoroacetamide derivatization by comparison with authentic standards. Additional experiments showed that strain RW1 formed a second metabolite, 2-methoxy-3,4,5,6-tetrachlorophenol, from the original degradation product, 3,4,5,6-tetrachlorocatechol, by methylation of one of the two hydroxy substituents.

Hoostal, M. J., G. S. Bullerjahn, et al. (2002). "Molecular assessment of the potential for in situ bioremediation of PCBs from aquatic sediments." *Hydrobiologia* 469(1-3): 59-65.

Polychlorinated biphenyls (PCBs) are a family of xenobiotic compounds that are ubiquitous and oftentimes persistent environmental pollutants. As such, PCBs are a common target of sediment remediation efforts. Microbial degradation of sediment pollutants such as PCBs offers an environmentally sound and economically favorable alternative to conventional means of remediation such as dredging. This project describes the development of a PCR-based assay to determine the potential for PCB bioremediation by the resident microbial consortium in contaminated sediments. Using PCR and RT-PCR of DNA and RNA, respectively, extracted from aquatic sediments collected from the western basin of Lake Erie and one of its tributaries, we were able to amplify the *bphA1* gene that encodes the large subunit of biphenyl dioxygenase. Since other studies have determined that the *BphA1* gene product dictates PCB congener specificity, this assay may prove to be a useful screen for endemic catabolic activities for PCB mixtures in aquatic sediments.

Kayser, M. F., Z. Ucurum, et al. (2002). "Dichloromethane metabolism and C-1 utilization genes in *Methylobacterium* strains." *Microbiology-Sgm* 148: 1915-1922.

The ability of methylotrophic alpha-proteobacteria to grow with dichloromethane (DCM) as source of carbon and energy has long been thought to depend solely on a single cytoplasmic enzyme, DCM dehalogenase, which converts DCM to formaldehyde, a central intermediate of methylotrophic growth. The gene *dcmA* encoding DCM dehalogenase of *Methylobacterium dichloromethanicum* DM4 was expressed from a plasmid in closely related *Methylobacterium* strains lacking this enzyme. The ability to grow with DCM could be conferred upon *Methylobacterium chloromethanicum* CM4, a chloromethane degrader, but not upon *Methylobacterium extorquens* AM1. In addition, growth of strain AM1 with methanol was impaired in the presence of DCM. The possibility that single-carbon (C) utilization pathways in dehalogenating *Methylobacterium* strains differed from those discovered in strain AM1 was addressed. Homologues of tetrahydrofolate-linked and tetrahydromethanopterin-linked C, utilization genes of strain AM1 were detected in both strain DM4 and strain CM4, and cloning and sequencing of

several of these genes from strain DM4 revealed very high sequence identity (96-5-99-7%) to the corresponding genes of strain AM1. The expression of transcriptional xylE fusions of selected genes of the tetrahydrofolate- and tetrahydromethanopterin-linked pathways from strain DM4 was investigated. The data obtained suggest that the expression levels of some C-1 utilization genes in *M. dichloromethanicum* DM4 grown with DCM may differ from those observed during growth with methanol.

Keppler, F., R. Borchers, et al. (2002). "Natural formation of vinyl chloride in the terrestrial environment." *Environmental Science & Technology* 36(11): 2479-2483.

Vinyl chloride is a highly reactive and toxic substance which is widely used in industry. It is the parent compound of poly(vinyl chloride) (PVC), one of the most important industrial polymers. Until now, it was thought that vinyl chloride found in the environment is exclusively man-made or results from the degradation of other anthropogenic substances, such as trichloroethylene and tetrachloroethylene. Here, we demonstrate that vinyl chloride also has natural sources. Soil air and ambient air from a rural area in Northern Germany were investigated for volatile chlorinated halocarbons. The concentrations of vinyl chloride in the soil air were significantly enhanced as compared to ambient air, indicating a natural formation of this compound in the soil. A series of laboratory experiments using different soils and model compounds was conducted, which clearly proved that vinyl chloride could be produced during soil processes. We propose that this highly reactive compound can be formed during the oxidative degradation of organic matter in soil, for example, in a reaction between humic substances, chloride ions and an oxidant (ferric ions or hydroxyl radicals). The redox-sensitive aromatic compounds in soil such as catechols and o-quinones can be degraded to CO₂, accompanied by the release of vinyl chloride and other volatile chlorinated compounds. This process could have started in the Late Silurian to Early Devonian, 400 million years ago, when the first soils on earth evolved.

Kim, I. S., L. A. Beaudette, et al. (2002). "Alterations in fatty acid composition and fluidity of cell membranes affect the accumulation of PCB congener 2,2',5,5'- tetrachlorobiphenyl by *Ralstonia eutropha* H850." *Journal of Chemical Technology and Biotechnology* 77(7): 793-799.

The effect of alterations in fatty acid composition and fluidity of cell membranes on the accumulation of PCB congener 2,2,5,5'-tetrachlorobiphenyl (TeCB) by *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*) H850 was studied. Cells of *R. eutropha* H850 grown on either biphenyl or fructose were used. Significant increases in saturated fatty acid composition and decreases in membrane fluidity of bacteria grown on biphenyl at 28degreesC were observed compared with those grown on fructose at 17 or 28degreesC. The ratio of saturated fatty acids to unsaturated fatty acids and membrane fluidity of *R. eutropha* H850 grown on biphenyl at 28degreesC resembled those of cells grown on fructose at 37degreesC. No inhibition effect of the uncoupler 2,4-dinitrophenol (2,4-DNP) on TeCB accumulation was observed, suggesting an energy-independent mechanism for TeCB accumulation in cells of *R. eutropha* H850. The amount of TeCB accumulated was considerably higher in *R. eutropha* H850 grown on fructose at 17 and 28degreesC than when grown on biphenyl at 28degreesC. Similar amounts of TeCB accumulated in bacteria grown on biphenyl at 28degreesC as compared with those grown on fructose at 37 C. These results suggest the alterations in fatty acid composition and membrane fluidity of *R. eutropha* H850 may affect the accumulation of TeCB. (C) 2002 Society of Chemical Industry.

Krauss, M. and W. Wilcke (2002). "Photochemical oxidation of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in soils - a tool to assess their degradability?" *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* 165(2): 173-178.

To determine the degradability of PAHs and PCBs for soil remediation or ecotoxicological risk assessment, a simple method is needed. We tested the suitability of photocatalytic oxidation for this purpose. We determined the concentrations of 20 PAHs and 12 PCBs in four mineral topsoil horizons, six organic horizons, and four particle-size fractions of each of three soils before and after UV irradiation with TiO₂ as a catalyst in suspension. Preliminary experiments showed that in dry soil no photooxidation occurred, but after 48 h of irradiation in suspension the PCB concentrations decreased by up to 40-50 %, while the PAH concentrations did not change significantly. In contrast to this, 95-100 % of PAH and PCB standards spiked on quartz sand were degraded within 8 h, indicating that sorption to organic matter limited degradation of PAHs and PCBs in soil suspensions. There was no difference in the degradation among different individual PAEs and PCBs, respectively, indicating that the degradation did not occur in dissolved state, but in association with soil organic matter. In all samples except one, the degradation of PCBs (10-80 % loss of initial concentrations) was higher than those of the

PAHs (0-40 % loss). This suggests that the accessibility of PCBs for OH- radicals generated during irradiation was higher, or the oxidation of PAHs was limited by the properties of the sorbing organic matter. Thus, the tested method was not suitable to predict biodegradability, because it did not reflect the differences in degradability of individual compounds.

Lowe, M., E. L. Madsen, et al. (2002). "Geochemistry and microbial diversity of a trichloroethene-contaminated Superfund site undergoing intrinsic in situ reductive dechlorination." *Fems Microbiology Ecology* 40(2): 123-134.

This study explored the geochemistry and microbial diversity of a Superfund site containing trichloroethene (TCE) and an unusual copollutant, tetra kis(2-ethylbutoxy)silane. Geochemical analysis of contaminated groundwater indicated subsurface anaerobiosis, reductive dechlorination of TCE to predominantly cis-1,2-dichloroethene, and (transient) accumulation of 2-ethylbutanol and 2-ethylbutyrate as a result of tetrakis(2-ethylbutoxy) silane breakdown. Comparative analysis of 106 16S rDNA and 61 16S-23S rDNA intergenic spacer region sequences - obtained from pristine and contaminated groundwater via DNA extraction, PCR amplification, cloning and sequencing revealed that the contaminated groundwater featured (i) a distinct microbial community, (ii) reduced species diversity, (iii) various anaerobes, and (iv) bacteria closely related to the TCE-dechlorinating, dichloroethene-accumulating genus *Dehalobacter*, whereas (v) the TCE-dechlorinating, ethene-producing species *Dehalococcoides ethenogenes* was not detectable. Thus, geochemical and molecular biological results were in excellent agreement in this first ecological field study linking in situ reductive dechlorination of TCE to metabolism of tetraalkoxysilanes. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Lu, X. X., G. H. Li, et al. (2002). "Volatile fatty acids as electron donors for the reductive dechlorination of chloroethenes." *Journal of Environmental Science and Health Part a- Toxic/Hazardous Substances & Environmental Engineering* 37(4): 439-449.

Uses of a mixture of six volatile fatty acids (VFAs) including acetic, propionic, butyric, isobutyric, valeric and isovaleric acids as electron donors for the reductive dechlorination of chloroethenes have been investigated by both microcosm and column studies. The fates of tetrachloroethene (PCE), cis-dichloroethene (cis-DCE) and vinyl chloride (VC) in the presence of VFAs and in the absence of VFAs were respectively documented. The results showed that VFAs stimulated complete reductive dechlorination of chloroethenes, either as direct substrates for the dechlorinating bacteria or via H₂ formed during VFAs-degradation. There were sequential utilizations of different VFAs by fermenting bacteria. In the microcosm, propionic acid was the first to be used, followed by acetic, butyric, isobutyric, valeric, and isovaleric acids, and their mean first-order degradation rates obtained were 0.128, 0.048, 0.016, 0.027, 0.025 and 0.003 day⁻¹, respectively. In the column, propionic acid was the first to be used, followed by butyric and valeric acids, and their calculated first-order degradation rates were 0.833, 0.403 and 0.260 day⁻¹, respectively.

Lyew, D., B. Tartakovsky, et al. (2002). "A microcosm test for potential mineralization of chlorinated compounds under coupled aerobic/anaerobic conditions." *Chemosphere* 47(7): 695-699.

In this study, the feasibility of using a mineralization test under coupled aerobic/anaerobic conditions was demonstrated. The coupling of anaerobic methanogenic and aerobic methanotrophic conditions in a microcosm required the presence of both a carbon source for anaerobic metabolism and oxygen for aerobic metabolism. These requirements were fulfilled by using a slow hydrolyzing organic matter along with intermittent addition of oxygen to the bottle headspace. Perchloroethylene (PCE) mineralization tests confirmed the effectiveness of the proposed methodology as well as PCE mineralization under coupled conditions. Crown Copyright (C) 2002 Published by Elsevier Science Ltd. All rights reserved.

Neumann, A., A. Seibert, et al. (2002). "Tetrachloroethene reductive dehalogenase of *Dehalospirillum multivorans*: substrate specificity of the native enzyme and its corrinoid cofactor." *Archives of Microbiology* 177(5): 420-426.

The substrate specificity of the tetrachloroethene reductive dehalogenase of *Dehalospirillum multivorans* and its corrinoid cofactor were studied. Besides reduced methyl viologen, titanium(III) citrate could serve as electron donor for reductive dehalogenation of tetrachloroethene (PCE) and trichloroethene to cis-1,2-dichloroethene. In addition to chlorinated ethenes, chlorinated propenes were reductively dechlorinated solely by

the native enzyme. trans-1,3- Dichloropropene, 1,1,3-trichloropropene and 2,3-dichloropropene were reduced to a mixture of mono-chloropropenes, 1,1- dichloropropene, and 2-chloropropene, respectively. Other halogenated compounds that were rapidly reduced by the enzyme were also dehalogenated abiotically by the heat-inactivated enzyme and by commercially available cyanocobalamin. The rate of this abiotic reaction was dependent on the number and type of halogen substituents and on the type of catalyst. The corrinoid cofactor purified from the tetrachloroethene dehalogenase of *D. multivorans* exhibited an activity about 50- fold higher than that of cyanocobalamin (vitamin B-12) with trichloro acetate as electron acceptor, indicating that the corrinoid cofactor of the PCE dehalogenase is not cyanocobalamin. Corrinoids catalyzed the rapid dehalogenation of trichloroacetic acid. The rate was proportional to the amount of, e.g. cyanocobalamin; therefore, the reductive dehalogenation assay can be used for the sensitive and rapid quantification of this cofactor.

Oberg, G. (2002). "The natural chlorine cycle - fitting the scattered pieces." *Applied Microbiology and Biotechnology* 58(5): 565-581.

Chlorine is one of the most abundant elements on the surface of the earth. Until recently, it was widely believed that all chlorinated organic compounds were xenobiotic, that chlorine does not participate in biological processes and that it is present in the environment only as chloride. However, over the years, research has revealed that chlorine takes part in a complex biogeochemical cycle, that it is one of the major elements of soil organic matter and that the amount of naturally formed organic chlorine present in the environment can be counted in tons per km². Interestingly enough, some of the pieces of the chlorine puzzle have actually been known for decades, but the information has been scattered among a number of different disciplines with little or no exchange of information. The lack of communication appears to be due to the fact that the points of departure in the various fields have not corresponded; a number of paradoxes are actually revealed when the known pieces of the chlorine puzzle are fit together. It appears as if a number of generally agreed statements or tacit understandings have guided perceptions, and that these have obstructed the understanding of the chlorine-cycle as a whole. The present review enlightens four paradoxes that spring up when some persistent tacit understandings are viewed in the light of recent work as well as earlier findings in other areas. The paradoxes illuminated in this paper are that it is generally agreed that: (1) chlorinated organic compounds are xenobiotic even though more than 1,000 naturally produced chlorinated compounds have been identified; (2) only a few, rather specialised, organisms are able to convert chloride to organic chlorine even though it appears as if the ability among organisms to transform chloride to organic chlorine is more the rule than the exception; (3) all chlorinated organic compounds are persistent and toxic even though the vast majority of naturally produced organic chlorine is neither persistent nor toxic; (4) chlorine is mainly found in its ionic form in the environment even though organic chlorine is as abundant or even more abundant than chloride in soil. Furthermore, the contours of the terrestrial chlorine cycle are outlined and put in a concrete form by constructing a rough chlorine budget over a small forested catchment. Finally, possible ecological roles of the turnover of chlorine are discussed.

Okeke, B. C., T. Siddique, et al. (2002). "Biodegradation of gamma-hexachlorocyclohexane (lindane) and alpha-hexachlorocyclohexane in water and a soil slurry by a *Pandoraea* species." *Journal of Agricultural and Food Chemistry* 50(9): 2548-2555.

Isomers of 1,2,3,4,5,6-hexachlorocyclohexane (HCH) were some of the most widely used pesticides. Despite reduction in their production and use, HCH isomers present a serious environmental hazard. In this study, two bacterial isolates (LIN-1 and LIN-3) that can grow on gamma-HCH as a sole source of carbon and energy were isolated from an enrichment culture. In liquid cultures of LIN-1 and LIN-3, 25.0 and 45.5% removal of gamma- HCH, respectively, were achieved in 2 weeks. LIN-3 was identified as *Pandoraea* sp. by 16S rRNA gene sequence analysis (99% identity). *Pandoraea* sp. substantially degraded both gamma- and alpha-HCH isomers at concentrations of 10-200 mg L⁻¹ in liquid cultures. After 8 weeks of incubation in liquid culture, 89.9 and 93.3% of the gamma- and alpha-HCH isomers declined, respectively, at an initial concentration of 150 mg L⁻¹. In soil slurry cultures of *Pandoraea* sp., simulating a soil slurry phase bioremediation treatment, substantial decreases in the levels of the HCH isomers were observed at concentrations of 50-200 mg L⁻¹. After 9 weeks, 59.6 and 53.3% biodegradations of gamma- and alpha-HCH isomers, respectively, were achieved at 150 mg L⁻¹. Using two 23-mer oligonucleotide primers targeting the 330 bp region of the 16S rRNA gene of *Pandoraea* sp., an approximately 330 bp PCR product was successfully amplified from DNA templates prepared from bacterial colonies and soil slurry culture. This system provides a direct and rapid PCR-based molecular tool for tracking *Pandoraea* sp. strain

LIN-3 in water and soils. These results have implied implications for the treatment of soils and water contaminated with HCH isomers.

Otyepka, M. and J. Damborsky (2002). "Functionally relevant motions of haloalkane dehalogenases occur in the specificity-modulating cap domains." *Protein Science* 11(5): 1206-1217.

One-nanosecond molecular dynamics trajectories of three haloalkane dehalogenases (Dh1A, LinB, and DhaA) are compared. The main domain was rigid in all three dehalogenases, whereas the substrate specificity-modulating cap domains showed considerably higher mobility. The functionally relevant motions were spread over the entire cap domain in Dh1A, whereas they were more localized in LinB and DhaA. The highest amplitude of essential motions of Dh1A was noted in the alpha4'-helix-loop- alpha4-helix region, formerly proposed to participate in the large conformation change needed for product release. The highest amplitude of essential motions of LinB and DhaA was observed in the random coil before helix 4, linking two domains of these proteins. This flexibility is the consequence of the modular composition of haloalkane dehalogenases. Two members of the catalytic triad, that is, the nucleophile and the base, showed a very high level of rigidity in all three dehalogenases. This rigidity is essential for their function. One of the halide-stabilizing residues, important for the catalysis, shows significantly higher flexibility in Dh1A compared with LinB and DhaA. Enhanced flexibility may be required for destabilization of the electrostatic interactions during the release of the halide ion from the deeply buried active site of Dh1A. The exchange of water molecules between the enzyme active site and bulk solvent was very different among the three dehalogenases. The differences could be related to the flexibility of the cap domains and to the number of entrance tunnels.

Parales, R. E. and C. S. Harwood (2002). "Bacterial chemotaxis to pollutants and plant-derived aromatic molecules." *Current Opinion in Microbiology* 5(3): 266-273.

There is accumulating evidence that motile bacteria are chemotactically attracted to environmental pollutants that they can degrade. Chemotaxis, the ability of motile bacteria to detect and respond to specific chemicals in the environment, can increase an organism's chances of locating useful sources of carbon, nitrogen and energy, and could thus play an important role in the biodegradation process. Recent evidence demonstrating that chemotaxis and biodegradation genes are coordinately regulated suggests that these processes are intimately linked in nature.

Pikkemaat, M. G., A. B. M. Linssen, et al. (2002). "Molecular dynamics simulations as a tool for improving protein stability." *Protein Engineering* 15(3): 185-192.

Haloalkane dehalogenase (Dh1A) was used as a model protein to explore the possibility to use molecular dynamics (MD) simulations as a tool to identify flexible regions in proteins that can serve as a target for stability enhancement by introduction of a disulfide bond. Dh1A consists of two domains: an alpha/beta-hydrolase fold main domain and a cap domain composed of five alpha-helices. MD simulations of Dh1A showed high mobility in a helix-loop-helix region in the cap domain, involving residues 184-211. A disulfide cross-link was engineered between residue 201 of this flexible region and residue 16 of the main domain. The mutant enzyme showed substantial changes in both thermal and urea denaturation. The oxidized form of the mutant enzyme showed an increase of the apparent transition temperature from 47.5 to 52.5 degrees C, whereas the T_m of the reduced mutant decreased by more than 8 degrees C compared to the wild-type enzyme. Urea denaturation results showed a similar trend. Measurement of the kinetic stability showed that the introduction of the disulfide bond caused a decrease in activation free energy of unfolding of 0.43 kcal mol⁻¹ compared to the wild-type enzyme and also indicated that the helix-loop-helix region was involved early in the unfolding process. The results show that MD simulations are capable of identifying mobile protein domains that can successfully be used as a target for stability enhancement by the introduction of a disulfide cross-link.

Richardson, R. E., V. K. Bhupathiraju, et al. (2002). "Phylogenetic characterization of microbial communities that reductively dechlorinate TCE based upon a combination of molecular techniques." *Environmental Science & Technology* 36(12): 2652-2662.

An anaerobic microbial consortium (referred to as ANAS) that reductively dechlorinates trichloroethene (TCE) completely to ethene with the transient production of cis-dichloroethene (cDCE) and vinyl chloride was enriched from contaminated soil obtained from Alameda Naval Air Station. ANAS uses lactate as its electron donor and has been functionally stable for over 2 years. Following a brief exposure to oxygen, a subculture

(designated VCC) derived from ANAS could dechlorinate TCE only to vinyl chloride with lactate as its electron donor. Three molecular methods were used concurrently to characterize the community structure of ANAS and VCC: clone library construction/clone sequencing, terminal restriction fragment length polymorphism (T-RFLP) analysis, and fluorescent in situ hybridization (FISH) with rRNA probes. The community structure of ANAS did not change significantly over the course of a single feeding/dechlorination cycle, and only minor fluctuations occurred over many feeding cycles spanning the course of 1 year. Clone libraries and T-RFLP analyses suggested that ANAS was dominated by populations belonging to three phylogenetic groups: Dehalococcoides species, Desulfovibrio species, and members of the Clostridiaceae (within the low G + C Gram-positives). FISH results suggest that members of the Cytophaga/Flavobacterium/Bacteroides (CFB) cluster and high G + C Gram-positives (HGCs) were numerically important in ANAS despite their under-representation in the clone libraries. Parallel analyses of VCC samples suggested that Dehalococcoides species and Clostridiaceae were only minor populations in this community. Instead, VCC had increased populations of organisms in the beta and gamma subclasses of the Proteobacteria as well as significant populations of organisms in the CFB cluster. It is possible that symbiotic interactions are occurring between some of ANAS's phylogenetic groups under the enrichment conditions, including interspecies hydrogen transfer from Desulfovibrio species to Dehalococcoides species. However, the nucleic acid-based analyses performed here would need to be supplemented with chemical species data in order to test any hypotheses about functional roles of various community members. Additionally, these results suggest that an organism outside the Dehalococcoides genus may be capable of dechlorinating cDCE to vinyl chloride.

Sakai, M., E. Masai, et al. (2002). "Diversity of 2,3-dihydroxybiphenyl dioxygenase genes in a strong PCB degrader, Rhodococcus sp strain RHA1." *Journal of Bioscience and Bioengineering* 93(4): 421-427.

Two 2,3-dihydroxybiphenyl (23DHBP) dioxygenase genes, bphC1 and etbC involved in the degradation of polychlorinated biphenyl(s) (PCBs) have been isolated and characterized from a strong PCB degrader, Rhodococcus sp. RHA1. In this study, four new 23DHBP dioxygenase genes, designated as bphC2, bphC3, bphC4, and bphC5 were isolated from RHA1, and their nucleotide sequences were determined. Based on amino acid sequence similarities, all of the newly isolated bphC genes could be categorized into type I along with BphC1 and EtbC (Eltis, L. D. and Bolin, J. T., *J. Bacteriol.*, 178, 5930-5937 (1996)). Six bphC genes, including bphC1, etbC, and four new genes, were expressed in *Escherichia coli* to determine their substrate specificity. The activities of BphC2, BphC3, BphC4, and BphC5 were found to be specific to 23DHBP, while BphC1 and EtbC exhibited activities towards compounds other than 23DHBP, including catechol (CAT) and 3- methylcatechol (3MC). RNA slot blot hybridization analysis indicated that only bphC5 was transcribed among the newly isolated bphC in RHA1 cells grown on biphenyl and ethylbenzene. The nucleotide sequence of the flanking region of each bphC revealed a homolog of the 2-hydroxy-6-oxo-6-phenylhexa-2,4- dienoate (HOPD) hydrolase gene, bphD, just upstream of bphC5. The bphC5 and putative bphD genes may constitute an operon and play a role in the degradation of biphenyl and PCBs together with bphC1 and etbC. In contrast, the bphC2, bphC3, and bphC4 genes may not be involved in biphenyl and PCB degradation.

Sakaki, T., R. Shinkyō, et al. (2002). "Biodegradation of polychlorinated dibenzo-p-dioxins by recombinant yeast expressing rat CYP1A subfamily." *Archives of Biochemistry and Biophysics* 401(1): 91-98.

Metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) by recombinant yeast cells expressing either rat CYP1A1 or CYP1A2 was examined. When each of the dibenzo-p-dioxins (DDs), mono-, di-, and tri-chloroDDs, was added to the cell culture of the recombinant yeast, a remarkable metabolism was observed. The metabolism contained multiple reactions such as hydroxylation at an unsubstituted position, hydroxylation with migration of a chloride substituent, hydroxylation with elimination of a chloride substituent, and opening of dioxin ring. The distinct difference was observed in substrate specificity and reaction specificity between CYP1A1 and CYP1A2. Kinetic analysis using microsomal fractions prepared from the recombinant yeast cells revealed that 2,7-dichloroDD and 2,3,7-trichloroDD were good substrates for both CYP1A1 and CYP1A2. When 2,3,7-trichloroDD was added to the yeast cells expressing each of rat CYP1A1 and CYP1A2, most of 2,3,7-trichloroDD was first converted to 8- hydroxy-2,3,7-trichloroDD, and further metabolized to more hydrophilic compounds whose ethereal bridges were cleaved. These findings give essential information on the metabolism of PCDDs in mammalian liver. In addition, this study indicates the possibility of application of microorganisms expressing mammalian cytochrome P450 to bioremediation of contaminated soils with dioxins. (C) 2002 Elsevier Science (USA). All rights reserved.

Sakurai, T., N. Suzuki, et al. (2002). "Examination of dioxin fluxes recorded in dated aquatic-sediment cores in the Kanto region of Japan using multivariate data analysis." *Chemosphere* 46(9-10): 1359-1365.

Past dioxin (coplanar polychlorinated biphenyl (Co-PCB), 2,3,7,8 -substituted polychlorinated dibenzo-p-dioxin (PCDD) and 2,3,7,8-substituted polychlorinated dibenzofuran (PCDF)) fluxes recorded in dated aquatic-sediment cores were analyzed using principal component analysis (PCA). The data set consisted of samples from four cores collected from the Kanto region of Japan. Time trends and spatial differences in the dioxin flux were examined, and the potential relationship to emission sources was investigated. Twenty-five compounds and 58 core slices, corresponding to the later half of the 20th century, were subjected to the analysis. The PCA of both log-transformed and maximum-value-standardized data successfully divided the dioxin compounds into a small number of groups, and three similar clusters of Co-PCBs, PCDDs and penta- to hepta- CDFs were identified. PCB formulations used in the past are judged to have been responsible for the major part of the Co- PCB flux recorded in the sediment cores. However, the relationship to emission sources needs further investigation. It is suggested that most 2,3,7,8 -substituted PCDDs and PCDFs are different from Co-PCBs in their emission sources or movements in the environment. The subcore clusters obtained from the PCA of log-transformed data show that the cores from different sampling areas exhibited distinct dioxin fluxes and compositions. Common time trends among the cores were more effectively summarized by the PCA of maximum-value-standardized data focusing on relative time trends. PC scores show that recently the flux of each dioxin compound in the four cores has been generally declining after having reached a peak. (C) 2002 Elsevier Science Ltd. All rights reserved.

Song, D. L., M. E. Conrad, et al. (2002). "Stable carbon isotope fractionation during enhanced in situ bioremediation of trichloroethene." *Environmental Science & Technology* 36(10): 2262-2268.

Time-series stable carbon isotope monitoring of volatile organic compounds (VOCs) at the Idaho National Engineering and Environmental Laboratory's (INEEL) field site Test Area North (TAN) was conducted during a pilot study to investigate the treatment potential of using lactate to stimulate in situ biologic reductive dechlorination of trichloroethene (TCE). The isotope ratios of TCE and its biodegradation by products, cis-dichloroethene (c-DCE), trans-dichloroethene (t-DCE), vinyl chloride (VC), and ethene, in groundwater samples collected during the pilot study were preconcentrated with a combination of purge-and-trap and cryogenic techniques in order to allow for reproducible isotopic measurements of the low concentrations of these compounds in the samples (down to 0.04 μM , or 5 ppb, of TCE). Compound-specific stable isotope monitoring of chlorinated solvents clearly differentiated between the effects of groundwater transport, dissolution of DNAPL at the source, and enhanced bioremediation. Isotope data from all wells within the zone of lactate influence exhibited large kinetic isotope effects during the reduction of c-DCE to VC and VC to ethene. Despite these large effects, the carbon isotope ratio of ethene in all these wells reached the carbon isotope ratios of the initial dissolved TCE, confirming the complete conversion of dissolved TCE to ethene. Conversely, the carbon isotope ratios of t-DCE were only marginally affected during the study, indicating that minimal biologic degradation of t-DCE was occurring.

Sota, M., M. Endo, et al. (2002). "Characterization of a class II defective transposon carrying two haloacetate dehalogenase genes from *Delftia acidovorans* plasmid pUO1." *Applied and Environmental Microbiology* 68(5): 2307-2315.

The two haloacetate dehalogenase genes, dehH1 and dehH2, on the 65-kb plasmid pUO1 from *Delftia acidovorans* strain B were found to be located on transposable elements. The dehH2 gene was carried on an 8.9-kb class I composite transposon (TnHad1) that was flanked by two directly repeated copies of IS1071, IS1071L and IS1071R. The dehH1 gene was also flanked by IS1071L and a truncated version of IS1071 (IS1071N). TnHad1, dehH1, and IS1071N were located on a 15.6-kb class II transposon (TnHad2) whose terminal inverted repeats and *res* site showed high homology with those of the Tn21-related transposons. TnHad2 was defective in transposition because of its lacking the transposase and resolvase genes. TnHad2 could transpose when the Tn21-encoded transposase and resolvase were supplied in trans. These results demonstrated that TnHad2 is a defective Tn21-related transposon carrying another class I catabolic transposon.

Studer, A., C. McAnulla, et al. (2002). "Chloromethane-induced genes define a third C-1 utilization pathway in *Methylobacterium chloromethanicum* CM4." *Journal of Bacteriology* 184(13): 3476-3484.

Methylobacterium chloromethanicum CM4 is an aerobic alpha- proteobacterium capable of growth with chloromethane as the sole carbon and energy source. Two proteins, CmuA and CmuB, were previously purified

and shown to catalyze the dehalogenation of chloromethane and the vitamin B-12-mediated transfer of the methyl group of chloromethane to tetrahydrofolate. Three genes located near *cmuA* and *cmuB*, designated *metF*, *folD* and *purU* and encoding homologs of methylene tetrahydrofolate (methylene-H(4)folate) reductase, methylene-H(4)folate dehydrogenase-methenyl-H(4)folate cyclohydrolase and formyl-H(4)folate hydrolase, respectively, suggested the existence of a chloromethane-specific oxidation pathway from methyl-tetrahydrofolate to formate in strain CM4. Hybridization and PCR analysis indicated that these genes were absent in *Methylobacterium extorquens* AM1, which is unable to grow with chloromethane. Studies with transcriptional *xylE* fusions demonstrated the chloromethane-dependent expression of these genes. Transcriptional start sites were mapped by primer extension and allowed to define three transcriptional units, each likely comprising several genes, that were specifically expressed during growth of strain CM4 with chloromethane. The DNA sequences of the deduced promoters display a high degree of sequence conservation but differ from the *Methylobacterium* promoters described thus far. As shown previously for *purU*, inactivation of the *metF* gene resulted in a CM4 mutant unable to grow with chloromethane. Methylene-H(4)folate reductase activity was detected in a cell extract of strain CM4 only in the presence of chloromethane but not in the *metF* mutant. Taken together, these data provide evidence that *M. chloromethanicum* CM4 requires a specific set of tetrahydrofolate-dependent enzymes for growth with chloromethane.

Susarla, S., V. F. Medina, et al. (2002). "Phytoremediation: An ecological solution to organic chemical contamination." *Ecological Engineering* 18(5): 647-658.

Phytoremediation is a promising new technology that uses plants to degrade, assimilate, metabolize, or detoxify metals, hydrocarbons, pesticides, and chlorinated solvents. In this review, in situ, in vivo and in vitro methods of application are described for remediation of these compounds. Phytoaccumulation, phytoextraction, phytostabilization, phytotransformation, phytovolatilization and rhizodegradation are discussed and the role of enzymes in transforming organic chemicals in plants is presented. The advantages and constraints of phytoremediation are provided. Our Conclusions is that phytoremediation prescriptions Must be site-specific; however, these applications have the potential for providing the most cost-effective and resource-conservative approach for remediating sites contaminated with a variety of hazardous chemicals. (C) 2002 Elsevier Science B.V. All rights reserved.

Suyama, A., M. Yamashita, et al. (2002). "Molecular characterization of the PceA reductive dehalogenase of *Desulfitobacterium* sp strain Y51." *Journal of Bacteriology* 184(13): 3419-3425.

The tetrachloroethene (PCE) reductive dehalogenase (encoded by the *pceA* gene and designated PceA dehalogenase) of *Desulfitobacterium* sp. strain Y51 was purified and characterized. The expression of the enzyme was highly induced in the presence of PCE and trichloroethene (TCE). The purified enzyme catalyzed the reductive dehalogenation of PCE via TCE to cis-1,2-dichloroethene at a specific activity of 113.6 nmol . min(-1) . mg of protein(-1). The apparent K-m values for PCE and TCE were 105.7 and 535.3 μ M, respectively. Chlorinated ethenes other than PCE and TCE were not dehalogenated. However, the enzyme exhibited dehalogenation activity for various chlorinated ethanes such as hexachloroethane, pentachloroethane, 1,1,1,2-tetrachloroethane, and 1,1,2,2- tetrachloroethane. The *pceA* gene of *Desulfitobacterium* sp. strain Y51 was identified in a 2.8-kb DNA fragment and used to express the protein in *Escherichia coli* for the preparation of antibodies. Immunoblot analyses located PceA in the periplasm of the cell.

Suzuki, M., S. Nakano, et al. (2002). "Brominated labdane-type diterpenoids from an Okinawan *Laurencia* sp." *Journal of Natural Products* 65(6): 801-804.

From an unidentified species of *Laurencia* collected from Okinawan waters two novel brominated metabolites, 1 and 2, along with known halogenated compounds, 2,10-dibromo-3-chloro- α -chamigrene (3), and microcladallene A (4), were isolated and identified. The structures of these new compounds were established, as ent-labdane-type bromoditerpenes, (1S,3R,5S,6S,8S,9S,10R,13R)-1-acetoxy-3-bromo-6-hydroxy-8,13- epoxy-labd-14-ene (1) and (3R,5S,6S,8S,9S,10R,13R)-3-bromo-6- hydroxy-8,13-epoxylabd14-en-1-one (2), by interpretation-of their spectroscopic data as well as by X-ray crystallographic analysis.

Tanaka, N., V. Dumay, et al. (2002). "Bromoperoxidase activity of vanadate-substituted acid phosphatases from *Shigella flexneri* and *Salmonella enterica* ser. typhimurium." *European Journal of Biochemistry* 269(8): 2162-2167.

Vanadium haloperoxidases and the bacterial class A nonspecific acid phosphatases have a conserved active site. It is shown that vanadate-substituted recombinant acid phosphatase from *Shigella flexneri* (PhoN-Sf) and *Salmonella enterica* ser. typhimurium (PhoN-Se) in the presence of H₂O₂ are able to oxidize bromide to hypobromous acid. Vanadate is essential for this activity. The kinetic parameters for the artificial bromoperoxidases have been determined. The K_m value for H₂O₂ is about the same as that for the vanadium bromoperoxidases from the seaweed *Ascophyllum nodosum*. However, the K_m value for Br⁻ is about 10-20 times higher, and the turnover values of about 3.4 min⁻¹ and 33 min⁻¹ for PhoN-Sf and PhoN-Se, respectively, are much slower, than those of the native bromoperoxidase. Thus, despite the striking similarity in the active-site structures of the vanadium haloperoxidases and the acid phosphatase, the turnover frequency is low, and clearly the active site of acid phosphatases is not optimized for haloperoxidase activity. Like the native vanadium bromoperoxidase, the vanadate-substituted PhoN-Sf and PhoN-Se catalyze the enantioselective sulfoxidation of thioanisole.

Totevova, S., M. Prouza, et al. (2002). "Characterization of polychlorinated biphenyl-degrading bacteria isolated from contaminated sites in Czechia." *Folia Microbiologica* 47(3): 247-254.

Biphenyl-utilizing polychlorinated biphenyls (PCB)-degrading bacteria were isolated from sites highly contaminated by PCBs, and their degradation abilities were determined using GC for typical commercial PCB mixtures (Delor 103 and Delor 106). Out of twelve strains which utilized biphenyl as a sole source of carbon and energy, strains *Pseudomonas alcaligenes* KP2 and *P. fluorescens* K P 12, characterized by the BIOLOG identification system and the NEFERM test, were shown to significantly co-metabolize the PCB mixture Delor 103. DNA-DNA hybridization was used to compare both strains with well-known PCB-degraders *Burkholderia cepacia* strain LB400 and *Ralstonia eutropha* strain H850. The strain KP12 employs the same meta-fission route for degradation of chlorobenzoates as a chlorobiphenyl degrader *Pseudomonas cepacia* P166. Both isolates KP2 and KP12 belong to different phylogenetic groups, which indicates that the same geographical location does not ensure the same ancestor of degradative enzymes. We confirmed that also highly chlorinated and the most toxic congeners, which are contained in commercial PCB mixtures, can be biotransformed by members of indigenous bacterial-soil community under aerobic conditions.

Tsang, J. S. H. and J. Sze (2002). "Sec-dependent and Sec-independent translocation of haloacid dehalogenase Chd1 of *Burkholderia cepacia* MBA4 in *Escherichia coli*." *Fems Microbiology Letters* 211(2): 259-264.

2-Haloacid dehalogenases are hydrolytic enzymes that cleave the halogen-carbon bond(s) in haloalkanoic acids. We have previously isolated a cryptic haloacid dehalogenase gene from *Burkholderia cepacia* MBA4 and expressed it in *Escherichia coli*. This recombinant protein is unusual in having a long leader sequence, a property of periplasmic enzymes. In this paper, we report the functional role of this leader sequence. Western blot analyses showed that Chd1 is translocated to the periplasm. The results on the expression of Chd1 in the presence of sodium azide suggested the cleavage of the leader to be Sec-dependent. Chimeras of Chd1 and green fluorescent protein demonstrated that the leader sequence is fully functional in translocating the fusion protein to the periplasm. The expression of the chimeras in Sec mutants supported the Sec-dependent translocation. Surprisingly, recombinant Chd1 and a chimera with no leader sequence were also found in the periplasm. (C) 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Microbiological Societies.

Verce, M. F., C. K. Gunsch, et al. (2002). "Cometabolism of cis-1,2-dichloroethene by aerobic cultures grown on vinyl chloride as the primary substrate." *Environmental Science & Technology* 36(10): 2171-2177.

An aerobic enrichment culture was grown on vinyl chloride (VC) as the sole source of carbon and energy. In the absence of VC, the enrichment culture cometabolized cis-1,2-dichloroethene (cDCE) and, to a lesser extent, trans-1,2-dichloroethene (tDCE), beginning with oxidation to the corresponding DCE-epoxides. When provided with VC (1.3 mM) and cDCE (0.2-0.3 mM), the enrichment culture cometabolized repeated additions of cDCE for over 85 days. Cometabolism of repeated additions of tDCE was also demonstrated but at a lower ratio of nongrowth substrate to VC. VC-grown *Pseudomonas aeruginosa* MF1 (previously isolated from the enrichment culture) also readily cometabolizes cDCE, with an observed transformation capacity (T-c,T-obs) of 0.82/mumol of

cDCE/mg of total suspended solids (TSS). When provided with VC and cDCE, MF1 did not begin cometabolizing cDCE until nearly all of the VC was consumed. The presence of cDCE reduces the maximum specific rate of VC utilization. A kinetic model was developed that describes these phenomena via Monod parameters for substrate and nongrowth substrate, plus inactivation and inhibition coefficients. MF1 did not show any cometabolic activity on tDCE or trichloroethene and very limited activity on 1,1-DCE ($T_c, T_{obs} = 2 \times 10^{-5}$ $\mu\text{mol/mg TSS}$). Above 40 μM , tDCE and TICE noticeably increased the maximum specific rate of VC utilization, even though neither compound was consumed during or after VC consumption. High concentrations of 1,1-DCE (950 μM) completely inhibited VC biodegradation. As there is currently no evidence for aerobic biodegradation of cDCE as a sole source of carbon and energy, the results of this study provide a potential explanation for in situ disappearance of cDCE when the only other significant substrate available is VC. It is fortuitous that the VC-grown cultures tested exhibit their highest cometabolic activity toward Oft, because, it is the predominant DCE isomer formed during anaerobic reductive dechlorination of trichloroethene and tetrachloroethene.

Witt, M. E., G. M. Klecka, et al. (2002). "Natural attenuation of chlorinated solvents at Area 6, Dover Air Force Base: groundwater biogeochemistry." *Journal of Contaminant Hydrology* 57(1-2): 61-80.

Monitored natural attenuation (MNA) has recently emerged as a viable groundwater remediation technology in the United States. Area 6 at Dover Air Force Base (Dover, DE) was chosen as a test site to examine the potential for MNA of tetrachloroethene (PCE) and trichloroethene (TCE) in groundwater and aquifer sediments. A "lines of evidence" approach was used to document the occurrence of natural attenuation. Chlorinated hydrocarbon and biogeochemical data were used to develop a site-specific conceptual model where both anaerobic and aerobic biological processes are responsible for the destruction of PCE, TCE, and daughter metabolites. An examination of groundwater biogeochemical data showed a region of depleted dissolved oxygen with elevated dissolved methane and hydrogen concentrations. Reductive dechlorination likely dominated in the anaerobic portion of the aquifer where PCE and TCE levels were observed to decrease with a simultaneous increase in cis-1,2-dichloroethene (cis-DCE), vinyl chloride (VC), ethene, and dissolved chloride. Near the anaerobic/aerobic interface, concentrations of cis-DCE and VC decreased to below detection limits, presumably due to aerobic biotransformation processes. Therefore, the contaminant and daughter product plumes present at the site appear to have been naturally attenuated by a combination of active anaerobic and aerobic biotransformation processes. (C) 2002 Elsevier Science B.V. All rights reserved.

Zhang, Z. L., H. S. Hong, et al. (2002). "Transport and fate of organochlorine pesticides in the River Wuchuan, Southeast China." *Journal of Environmental Monitoring* 4(3): 435-441.

Persistent organic pollutants (POPs) such as chlorinated pesticides are of global concern due to their widespread occurrence, persistence, bioaccumulation and toxicity to animals and human. This paper summarises recent research on 18 chlorinated pesticides in an important catchment in China, by determining their concentrations and behaviour in water, sediment, soil and plants. The concentrations of: the total pesticides were in the ranges 187-893 ng l^{-1} in river water; 8.53-210 ng g^{-1} dry weight in soil, 2.66-13.45 ng g^{-1} dry weight in river sediment, and 651-2823 ng g^{-1} dry weight in plants. The predominance of beta-HCH as the major isomer of HCHs in all water, soil, sediment and plant samples was clearly observed, due to beta-HCH's resistance to biodegradation. On average beta-HCH accounted for 44%, 53%, 50%, and 46% of the total HCH concentration in water, soil, sediment and plant, respectively. Of the DDTs, DDE accounted for 48%, 43%, 53%, 55% of the total DDT, which suggested that DDT had been transformed to its metabolites, DDE and DDD, of which DDE was the more stable. The chlorinated pesticide levels in the River Wuchuan were generally below the guideline values in China, but some sites displayed levels in excess of EC Environmental Quality Standards for HCHs and DDTs. The results therefore provide important information on the current contamination status of a key agricultural watershed in China, and point to the need for urgent actions to evaluate the long-term fate and toxicity of such persistent compounds and an appropriate remediation strategy.